SYNTHESIS CHARACTERIZATION AND ANTIDIABETIC EVALUATION OF NOVEL COUMARIN ANALOGUES

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Summary

In the present research work we have undertaken synthesis of novel coumarin analogues. And the synthesized compounds were characterized by various spectrochemical methods including some of the important physicochemical parameters.

Eight of the synthesized compounds have shown free radical scavenging activity by DPPH· and Nitric oxide scavenging activity and none of the compounds shown effective antidiabetic activity.

Key words--- Diabetic, coumarin,DPPH·,Free radical, salicylates,Nitric oxide

Introduction

Diabetes mellitus is a group of metabolic diseases, characterized by high blood sugar levels that result from defects in insulin secretion, or action, or both. Type 1 diabetes previously known as insulin-dependent or childhood-onset diabetes is characterized by a lack of insulin production. Type 2 diabetes formerly called non-insulin-dependent or adult-onset diabetes is caused by the body's ineffective use of insulin. Type 2 diabetes is often associated with obesity, hypertension, hyperlipidaemia and the condition is often called metabolic syndrome formerly known as Reaven's syndrome[1.]

According to current reviews, there are two major underlying causes of the metabolic syndrome: obesity especially abdominal obesity and insulin resistance[2]. These two parameters are closely and reciprocally interrelated. It is well established that obesity can cause insulin resistance, but underlying genetic forms of insulin resistance seem to increase susceptibility for the syndrome by recapitulating the metabolic abnormalities of abdominal obesity. Type 2 diabetes modulate the risk for cardiovascular diseases and other metabolic conditions.

Conflicting results on the effects of salicylates on glucose tolerance in subjects with normal glucose tolerance or Type 2 diabetes have been reported.[5] The administration of triflusal which is chemically 2-hydroxy-4-trifluoromethylbenzoic acid led to decreased fasting serum glucose concentration in the study subjects. It was suggested that this effect was mediated through increased insulin secretion induced by salicylate directly on the beta-cell. In 1876 Ebstein concluded that sodium salicylate could make the symptoms of diabetes mellitus totally disappear[4]. Similarly, in 1901 Williamson found that "sodium salicylate had a definite influence in greatly diminishing the sugar excretion". The effect was rediscovered in 1957 when an insulin-treated diabetic, given high dose aspirin to treat the arthritis associated with rheumatic fever, no longer required daily insulin injections. While drugs that secondarily alter

the inflammatory process are undoubtly of great clinical importance, several lines of evidence suggest it might also be possible to directly target inflammation with pharmacological interventions to treat and/or prevent insulin resistance and Type 2 diabetes and modulate risk for cardiovascular diseases and other metabolic conditions. Thus our present study was focussed on synthesizing salicylate derivatives of coumarins as coumarins having benzopyrones are well known for exhibiting interesting biological activities owing to the presence of benzopyrone 2 one

Coumarin is a chemical compound, a toxin found in many plants, notably in high concentration in the tonka bean (*Dipteryx odorata*), vanilla grass (*Anthoxanthum odoratum*), woodruff (*Galium odoratum*), mullein (*Verbascum* spp), and sweet grass (*Hierochloe odorata*). It has clinical medical value as the precursor for several anticoagulants, notably warfarin. When ingested, coumarin acts as a blood thinner, and it also appears to be effective in treating some tumors. Coumarin has fungicidal properties as well. Studies have been designed to examine the hypoglycaemic effect of *Clausena anisata* (Willd) Hook [family: Rutaceae] root methanolic extract in normal (normoglycaemic) and in streptozotocin-treated diabetic rats[14]. While it is possible that the hypoglycaemic effect of the plant extract may be due, at least in part, to its terpenoid and coumarin contents, the mechanism of its hypoglycaemic action remains largely speculative, and is unlikely to be due to the stimulation of pancreatic β-cells and subsequent secretion of insulin[14]. Although *Clausena anisata* root methanolic extract is less potent than insulin as an antidiabetic agent. 27853, and *Staphylococcus aureus* ATCC 25923[15]. The inhibitory effects of coumarins were affected by their substitution patterns.

Despite the fact that there are so many synthetic and herbal drugs available, there is no proper treatment for diabetes. This inculcated the need for discovering newer and better molecules to treat diabetes in terms of controlling blood glucose as well as to reduce the complications in order to improve the quality of life.

Literature review of the compounds having benzopyrone moiety and the various chemical modifications on benzopyrones supported different biological activities such as antibacterial, antihypertensive, anticholesteromic, antiinflamatory and hypoglycaemic activities.

Materials and Methods

All the starting materials were procured from authentic sources and purity has been checked by TLC. The equipments were used according to the SOP and results were documented.

Synthesis of coumarins

Synthesis of 4, 6 - Dimethyl-7- hydroxy coumarin

Procedure

In a 100ml conical flask, 25 ml of concentrated sulphuric acid was added and kept on an ice bath. When the temperature was reduced below 10˚C, a solution of 3.23g of orcinol in 3.25 ml of redistilled ethyl acetoacetate was then added drop wise with stirring. The temperature of the mixture was maintained below 10˚C by using ice bath for 2 hours .Further, the reaction mixture was kept aside at room temperature for about 18 hours and was poured into a mixture of 50g of crushed ice and 75 ml of water with stirring. The precipitate was collected by suction filtration and was washed with three 2.5ml portions of cold water. The solid was dissolved in 50 ml of 5% NaOH solution, filtered and added to 10 ml dilute 2M sulphuric acid with vigorous stirring until the

solution was acidic to litmus. The crude product was collected by filtration at the pump, washed with four 2.5 ml portions of cold water and dried at 100˚C.

Synthesis of 4, 8- Dimethyl-7- hydroxyl coumarin

Procedure

In a 250ml conical flask, 50 ml of concentrated sulphuric acid was added and kept on an ice bath. When the temperature was reduced below 10˚C, a solution of 5.65g of 2-Methyl resorcinol in 6.5 ml of redistilled ethyl acetoacetate was then added drop wise with stirring. The temperature of the mixture was maintained below 10˚ by using ice bath for 2 hours. Further, the reaction mixture was kept aside at room temperature for about 18 hours and was poured into a mixture 100g of crushed ice and 150 ml of water with stirring. The precipitate was collected by suction filtration and was washed with three 2.5ml portions of cold water. The solid was dissolved in 100ml of 5% NaOH solution, filtered and added to 15 ml dilute 2M sulphuric acid with vigorous stirring until the solution was acidic to litmus. The crude product was collected by filtration at the pump, washed with four 2.5 ml portions of cold water and dried at 100˚C.

Synthesis of 6-Nitro-3- acetyl coumarin[62]

Procedure

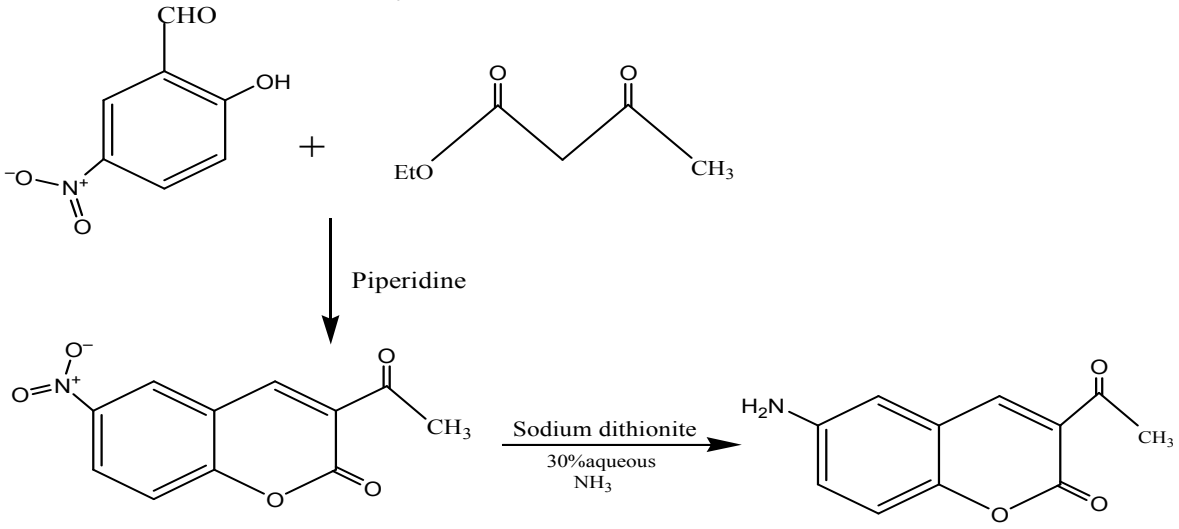
5g of 5-Nitro salicylaldehyde was dissolved in 10 ml of ethyl acetoacetate with the addition of a small quantity of absolute alcohol and the mixture was heated. The hot solution was cooled to about 40˚C and 1ml of piperidine was then added. When the liquid became viscous, scratching was done and on cooling the solid was separated. Further, the solid was triturated with a small quantity of absolute alcohol and was filtered. The residue was washed with cold alcohol until it was freed from colouring matter and was recrystallised from alcohol-acetone mixture as pale yellow needles.

Synthesis of 6-amino-3- acetyl coumarin 63

Procedure

A solution of 2.8g of sodium hydrosulfite in 12 ml water was added rapidly to a stirred suspension of 1g of 6-Nitro-3-acetyl coumarin in 8ml of 30% aqueous ammonia. The reaction mixture was warmed spontaneously and as the suspended solid began to dissolve, was boiled for 15 minutes. The mixture was then evaporated under reduced pressure and was triturated with water and extracted with ethyl acetate to get an orange coloured solid.

General scheme for the synthesis of coumarins



CHARACTERIZATION

The synthesized compounds were characterized by NMR, MASS ,IR,UV methods

Log p value of all of the synthesized compounds were determined by standard methods.

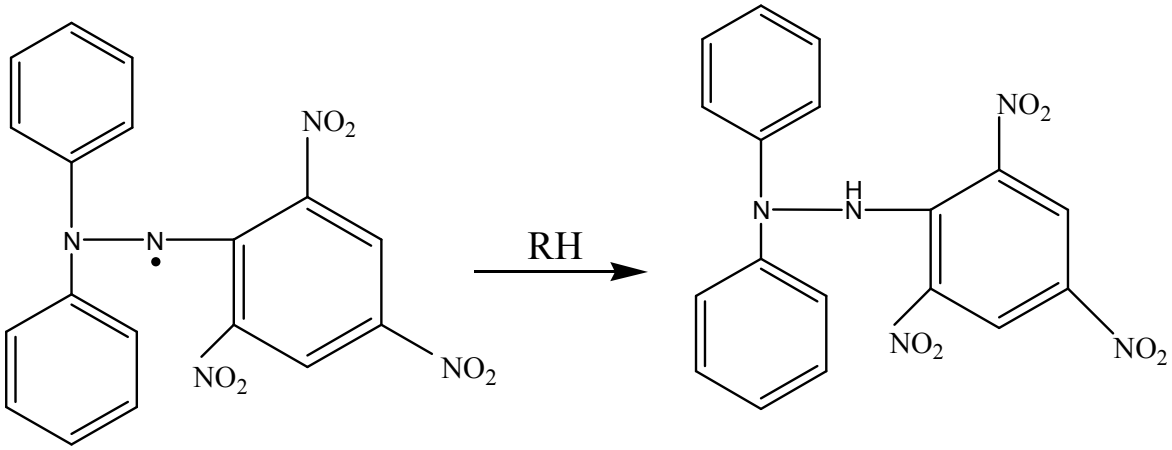
BIOLOGICAL ACTIVITY

Antioxidant activity

DPPH· Method

Principle

DPPH· 1, 1-diphenyl-2-picryl hydrazyl radical is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. Due to its odd electron, the methanolic solution of DPPH· showd a strong absorption band at 517nm. DPPH· radical reacts with suitable reducing agents, resulting in the pairing of electron and subsequent loss of colour. The colour decreases stochiometrically with the number of electrons taken up and the decrease in the absorbance can be directly measured and compared with that of the standard ascorbic acid. The samples may not be necessarily freely available to react with DPPH·, hence they react at different rates and the reaction will often not go to completion in a reasonable assay time. Therefore, the sample size that can lower the initial absorbance of DPPH· solution by 50% can been chosen as the endpoint for measuring the antioxidant activity. This method is widely reported for screening of antioxidant activity.



Procedure

- **DPPH· solution**

The DPPH· stock solution was prepared by dissolving 39 mg of the DPPH· in 50 ml of methanol.

- **Standard solution**

Ascorbic acid was used a standard free radical scavenger. This was prepared by dissolving 1mg of ascorbic acid in 10 ml of methanol to get a 100µg/ml stock solution.Serial dilutions were then made so as to get concentrations of 50 µg/ml, 25µg/ml, 12.5µg/ml and 6.25µg/ml each.

- **Test solution**

The test solutions of the test compounds were prepared in methanol, by dissolving 1 mg of the test compound in 1ml methanol to get 1000µg/ml stock solution. Serial dilutions were then made so as to get concentrations of 500µg/ml, 250µg/ml, 125µg/ml, 61.25µg/ml, 30.63µg/ml, 15.3µg/ml and 7.65µg/ml each.

- **Method**

The assay was carried out in a 96 well microtitre plate. To 100µl of methanol solution, 100µl of each of the test sample or the standard solution was added separately in wells of the microtitre plate in triplicate. Control was prepared by adding 100µl methanol in 100µl DPPH· solution. The plates were incubated at 37˚C for 20 minutes and the absorbance of each solution was measured at 540nm using ELISA reader against the corresponding test and standard blanks and the remaining DPPH· was calculated.IC_{50} is the concentration of the sample required to scavenge 50 % of DPPH· free radicals.

$$\% \text{ inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

a. Nitric oxide radical inhibition assay

Principle

Nitric oxide is produced in various types of cells and is well studied in vascular endothelium. While this species is not too reactive ,even antioxidant under physiological concentrations upto 100 nM, it reacts rapidly with oxygen to yield nitrogen dioxide radical which in turn may react with nitric oxide radical to yield nitrogen trioxide. Nitric oxide is naturally formed in activated macrophages and endothelial cells and is considered as an active agent in several pathologies based on inflammation, organ reperfusion and also may play an important role in atherosclerosis.

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be used by the use of Griess Illosvoy reaction. In the present investigation, Griess Illosvoy reagent is modified by using 0.1%w/v Naphthyl ethylene diamine dihydrochloride instead of 5% 1-naphthylamine.Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide.

Chemicals and Reagents

1. Sodium nitroprusside solution: 0.2998g of sodium nitroprusside was dissolved in 100ml of distilled water.
2. Naphthyl ethylene diamine dihydrochloride: 0.1% of the solution was made up with 20% glacial acetic acid and made up the volume to 100ml.
3. Phosphate buffer saline,pH adjusted to 7.4
4. DMSO distilled

Method

600 µl of the reaction mixture containing 400µl sodium nitroprusside, 100µl of phosphate buffer, 100µl of drug solution of different concentrations were taken in a micro centrifuge tube and incubated at 25˚C for 150 minutes. After incubation to 50µl of the reaction mixture ,100µl of sulphanilic acid was added in microtitre plate. It was mixed well and allowed to stand for diazotisation for 5 minutes .To this 100µl of Naphthyl ethylene diamine dihydrochloride was added and allowed to stand for 30 minutes in diffused light at room temperature. The absorbance was measured at 540 nm using ELISA reader against corresponding blank solution.IC_{50} is the concentration of the sample required to inhibit 50% of nitric oxide radical.

Antidiabetic activity

The various *in vitro* andiabetic activity are:

- Advanced glycation end product inhibition assay
- Alpha glucosidase inhibition assay
- Alpha amylase inhibition assay
- Non enzymatic glycosylation of haemoglobin method

In the present work, *in vitro* advanced glycation end product inhibition assay was performed and non enzymatic glycosylation by haemoglobin method was attempted.

1. Advanced glycation end product inhibition assay[68]

Principle

Non- enzymatic reaction between reducing sugar and amino group of proteins, also known as Maillard reaction, leads to the formation of glycated protein formed termed as Amadori product. Further, rearrangement, oxidation and reduction of the Amadori

product result s in the formation of several advanced glycation end products such as pentosidine carboxymethyllysine,crossline and and pyralline. Some of these products can react with a free amino group nearby and form crosslinking between proteins. The cross linked protein are postulated to confer pathological conditions in diabetes and aging such as arterial stiffness and decreased myocardial compliance resulting from loss of collagen elasticity .Thus, agents that inhibit the formation of advanced glycation end products are purported to have therapeutic potentials in patients with diabetes and age related diseases.

The oxidation process is believed to play an important role in advanced glycation end products formation. Further, oxidation of Amadori products leads to the formation of intermediate carbonyl compounds that can react with the nearby lysine or arginine residues to form protein link and advanced glycation end products. Therefore, agents with antioxidative or metal chelating property may retard the process of advanced glycation end products formation by preventing further oxidation of Amadori product and metal catalyzed glucose oxidation. In this regard, several natural compounds known to possess antioxidative property, such as curcumin, rutin, garcinol and flavonoids rich extracts, have been shown to prevent advanced glycation end products formation *in vitro and in vivo*.

Materials

- Bovine serum albumin
- glucose
- 100% w/v trichloroacetic acid
- Alkaline phosphate-buffered saline: 137mM NaCl, 8.1mM Na_2HPO_4, 2.68mM KCl, and 1.47mM KH_2PO_4 was adjusted to pH 10 with 0.25N NaOH.

Procedure

AGE inhibition assay:

The measurement of fluorescent material based on AGEs in order to detect the inhibitory effect of test samples on the Maillard reaction was performed as follows :

- Dissolved the test compounds in DMSO.
- The reaction mixtures, containing 400 µg bovine serum albumin, 200mM D-Glucose and 10 µl of test sample solution or DMSO was incubated in a total volume of 500µl of 50mM phosphate buffer pH 7.4, at 60 °C for 72 hours.
- The blank, which contained no test compound, was kept at 4 °C until measurement.
- 250µl of aliquots was cooled and transferred to 1.5 ml plastic tubes.
- 50µl of trichloroacetic acid was added to each tube and stirred.
- The supernatant was removed after centrifugation at 15,000 rpm at 4 °C for 4 minutes
- The advanced glycation end products and bovine serum albumin precipitate was dissolved with 2ml of alkaline phosphate buffer saline.
- These solutions were monitored by using spectrofluorometer to compare their fluorescence spectra and the changes in fluorescence intensity at excitation wavelength at 370 nm and emission wavelength at 440nm, based on advanced glycation end products.
- Calculated the % inhibition by

$$\% \text{ inhibition} = \frac{\text{Control} - \text{Test}}{\text{Test}} \times 100$$

2. Non enzymatic glycosylation of haemoglobin method

Procedure:

The antioxidant activities of the test compound were investigated by estimating degree of non enzymatic haemoglobin glycosylation, measured colorimetrically. Haemoglobin, 60 mg/100 ml in 0.01 M phosphate buffer at pH 7.4 was incubated in presence of 2 g/100 ml concentration of glucose for 72 hours, in order to find out the best condition for haemoglobin glycosylation. The assay was performed by adding 1 ml of glucose solution, 1 ml of haemoglobin solution, and 1 ml of gentamycin 20 mg/ 100 ml, in 0.01 M phosphate buffer at pH 7.4. The mixture was incubated in dark at room temperature for 72 hours. The degree of glycosylation of haemoglobin in the presence of different concentration of the test compound and their absence, were measured colorimetrically at 520 nm.

Results And Discussions

Antioxidant activity

Antioxidant activity is a prerequisite for performing many related biological activities, including anticancer, antiallergic, anti inflammatory, antidiabetic etc.

1) DPPH· radical scavenging method

The antioxidant activity of the synthesised test compounds JAK 1 to JAK 8 was done using DPPH· radical scavenging method. Ascorbic acid was used as the standard. Table 9, indicates the antioxidant activity and IC_{50} values of the synthesised test compounds JAK 1 to JAK 8.

Out of the 8 synthesised test compounds, the test compounds such as, JAK 6, JAK 7 and JAK 8 showed values at 41.6, 60.3 and 50.85 respectively when compared to that of Ascorbic acid at IC_{50} value of 3.72µg/ml and showed inhibitory concentration for 50% inhibition below 100 µg/ml. It is seen from the table 9 that the parent compound JAK 6 showed values at 41.6 for DPPH· radical scavenging activity than the salicylate derivatives JAK 7 and JAK 8. However, carboxamide salicylate derivative, JAK 8 showed values at 79.42 for DPPH ·radical scavenging activity than the ester salicylate derivative .The test compounds such as JAK 1, JAK 2 and JAK 4 showed antioxidant activity at 105, 256 and 215 respectively when compared to that of Ascorbic acid at IC_{50} value of 4.3 µg/ml and showed inhibitory concentration for 50% inhibition above 100 µg/ml . It is seen from the table 9 that the benzoate derivative JAK 1 showed values at 61.93 for DPPH radical scavenging activity than salicylate derivative JAK 2 .The test compounds such as JAK 1,JAK 2,JAK 4, JAK 6,JAK 7 and JAK 8 showing DPPH radical scavenging activity was found worthwhile to be tested for their antidiabetic activity. However the IC_{50} of the test compounds JAK 3 and JAK 5 was found to be very high.

NITRIC OXIDE SCAVENGING ACTIVITY

The antioxidant activity of the synthesised test compounds, JAK 1 to JAK 8 was done by using Nitric oxide radical scavenging method. Ascorbic acid was used as the standard. Table 10, indicates the antioxidant activity and IC_{50} values of the synthesised test compounds JAK 1 to JAK 8.

Out of the 8 synthesised test compounds, the test compounds such as, JAK 1to JAK 4 showed antioxidant activity compared to that of Ascorbic acid at IC_{50} value of 4.3 µg/ml and showed inhibitory concentration for 50% inhibition below 100 µg/ml. It is seen from the table 10 that the benzoate derivative JAK 1 showed values at 23.95 for nitric oxide scavenging activity than salicylate derivative JAK 2 . The test compounds JAK 5, JAK 6 and JAK 7 showed antioxidant activity at 150.34 and 225 respectively when compared to that of Ascorbic acid at IC_{50} value of 4.3 µg/ml and showed inhibitory concentration for 50% inhibition above 100 µg/ml . It is seen from the table 10 that the parent compound JAK 4 showed values at 63.44 for nitric oxide radical scavenging activity than the carboxamide salicylate derivative JAK 5 whereas the parent compound JAK 6 and the ester salicylate derivative JAK 7 possessed similar IC_{50} values at 225 as shown in the table 10. The test compounds JAK 1to JAK 7 showing nitric oxide scavenging activity was found worthwhile to be tested for their antidiabetic activity.However, the IC_{50} of the test compound JAK 8 was found to be very high.

Antidiabetic activity

The different *in vitro* antidiabetic activity performed based on

1) Non enzymatic glycosylation by haemoglobin method

The synthesised test compound JAK 2 was screened for its antidiabetic activity *in vitro* non enzymatic glycosylation by haemoglobin method . α-Tocopherol acetate was taken as the standard as shown in the table 12. However, neither the test compound nor the standard showed any antidiabetic activity.

Table 12 showing *in vitro* non enzymatic glycosylation by haemoglobin method

Comp.code	Conc(µg/ml)	Absorbance
JAK 2	1250	0.879
	625	0.134
	312.5	0.103
	156.	0.078

Standard	Conc	Absorbance
α-Tocopherol	10 mM	1.960
	2000 µM	0.465
	800 µM	0.224
	400 µM	0.150

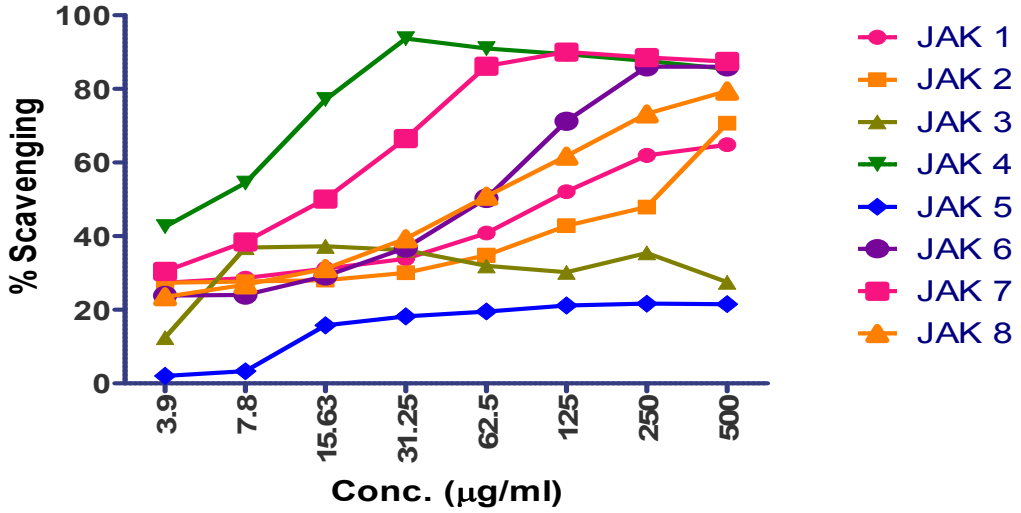
Summary and Conclusion

- The parent test compounds coumarins were synthesized
- . All the test compounds were purified by recrystallisation using solvents ,such as ethanol, methanol, acetone, ethyl acetate and glacial acetic acid and the purity was checked by melting point determination and TLC.
- All the purified test compounds including their parents were characterised by spectral studies by using UV, IR, ^{1}H NMR and Mass spectra to support their structures.
- Yields of the final test compounds were obtained in the range of 68 to 91%.
- The solubility profile and the UV absorption characteristics of the test compounds were determined by partition coefficient experiment and their λ_{max} and ε_{max} were determined.
- The λ_{max} and ε_{max} values obtained for the test compounds , were found to be in range of 256 to 328 nm and 2.09×10^3 to 2.68×10^4 $mol^{-1}cm^{-1}$ respectively.
- The IR spectra of the test compounds were characterised by the presence of specific functional groups present in the molecule.
- The ^{1}H NMR spectra of the test compounds were characterised and supported their structures.
- The mass spectra of the test compounds were characterised by the presence of molecular ion peak and its fragments.
- Log P values obtained for the test compounds , were found to be in the range of 0.36 to 1.92.
- The antioxidant activity of the test compounds were done using DPPH· radical scavenging activity and Nitric oxide scavenging activity.
- The *in vitro* antidiabetic activity of the test compounds were performed using *in vitro* non enzymatic glycosylation by haemoglobin method and advanced glycation end products inhibition .

Table 9 showing DPPH radical scavenging

Conc µg/ml	% DPPH RADICAL SCAVENGING							
	JAK 1	JAK 2	JAK 3	JAK 4	JAK 5	JAK 6	JAK 17	JAK 18
3.9	27.3	27.2	12.41	42.6	2	23.86	30.42	23.5
7.8	28.6	27.68	36.88	54.43	3.28	24	38.43	26.78
15.63	31.14	28	37.23	77.13	15.75	29.14	50	31.15
31.25	33.88	30	36.17	93.62	18.21	36.79	88.52	39.34

62.5	40.8	34.8	31.91	90.96	19.49	50.27	89.98	50.82
125	52	42.8	30.14	89.36	21.13	71.22	87.43	61.75
250	61.93	47.9	35.46	87.59	21.68	85.92	66.48	73.22
500	64.85	70.67	27.48	85.46	21.49	85.97	86.16	79.42
IC_{50}	**105**	**256**	**-**	**215**	**-**	**41.6**	**60.3**	**50.85**



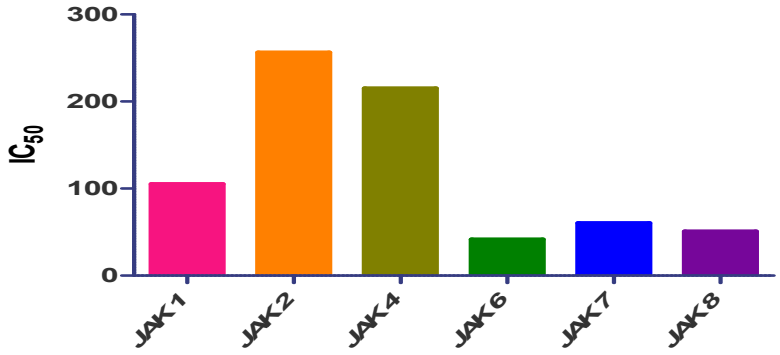


Table 10 showing Nitric oxide scavenging activity

Conc µg/ml	% NITRIC OXIDE SCAVENGING							
	JAK 1	JAK2	JAK3	JAK4	JAK5	JAK6	JAK17	JAK18
3	23.35	22.9	31.28	27.75	0.44	6.17	2.64	0.66
5	28.19	51.76	47.14	32.82	10.57	8.81	7.70	2.86
10	57.27	60.79	52.86	36.35	19.16	11.45	9.06	5.73
21	62.11	61.23	53.74	37.88	21.59	12.11	11.45	7.49
42	63.22	62.11	56.39	42.29	23.35	15.86	12.56	9.03
83	64.76	63.88	66.96	47.36	23.79	17.62	23.79	10.13

Table 12 showing *in vitro* non enzymatic glycosylation by haemoglobin method

Comp.code	Conc(µg/ml)	Absorbance
JAK 2	1250	0.879
	625	0.134
	312.5	0.103
	156.	0.078

Standard	Conc	Absorbance
α-Tocopherol	10 mM	1.960
	2000 µM	0.465
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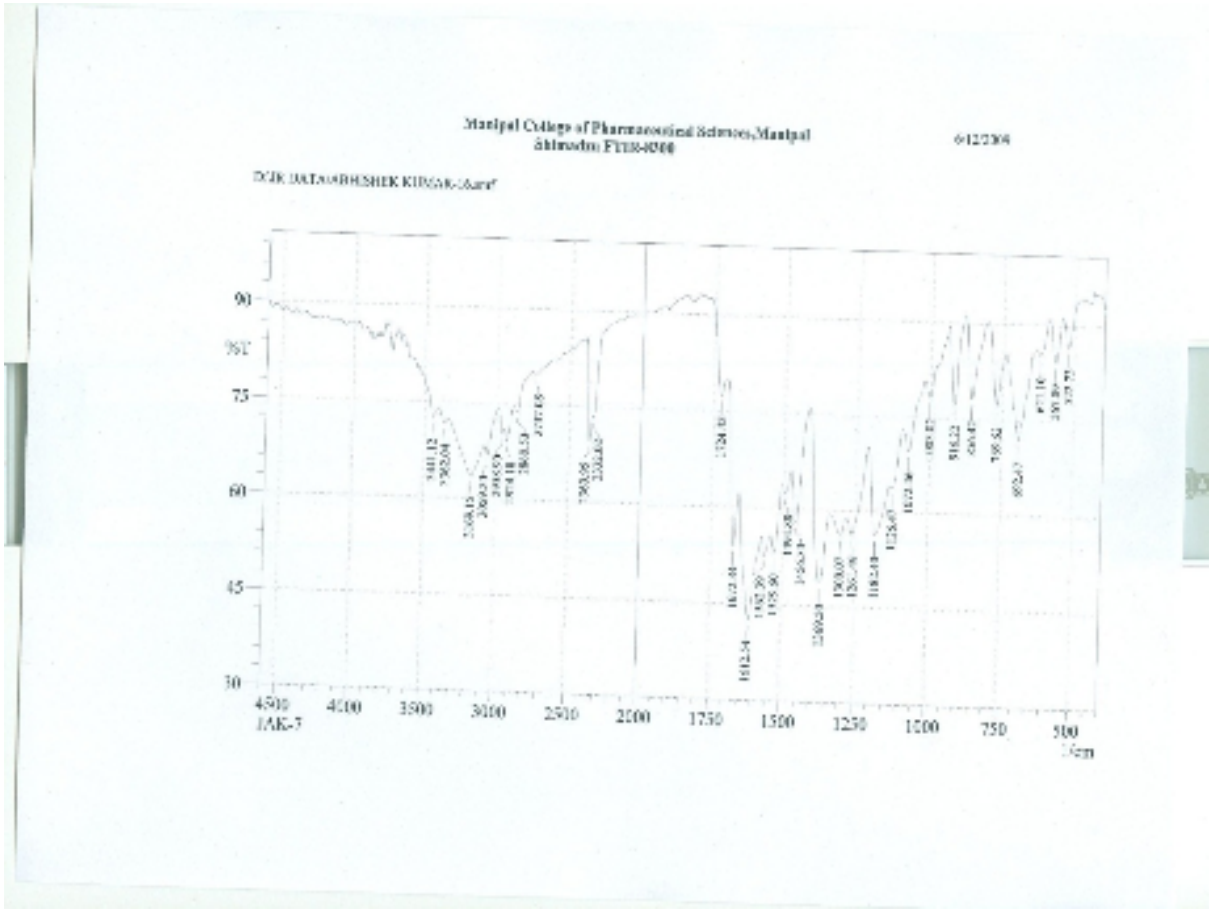
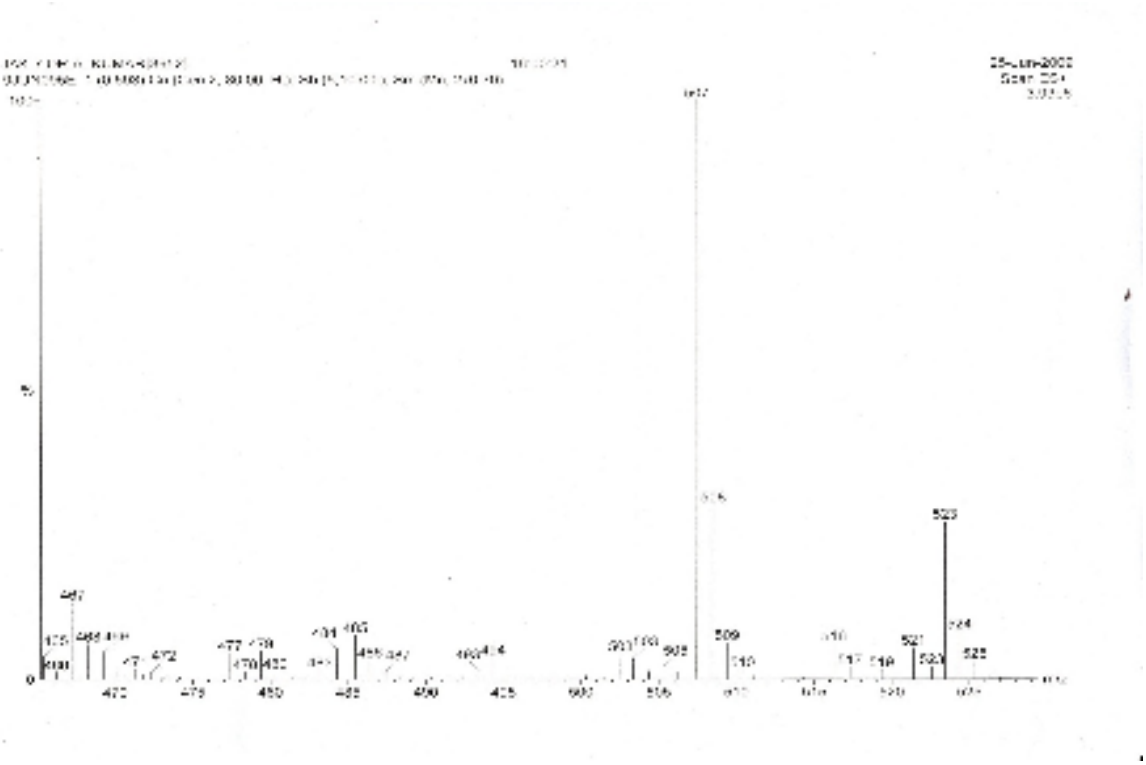
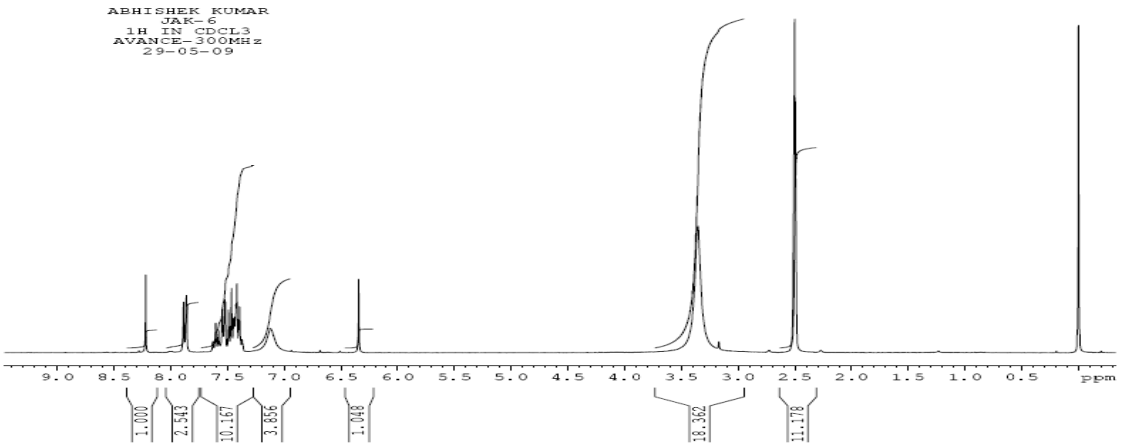
Spectral datas






ABHISHEK KUMAR
JAK-6
1H IN CDCL3
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9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.000
2.543
10.167
3.856
1.048
18.362
11.178

Conclusion

- Yields of the intermediates and the final test compounds obtained were found to be in the range of 36-86% and 68 to 91% respectively .
- 5 compounds, incuding their parents and final products are totally new and the procedure was standardised to improve the yield and all the physical constants were fixed for them.
- Out of the eight test compounds tested for their DPPH· radical scavenging activity, 6 compounds , JAK 1, JAK 2, JAK 4 showed antioxidant activity above 100µg/ml and JAK 6, JAK 7 and JAK 8 showed antioxidant activity below 100µg/ml when compared to that of standard Ascorbic acid at IC_{50} value of 3.72µg/ml .
- Out of the eight test compounds tested for their Nitric oxide radical scavenging activity, 7 test compounds , JAK 1 to JAK 4 showed antioxidant activity below 100µg/ml and JAK 5, JAK 6, JAK 7 showed antioxidant activity above 100µg/ml when compared to that of standard Ascorbic acid at IC_{50} value of 4.73 µg/ml.
- The test compound JAK 2 when screened for its antidiabetic activity *in vitro* non enzymatic glycosylation by haemoglobin method did not show any result for the test compound and the standard α- Tocopherol acetate , this method needs to be standardised further to obtain the results.
- Out of the eight test compounds screened for its antidiabetic activity by advanced glycation end products inhibition, none of the test compounds including the standard Quercetin showed any antidiabetic activity.

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