Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 534-541| ISSN:2251-6727

Synthesis, techniques, Anti-oxidant, Anti-diabetic and Anti-inflammatory activities of xanthene derivatives

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KEYWORDS:

IR, NMR**,** Anti-Oxidant, Anti-diabetic, Anti- inflammatory

ABSTRACT

The synthesised xanthene derivatives was analysed by various techniques like IR, NMR and mass spectral analysis. The biological studies like Anti-Oxidant activity carried out by DPPH scavenging assay method. The synthesised compounds like **3, 7** and **9** was better Anti-diabetic and Anti- inflammatory activities.

1. Introduction

An proficient, outstanding, and reusable medium for the fusion of 14-aryl-14H-dibenzo xanthene derivatives by one-pot mixture of β-naphthol with various aromatic aldehyde derivatives beneath solvent free state. Simple workup procedure, short reaction time, high yield, and reusability of the catalyst are the characteristic features of these reactions.[1] Fresh sulfonic acid functionalized imidazolium salts (SAFIS), as a innovative class of ionic liquids, are synthesize by ecological and undemanding actions, and used as extremely resourceful and reusable catalysts to encourage the subsequent one-pot multicomponent organic transformations.[2] The catalysts to be examine in a three constituent response to afford benzoxanthene derivative. The template-containing Zn/MCM-41 show predominantly the utmost activity. This action is due to both Lewis acid sites and the ionic template.[3]

The synthesis of 12-aryl -8, 9,10,12-tetrahydrobenzo [a] xanthen-11-one derivatives (ATXOs) via threecomponent reaction of aldehydes, 2-naphthol and 5,5-dimethyl-1,3-cyclohexadione beneath solvent-free conditions. This medium can as well used for the research of quinoxaline derivatives in a combination of H_2O and CH_3CN at 50 8C.[4,5] Synthesis of 1,8-dioxo-octahydroxanthene, 14H-dibenzo [a,j]xanthene, 12-aryl-tetrahydrobenzo [a]xanthenes-11-one and 13-aryl-5Hdibenzo[b,i]xanthene-5,7,12, 14(13H)-tetraone derivative in the existence of a catalytic quantity of nano-iron oxide encapsulate silica particle behavior of sulfonic acid. The consequences there is an proficient, environmentally gracious and magnetically recoverable medium beneath solvent-free conditions at 110–130 °C.[6-9] An efficient synthesis of biologically active 14-aryl-14H-dibenzoxanthenes has been achieved through a one-pot condensation of aryl aldehydes and β-naphthol below solvent-free situation in the existence of sulfonic acid functionalized silica (SiO_2-Pr-SO_3H), as an capable heterogeneous rock-solid acid medium with outstanding yield and tiny reaction time.[10-14]

2. Experimental

2.1. Synthetic route of xanthene derivatives

A mixture of 5,5-dimethylcyclohexane-1,3-dione, substituted benzaldehyde, acetic acid medium. The reaction mixture was refluxed for 6 hours and the completion of the reaction was monitored by thin layer chromatography technique using benzene and ethyl acetate (9:1) as the eluent. The resultant material was purified by column chromatography. The schematic representation of synthetic mode of xanthene derivatives (1-10) is represented in Scheme 1.

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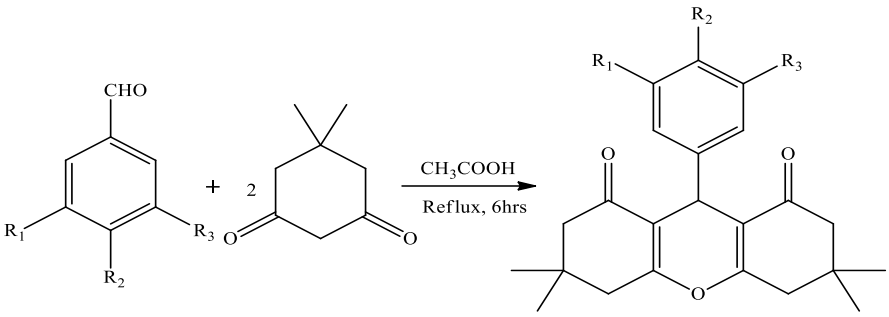
JCHR (2023) 13(4s), 534-541| ISSN:2251-6727



Compounds	R_1	R_2	R_3
1	H	$-CH_3$	H
2	$-OCH_3$	-OH	$-OCH_3$
3	H	-Br	H
4	H	$-OCH_3$	-OH
5	H	H	$-OCH_3$
6	$-OCH_3$	$-OCH_3$	$-OCH_3$
7	H	H	-OH
8	H	-Cl	H
9	H	$-OCH_3$	$-OCH_3$
10	H	$-N-(CH_3)_2$	H

Scheme 1. Synthetic route of xanthene derivatives

2.2. Spectral Measurements

The ^1H and ^{13}C NMR spectra of the synthesized compounds in DMSO were recorded on a Bruker AMX 400 MHz NMR spectrometer. The ^1H and ^{13}C NMR spectra were recorded to TMS as an internal standard and the central line of DMSO. Infrared spectra were recorded on a JASCO FT-IR-5300 Spectrometer in the range 4000–400 cm-1using KBr pellets.

2.3. DPPH radical scavenging assay

The free radical scavenging activity of the synthesized compounds were evaluated by 1,1-diphenyl-2-picrylhydrazil (DPPH) according to the previously reported method. Briefly, a 0.0001M solution of DPPH in DMSO was prepared and 10mL of this solution was added to 100mL of the solutions of all compounds in DMSO at different concentrations (200, 400, 600, 800, 1000 µL). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. Then their absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as a reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated by using the following formula:

DPPH scavenging effect (% inhibition) = $[A_0-A_t/ A_0]$ x100, Where A_0 is the absorbance of control and A_t is the absorbance of tested samples at particular time. IC_{50} value is the concentration of the compound required to inhibit 50% of DPPH• production.

2.4. Anti- inflammatory activity

The reaction was consisting of test extracts and 1% solution of bovine albumin fraction, pH of the reaction was used to little amount at 37 °C HCl. The extracts was incubated at 37 °C for 20 min and then animated to 51 °C for 20 min after cooling the turbidity was deducted spectrophotometrically at 660 nm and Aspirin was used as a standard drug.

% of inhibition = (OD of Control - OD of Sample / OD of Control) X 100.

3. Result and discussion

3.1. IR, NMR and Mass Spectral Analysis

3,3,6,6-tetramethyl-9-(p-tolyl)-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (1)

M.F.: C24H28O3: IR (cm^{-1}); 1663.60 (C=O); 3037.87 – 2874.79 (Aromatic C-H); 1625.26 (C=C) (Figure 1). ^1H NMR (DMSO, ppm); δ: 7.04 (dd, 9.20 MHz, 4H), 0.89 (s, 6H), 1.03 (s, 6H), 2.23 (s, 3H (C23 Protons)), 2.08 (s, 4H), 2.51 (s, 4H), 4.47 (s, 1H) (Figure 2). ^{13}C NMR (DMSO, ppm); δ: 20.95, 26.74, 29.14, 31.22, 32.27, 38.93, 50.45, 114.96, (128.38, 128.93, 135.78, 141.75, 163.52 for aromatic carbons), 197.06 (C=O) (Figure 3).

9-(4-hydroxy-3,5-dimethoxyphenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (2)

M.F.: C25H30O6: IR (cm^{-1}); 1661.67 (C=O); 3012.74 – 2871.98 (Aromatic C-H); 1617.09 (C=C). ^1H NMR (DMSO, ppm); δ: 0.87 (s, 6H, CH_3), 1.00 (s, 6H, CH_3),

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2.07 (s, 4H), 2.50 (s, 4H), 3.64 (s, 1H), 4.01 (s, 6H for methoxy groups), 6.34 (s, 1H (C23 – for OH - group)), 8.38 (s, 12H for aromatic protons) ^{13}C NMR (DMSO, ppm); δ: 26.69, 29.26, 31.19, 32.31, 50.54, (56.42 for methoxy carbons (C21, 22)), 106.17, 115.04, (134.63, 134.99, 147.94, 163.22, Aromatic carbons), 196.66 (C=O).

9-(4-bromophenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (3)

M.F.: C23H25BrO3: IR (cm^{-1}); 1661.52 (C=O); 2953.12 – 2875.33 (Aromatic C-H); 1625.62 (C=C). ^{1}H NMR (DMSO, ppm); δ: 0.90 (s, 6H, -CH_3), 1.04 (s, 6H, -CH_3), 2.10 (s, 4H), 2.51 (s, 4H), 4.48 (s, 1H), 7.13 (d, J = 8.4Hz, 2H), 7.42 (d, J = 8.0Hz, 2H) for aromatic protons. ^{13}C NMR (DMSO, ppm); δ: 26.94, 29.63, 31.41, 32.32, 50.43, 114.42, (128.30, 131.19, 143.32, 163.60 for aromatic carbons), 196.65 (C=O).

9-(3-hydroxy-4-methoxyphenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (4)

M.F.: C24H28O5: IR (cm^{-1}); 1666.00 (C=O); 2955.56 – 2896.74 (Aromatic C-H); 1626.13 (C=C). ^{1}H NMR (DMSO, ppm); δ: 0.92 (s, 6H), 1.03 (s, 6H), 2.28 (s, 2H), 2.10 (s, 2H), 2.51 (s, 4H), 3.68 (s, 3H), 4.38 (s, 1H), 6.64 (d, J=1.6Hz, 1H), 6.73 (d, J=8.4Hz, 1H), 8.80 (s, 1H for hydroxyl group). ^{13}C NMR (DMSO, ppm); δ: 26.97, 29.16, 30.72, 32.32, 50.54, (55.93 for –OCH_3 carbon (C23)), 112.05, 115.15, 116.22, 118.90, (137.43, 146.23, 146.40, 163.03 for aromatic carbons), 196.57 (C=O).

9-(3-methoxyphenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (5)

M.F.: C24H28O4: IR (cm^{-1}); 1667.73 (C=O); 2956.38 – 2872.42 (Aromatic C-H); 1628.27 (C=C). ^{1}H NMR (DMSO, ppm); δ: 0.90 (s, 6H), 1.03 (s, 6H), 2.25 (s, 2H), 2.10 (s, 2H), 2.51 (s, 4H), 3.68 (s, 3H for -OCH_3 protons), 4.49 (s, 1H), 6.69 (d, J=7.2Hz, 1H), 6.73 (d, J=8.0Hz, 1H), 7.25 (t, J=8.0Hz, 1H), 7.14 (s, 1H). ^{13}C NMR (DMSO, ppm); δ: 26.87, 29.13, 31.38, 32.31, 50.49, (55.31 for –OCH_3 carbon), (111.54, 114.77, 114.85, 120.71, 129.37, 146.19, 159.31, 163.49 for aromatic carbons) 196.60 (C=O).

3,3,6,6-tetramethyl-9-(3,4,5-trimethoxyphenyl)-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (6)

M.F.: C26H32O6: IR (cm^{-1}); 1667.68 (C=O); 2954.84 – 2876.42 (Aromatic C-H); 1625.29 (C=C). ^{1}H NMR (DMSO, ppm); δ: 0.92 (s, 6H), 1.04 (s, 6H), 2.07 (s, 4H), 2.25 (s, 4H), 3.67 (s, 9H for -OCH_3 protons), 4.46 (s, 1H), 6.72 (s, 2H). ^{13}C NMR (DMSO, ppm); δ: 27.5, 32.3, 38.9, 39.6, 51.5, 56.1, 60.8, 106.4, 113.9, 136.2, 136.5, 152.8, 155.0, 198.9.

9-(3-hydroxyphenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (7)

M.F.: C23H26O4: IR (cm^{-1}); 1658.50 (C=O); 2962.69 – 2875.34 (Aromatic C-H); 1617.21 (C=C). ^{1}H NMR (DMSO, ppm); δ: 0.91 (s, 6H), 1.03 (s, 6H), 2.11 (s, 2H), 2.28 (s, 2H), 2.51 (s, 4H), 4.44 (s, 1H), 6.49 (d, J=7.2Hz, 1H), 6.55 (d, J=7.2Hz, 1H), 6.64 (s, 1H), 6.98 (t, 1H), 9.25 (s, 1H for –OH group). ^{13}C NMR (DMSO, ppm); δ: 26.90, 29.16, 31.44, 32.32, 50.51, 113.62, (114.95, 115.87, 118.19, 146.04, 157.32, 163.33 for aromatic carbons), 196.58 (C=O).

9-(4-chlorophenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (8)

M.F.: C23H25ClO_3: IR (cm^{-1}); 1661.59 (C=O); 2953.05 – 2875.23 (Aromatic C-H); 1626.18 (C=C). ^{1}H NMR (DMSO, ppm); δ: 0.89 (s, 6H), 1.03 (s, 6H), 2.05 (s, 2H), 2.25 (s, 2H), 2.51 (s, 4H), 4.49 (s, 1H), 7.18 (d, J=8.4Hz, 2H), 7.28 (d, J=8.4Hz, 2H). ^{13}C NMR (DMSO, ppm); δ: 26.94, 29.07, 31.41, 32.32, 50.43, 114.42, (128.30, 130.39, 131.19, 143.72 for aromatic carbons), 163.60, 196.65 (C=O).

9-(3,4-dimethoxyphenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (9)

M.F.: C25H30O5: IR (cm^{-1}); 1665.38 (C=O); 3006.99 – 2873.89 (Aromatic C-H); 1622.33 (C=C). ^{1}H NMR (DMSO, ppm); δ: 0.92 (s, 6H), 1.04 (s, 6H), 2.11 (s, 2H), 2.29 (s, 2H), 2.50 (s, 4H), 3.67 (s, 6H for –OCH_3 protons), 4.46 (s, 1H), 6.66 (d, J=6.4Hz, 1H), 6.79 (d, J=8.4Hz, 1H), 6.72 (s, 1H). ^{13}C NMR (DMSO, ppm); δ: 26.84, 29.18, 31.01, 32.31, 50.52, 55.89, 111.78, 112.57, (115.0, 120.52, 137.31, 147.70, 148.53 for aromatic carbons), 163.21, 196.60 (C=O).

9-(4-(dimethylamino)phenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (10)

M.F.: C25H31NO3: IR (cm^{-1}); 1660.70 (C=O); 2965.62 – 2872.99 (Aromatic C-H); 1611.07 (C=C). ^{1}H NMR (DMSO, ppm); δ: 0.89 (s, 6H), 1.03 (s, 6H), 2.08 (s, 4H), 2.51 (s, 4H), 3.06 (s, 6H for –CH_3 protons), 4.47 (s, 1H), 6.94 (d, J=8.0Hz, 2H), 7.14 (d, J=9.5Hz, 2H). ^{13}C NMR (DMSO, ppm); δ: 26.34, 28.08, 32.62, 45.86, 50.43, 112.05, 113.31, (120.56, 136.33, 146.40 for aromatic carbons), 163.03, 189.17 (C=O).

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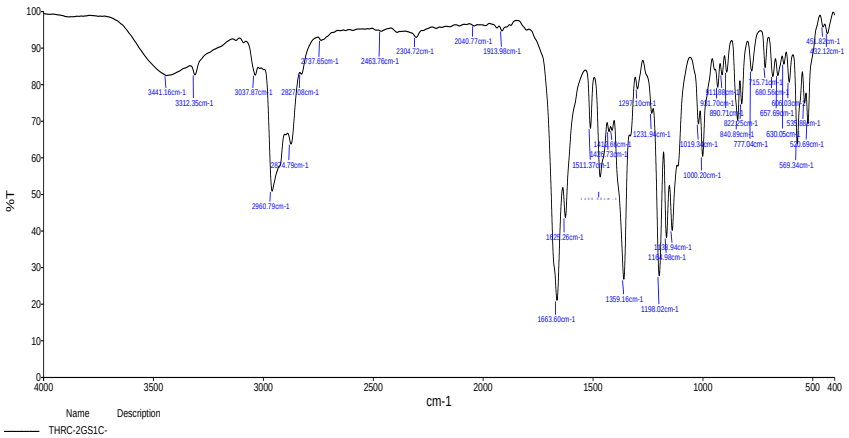

Figure 1. Representative FT-IR spectrum of compound **1**

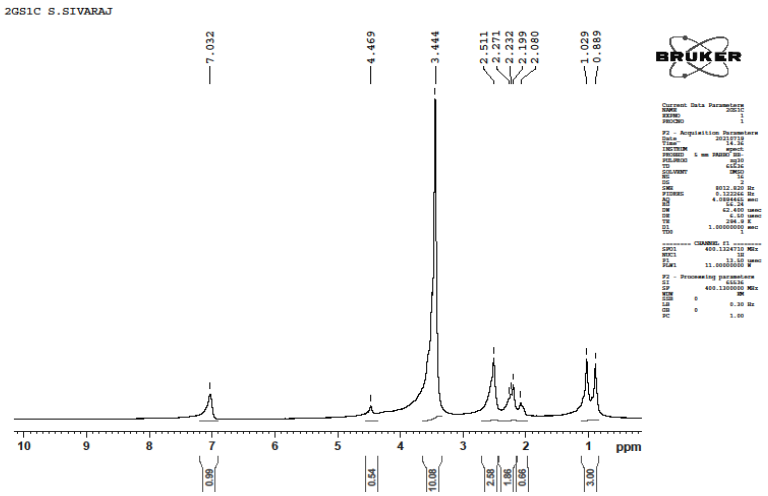

Figure 2. Representative ^{1}H NMR spectrum of compound **1**

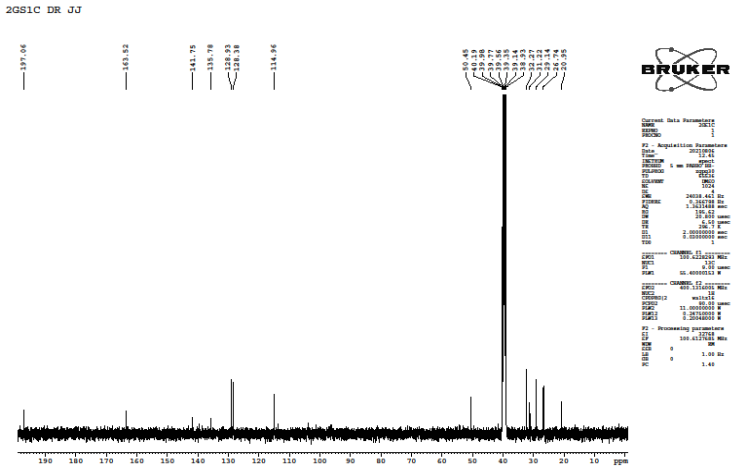

Figure 3. Representative ^{13}C NMR spectrum of compound **1**

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3.2. Anti-Oxidant activity by DPPH scavenging assay method

The percentage activity of DMSO solution of xanthene derivatives **1-10** were examined and compared with the internal standard Ascorbic acid. The compounds **7** and **9** of the result show the highest anti-oxidant activity with than compare Ascorbic acid the IC_{50} values are 140.0, 95.83 mg/ml, respectively. The most potent of compounds with hydroxyl and methoxy group as substituent showing good antioxidant activity even at very low concentration. Cytotoxicity of compound **7** for 200 mg/ml as 50%, 400 mg/ml as 65%, 600 mg/ml as 75%, 800 mg/ml as 85% and 1000 mg/ml as 90% whereas **9** for 200 mg/ml as 54%, 400 mg/ml as 66%, 600 mg/ml as 76%, 800 mg/ml as 80% and 1000 mg/ml as 95%, respectively. All the inhibition values are compared with Ascorbic acid for 200, 400, 600, 800 and 1000 mg/ml concentrations. Hence, this assay provided in sequence on the reactivity of the samples with a constant free radical. A part of the examination on the method of the anti-oxidant activity, capability of the compound to inhibit DPPH scavenging assay was studied. Among the **10** compounds, compound **7** and **9** has highest anti-oxidant activity than all others. The graphical representation of percentage inhibition at different concentrations (Table 1) of compounds **1-10** are shown in Figure 4.

Table 1. Anti-oxidant activities of compound **1-10** by DPPH scavenging assay method

S.No.	Concentration of the Sample (mg /ml)	% of Inhibition										
		1	2	3	4	5	6	7	8	9	10	Ascorbic acid
1	200	23	16	35	30	20	20	50	15	54	24	40
2	400	46	25	45	40	30	35	65	26	66	30	50
3	600	55	35	65	55	40	42	75	35	76	46	60
4	800	66	46	85	68	50	56	85	49	80	55	75
5	1000	70	56	90	70	60	66	90	59	95	60	85
IC_{50} value		564.91	885.14	413.33	551.85	800	709.73	140	837.83	95.83	744.32	391.30

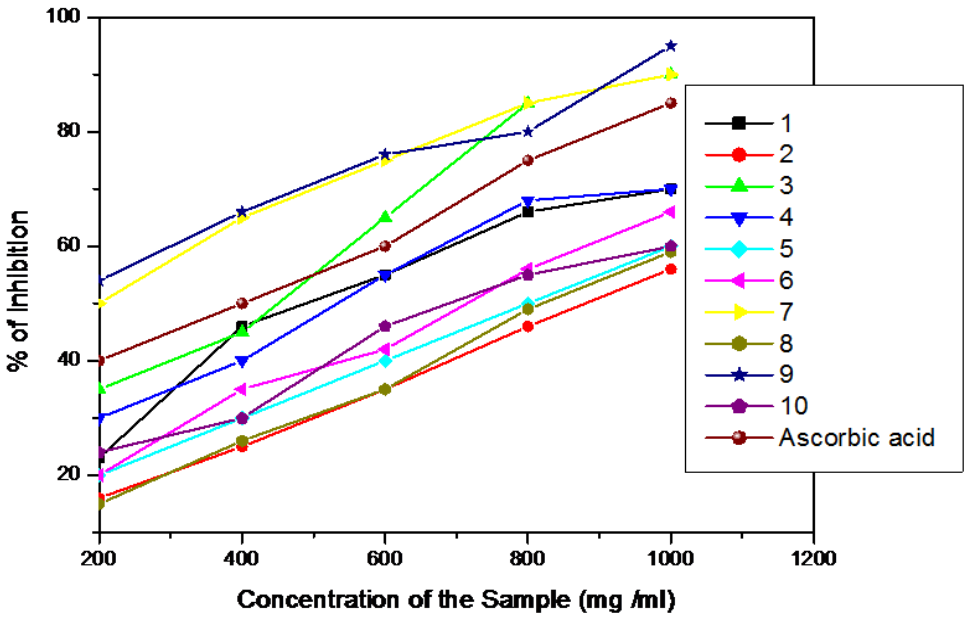

Figure 4. The percentage **of** inhibition at different concentrations of compounds **1-10**

3.3. Anti-diabetic activity of compounds 1-10

Diabetes mellitus is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. A therapeutic approach to decrease the hyper glycaemia is to inhibit the carbohydrate digesting enzymes, thereby preventing the breakdown of carbohydrates into monosaccharides which is a main cause of increasing blood glucose level. Therefore, developing compounds having inhibitory activities towards carbohydrate hydrolyzing enzymes may be a useful way to manage diabetes. The results suggest that compounds **9, 7** and **3** have higher activity than compared to standard ascarbose one and the IC_{50} values are 3.33, 7.62, and 11.75 are shown in table (**Table 2**). The compounds **9, 7** and **3** are used with a proper diet and control high blood sugar in people with type diabetes and compound **1** and **4** has moderated activity were observed and lower diabetic activity

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observed at remaining moieties **2, 5, 6, 8** and **10** was shown in **figure 5.** Hence, the biomolecules likely enhanced the anti-diabetic potential of the synthesized compounds. However, the foregoing results suggest that the synthesized xanthene derivatives are potentially better anti-diabetic particles at inhibiting carbohydrate digesting enzymes, and could prove an effective approach in the diabetes care.

Table 2. Anti-diabetic activity of compounds **1-10**

S.No.	Concentration of the Sample (mg/ml)	% of inhibition										
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Ascarbose**
1	20	25	14	55	25	19	12	56	19	58	20	50
2	40	35	20	65	35	25	23	66	29	69	30.5	60
3	60	45	30	70	46	36	33	75	38	76	40.9	70
4	80	60	45	82	58	46	46	85	49	85	50.7	80
5	100	70	55	95	68	58	60	95	59	98	60	90
IC_{50} value		65.21	92.14	11.75	66.60	86.66	85.54	7.62	82.4	3.33	79.12	20

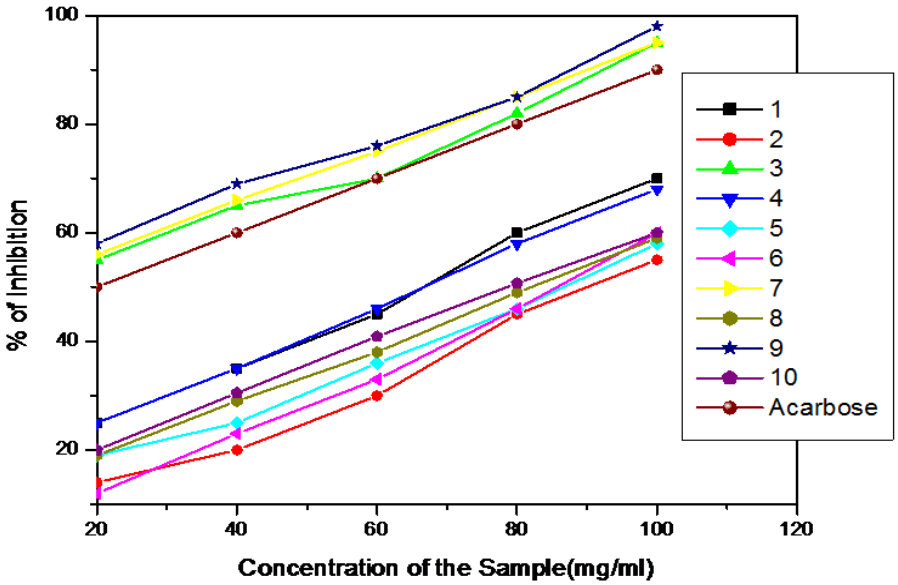

Figure 5. The Anti-diabetic activity at different concentrations of compounds **1-10**

3.6. Anti- inflammatory activity

The Albumen denaturation is a well recognized cause of inflammation. Production of autoantigen in confident arthritic sickness is owing to denaturation of protein. The method of denaturation involves a modification in electrostatic hydrogen, hydrophobic and disulfide bonding. Aspirin was used since a standard anti-inflammation drug as shown in Table 3 and Figure 6. The protein denaturation technique was agreed at 100µg/ml 200µg/ml, 300µg/ml, 400µg/ml and 500 µg/ml. The synthetic compounds **3, 7** and **9** shows better activity the compared to standard aspirin. compound **3** the % of cytotoxicity for 100 µg/ml as 45 %, 200 µg/ml as 56%, 300 µg/ml as 66%, 400 µg/ml as 78 and 500 µg/ml as 88% and compound **7** and **9** % of cytotoxicity for 100 µg/ml as 50 %, 200 µg/ml as 60%, 300 µg/ml as 70.3%, 400 µg/ml as 80.6 % and 500 µg/ml as 95 %; cytotoxicity for 100 µg/ml as 50%, 200 µg/ml as 60%, 300 µg/ml as 70%, 400 µg/ml as 80% and 500 µg/ml as 98%.

These inhibition values are compared with Aspirin for all other compounds lower activities. Albumen Denaturation show significant change when the concentrations are 100, 200, 300, 400 and 500 µg/ml for the compound isolated and % of cytotoxicity values are compared with Aspirin. The in-vitro studies of Anti- inflammatory activity indicates that the inhibition percentage of compound by Albumen Denaturation method. Inhibition percentage is higher in the compounds **3, 7** and **9** compared to other compounds.

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Table 3. Anti-inflammatory activity of compounds **1-10**

S.No.	% of Protein Denaturation											
	Concentration of the Sample (µg /ml)	1	2	3	4	5	6	7	8	9	10	Aspirin
1	100	25	12	45	23	30	30	50	28	50	18	40
2	200	36	24	56	45	40.3	40.6	60	36	60	28	55.0
3	300	45	36	66.0	55	50.6	55	70.3	46	70	39	66
4	400	60	45	78	65	66	66	80.6	58	80	46	76
5	500	70	55	88	75	76	76	95	66	98	59	90
IC_{50} value		324.56	445.79	146.29	279.03	278.07	270.01	108.49	332.65	113.79	420	172.72

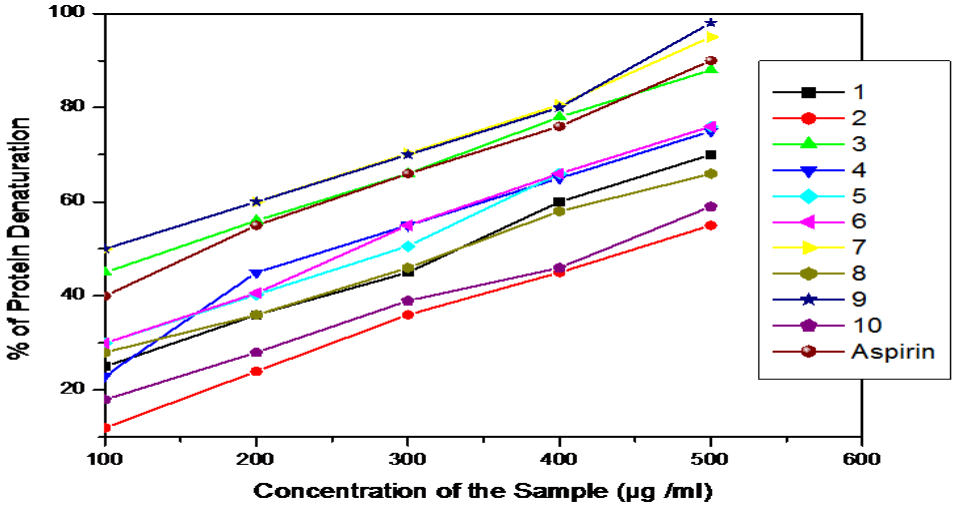

Figure 6. Anti-inflammatory activity of compounds **1-10**

4. Conclusion

Anti-oxidant activity compounds 7 and 9 show the highest anti-oxidant activity with than compare Ascorbic acid the IC_{50} values are 140.0, 95.83 mg/ml, respectively. The most potent of compounds with hydroxyl and methoxy group as substituent showing good antioxidant activity even at very low concentration. Anti-diabetic activity of compounds 9, 7 and 3 has higher activity than compared to standard ascarbose one and the IC_{50} values are 3.33, 7.62, and 11.75. The synthesized xanthene derivatives are potentially better anti-diabetic particles at inhibiting carbohydrate digesting enzymes, and could prove an effective approach in the diabetes care. Anti-inflammatory activity shows the Aspirin was used since a standard anti-inflammation drug compounds 3, 7 and 9 shows better activity the compared to standard aspirin. The inhibition percentage is higher in the compounds 3, 7 and 9 compared to other compounds.

Acknowledgement

Special thanks to Mr. M. Santhosh Kumar, Research Scholar, Department of Microbiology, Thiruvalluvar University, Serkadu, Vellore, for helping the biological activities. Mr. S. Sivaraj, Research Scholar, Department of Chemistry, Annamalai University, for helping the NMR facility. Next I thanks to St. Joseph College, Tiruchirappalli, for providing IR facility.

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