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Antiglycation and anticancer activity of some newer synthetic flavones

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ABSTRACT

Flavonoids have been used for prevention and treatment for diabetes and cancer.Several attempts have been made earlier to substitute the γ-benzopyronepharmacophore in the flavonoid ring. We have substituted α-position in the γ-benzopyrone ring with a thiophenyl group.Thus the newer chalcones (JPC-1 and JPC-19), 3-hydroxy flavones (JPC-2 and JPC-20) and many new substituted 3-benzyloxy derivatives of flavones were synthesized, purified, characterized by their spectral studies, and their logs P, ε_{max}values were determined. The synthesized compounds were screened for in vitro antiglycation and anticancer activities. In the in vitroglycosylation usinghaemoglobin-glucose method, test compounds JPC-6 and JPC-10 showed activity less than 100µg/ml when compared to that of α-tocopherol acetate. All the other test compounds showed antiglycation activity more than 100µg/ml.In the BSA-glucose assay, JPC-8 showed antiglycation activity less than100 µg/mland was comparable to quercetin. Test compounds JPC-10 and JPC-13 showed significant in vitroanticancer activity with IC_{50}values 43.50 and 67.44 µg/ml respectively, when they were tested on Ehrlich's Ascitic Carcinoma (EAC)cells by trypan blue exclusion assay.

Key words: Antiglycation,Anticancer,Flavones, Chalcones

INTRODUCTION

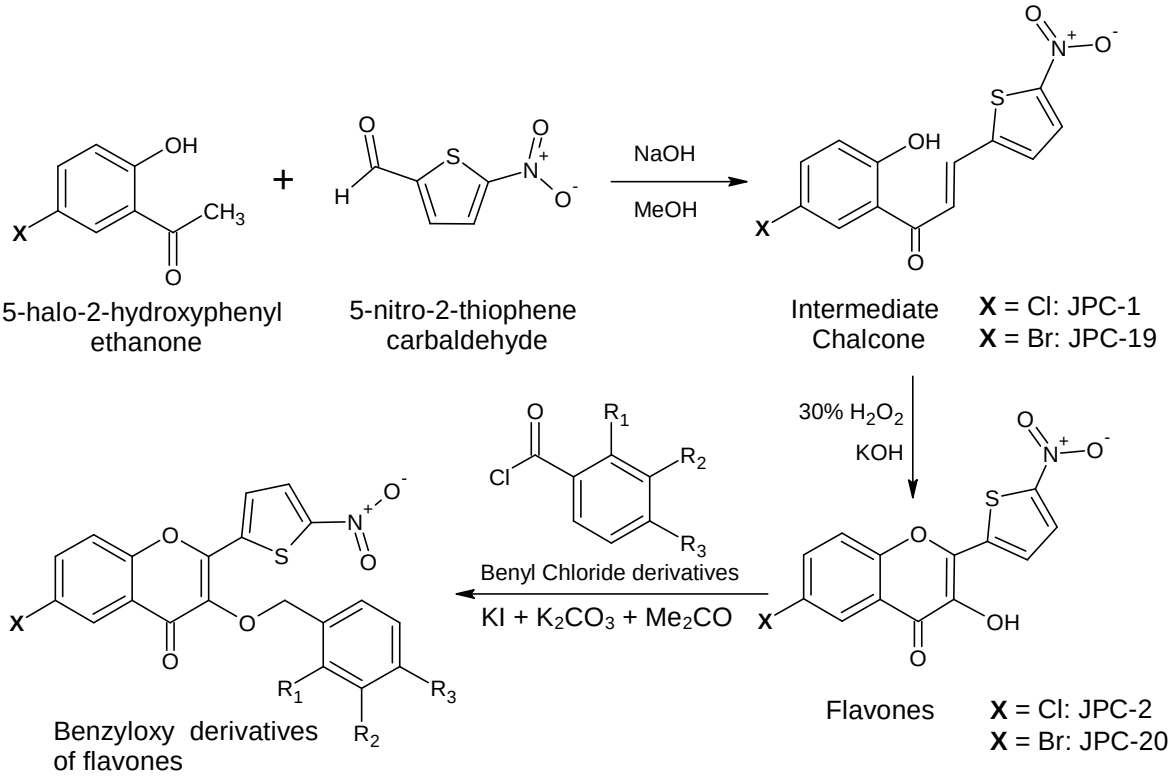
Theoxidative stress play an important role inpathogenesis and progression of diabetes mellitus, cancer and related metabolic complications[1]. Free radicals are formed disproportionately in diabetes by increased glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance[2]. Flavonoids have been reported for antidiabetic and anticancer activity[3-4]. Some of the flavonoids were reported for their antiglycation activity whereby they have ameliorated diabetes and prevented diabetes related metabolic complications[5]. Similarly, flavonoids have been exploited for the treatment and prevention of cancer and one of the flavonoids, flavopiridol was approved for therapyin leukemia where, it acts by inhibiting CDKs in leukemia cells[6].

All flavonoids have γ-benzopyrone as a pharmacophore with phenyl ring attached at α or β-position[7]. Earlier we have reported several such synthetic flavonoids and their biological activity[8-10]. In the present study, we have introducedthiophenyl moiety at α-position of γ-benzopyrone, to study the enhanced biological activity.The newly synthesized flavonoids are further screened fortheiranti-glycation and anticancer activity.

MATERIALS AND METHODS

Chemicals and instruments

Chemicals were purchasedfrom Aldrich, Himedia, Merc andRankem. All the chemicals were of AR and LR grade and solvents were of HPLC grade. Melting points were determined on a melting point apparatus (Shital Scientific Industries, Mumbai) and are uncorrected. The reactions were monitored by TLC and the Rf values were determined on pre-coated TLC plates. $\lambda_{max}$and ε_{max} for the test compounds were obtained on UV–visible spectrophotometer (Shimadzu UV-Visible spectrophotometer UV-1650 PC) in methanol (HPLC grade). The FTIR studies were done on Shimadzu FTIR 8310. ^{1}H NMR spectra were taken on a NMR (AMX 400) and the mass spectra were recorded on QP5050-GC-MS (Shimadzu, Japan).LogP values for the test compounds were determined using shake flask method[9].



Compound	X	R_1	R_2	R_3
JPC-3	-Cl	-H	-H	$-NO_2$
JPC-4	-Cl	$-NO_2$	-H	-H
JPC-5	-Cl	-H	-H	-Cl
JPC-6	-Cl	-H	$-NO_2$	-H
JPC-7	-Cl	-Cl	-H	-H
JPC-8	-Cl	-H	-H	$-OCH_3$
JPC-9	-Cl	-H	$-OCH_3$	-H
JPC-10	-Cl	-H	-H	$-CH_3$

Compound	X	R_1	R_2	R_3
JPC-11	-Cl	-H	-H	-F
JPC-12	-Cl	-F	-H	-H
JPC-13	-Br	-H	-H	$-NO_2$
JPC-14	-Br	-H	$-NO_2$	-H
JPC-15	-Br	$-NO_2$	-H	-H
JPC-16	-Br	-H	-H	-Cl
JPC-17	-Br	-Cl	-H	-H
JPC-18	-Br	-H	-H	-F

Fig.1. Scheme of synthesis for chalcones and flavones

General procedure for the synthesis

Equi-molar concentrations of 5-halo-2-hydroxyphenyl ethanoneand 5-nitro-2-thiophene carbaldehyde were stirred in an ice-cold condition in the presence ofNaOHfor 5 hours and it was then poured into an ice cold HCl, whichgave intermediate chalcones (JPC-1 and JPC-19; Fig.1). The Intermediate chalcones were then purified by recrystallization and were further used for the preparation ofthe corresponding 3-hydroxyflavones.The intermediate

chalcones was dissolved in methanoland NaOH. Further, resulting solution was cooled and stirred at ice-cold condition with drop-wise addition of 30% H_2O_2.The final solution wasstirred for 5 hours and the mixture was then poured onto ice-cold HClto get the corresponding 3-hydroxyflavone derivative (JPC-2 and JPC-20; Fig. 1).

To a suspension of the 3-hydroxyflavone derivative, benzyl halides, KI, and freshly ignited anhydrous K_2CO_3, dry acetone was added and refluxed for 5 hours. The reaction mixture was then filtered, evaporated and was subjected to percolation by passing through column of silica-gel to obtain corresponding flavone derivatives (JPC-3 to JPC18). All the flavones and chalcones were then purified by recrystallization and they were obtained in high purity and the structure was later confirmed by melting point, UV, IR, Mass and NMR spectral studies.

BIOLOGICAL ACTIVITY

Antiglycation activity

The bovine serum albumin (BSA)-glucose assay was carried out in order to quantify anti-glycosylation activity of test compounds[8]. The inhibition of glycosylation by test compounds was calculated and expressed as IC_{50} with quercetin as standards.

In hemoglobin-glucose assay, solution of test compounds at different concentrations was incubated in a mixture containing glucose (2%), hemoglobin (0.6%) and gentamycin (0.2%) in 10 mM phosphate buffer (pH 7.4). The reaction mixtures were then incubated in dark at room temperature for 72 hours with intermittent shaking and glycosylated hemoglobin was determined using NBT-reagent [11]. The antihemoglobin-glucose glycation was determined using α- tocopherol acetate as standard.

Anticancer activity by trypan blue exclusion assay

The cancer cell lines [Ehrlich's Ascitic Carcinoma (EAC)], wereobtained from National Centre for Cell Sciences (NCCS), Pune, and cultured at the cell lab facilities of Department of Pharmacology, Manipal College of Pharmaceutical Sciences and further, they were maintained as ascitis tumor by serial transplantation in Swiss albino mice.The ascitic fluid was withdrawn from the peritoneal fluid, washed with PBS and cell viability was then checked by trypan blue dye using haemocytometer.The stock cell suspension of 1 $X10^4$ was made with phosphate buffer saline (PBS) from which, 0.1 ml of the suspension was taken in sterile test tubes and incubated with 0.1 ml of varying concentrations of test compounds in 0.1% DMSO and 0.7 ml of PBS for 3 hours at 37 °C[12]. After incubation,0.1 ml of 1% w/v trypan blue solution was added and mixed well. The total number of dead and living cells was then counted usinghaemocytometer and the percentage viability was calculated as follows:

Percentage viable cells=(Number of unstained cells/ Total number of cells) ×100

Table 1: Showing physicochemical properties of different substituted flavone *

Comp Code	MF	% Yield	MP(°C)	Rf[#]	λ_{max}	ε_{max} (l mol/cm)	logP
JPC-1	$C_{13}H_8ClNO_4S$	56	68	0.46	372	23687.48	2.84
JPC-2	$C_{13}H_6ClNO_5S$	47	86	0.35	295	14852.44	2.38
JPC-3	$C_{20}H_{11}ClN_2O_7S$	39	106	0.86	271	1191.61	1.78
JPC-4	$C_{20}H_{11}ClN_2O_7S$	34	116	0.87	264	11162.08	1.96
JPC-5	$C_{20}H_{11}Cl_2NO_5S$	25	104	0.51	260	10190.28	1.29
JPC-6	$C_{20}H_{11}ClNO_5S$	28	112	0.89	289	13024.00	1.58
JPC-7	$C_{20}H_{11}Cl_2NO_5S$	29	102	0.55	259	10816.42	1.19
JPC-8	$C_{21}H_{14}ClNO_6S$	28	106	0.43	280	12461.58	1.64
JPC-9	$C_{21}H_{14}ClNO_6S$	30	110	0.47	275	11046.31	1.76
JPC-10	$C_{21}H_{14}ClNO_5S$	35	108	0.59	262	10238.30	2.75
JPC-11	$C_{20}H_{11}ClFNO_5S$	31	104	0.39	263	10270.06	1.9
JPC-12	$C_{20}H_{11}ClFNO_5S$	33	104	0.37	264	10540.09	1.95
JPC-13	$C_{20}H_{11}BrN_2O_7S$	37	118	0.72	284	13721.64	1.78
JPC-14	$C_{20}H_{11}BrN_2O_7S$	35	122	0.77	270	12656.08	-
JPC-15	$C_{20}H_{11}BrN_2O_7S$	33	114	0.73	290	14127.29	-
JPC-16	$C_{20}H_{11}BrClNO_5S$	32	132	0.42	264	10538.01	-
JPC-17	$C_{20}H_{11}BrClNO_5S$	31	128	0.44	260	11805.46	-
JPC-18	$C_{20}H_{11}BrFNO_5S$	28	130	0.42	265	11728.24	-
JPC-19	$C_{13}H_8BrNO_4S$	54	76	0.43	369	22578.31	-
JPC-20	$C_{20}H_8BrNO_5S$	44	90	0.54	285	15346.92	-

** Solvents used for recrystallization of the test compounds was methanol except for the compounds JPC-1, JPC-2, JPC-19 and JPC-20, it was glacial acetic acid.*

[#] Solvent system: n-hexane: ethyl acetate (6:2) for JPC-1, JPC-2, JPC-19, JPC-20; chloroform: ethyl acetate (7:3) for JPC-3, JPC-4, JPC-5, JPC-6, JPC-8, JPC-9, JPC-10, JPC-13, JPC-14; chloroform: acetone (7:3) for JPC-7, JPC-11, JPC-12, JPC-16, JPC-17, JPC-18.

RESULTS AND DISCUSSION

Chemistry

The newer chalcones and 3-hydroxy flavones were synthesized with yield, ranging from 25 to 55%. The logP values were in the range between 1.5 and 3 as shown in the table 1.

Spectral data for the representative molecule

6-chloro-3-[(4-methoxybenyl)oxy]-2-(5-nitrothiophen-2-yl)-4H-chromen-4-one (JPC-8): IR (KBr) (cm^{-1}): 1645.33 (C-O-CH_2), 1502.26 [Ar-NO_2 (N=O)], 752 (C-Cl);^{1}HNMR (DMSO-d6): δ 3.72 (s 12), δ 0.0082 (s 3); MS: m/z 444 (M^+).

BIOLOGICAL ACTIVITY

Antiglycation activity: The test compounds were subjected for their antiglycation activity and compared with the standard quercetin. In BSA-glucose antiglycation assay, the synthetic compounds JPC-3, JPC-8, JPC-10 and JPC-12 showed IC_{50} values at 340, 30, 170 and 229 µg/ml respectively as shown in table 2 and were significantly higher than that of the standardquercetin (IC_{50} = 354 µg/ml).Further, in haemoglobin-glucose assay JPC-6 and JPC-10 showed considerable activity and theIC_{50} was less than 100 µg/ml, compared to that of the standard α-tocopherol acetate(IC_{50}:12.7µg/ml).All remaining test compounds showed antiglycation activity more than 100 µg/ml as shown in table 2.

Anticancer activity: The test compounds synthesized were subjected for their anticancer activity by trypan blue dye exclusion assay using EAC cells.Trypan blue dye exclusion assay was performed to assess the preliminary anticancer activity of the synthesized compounds. Test Compounds, JPC-10 and JPC-13 were found to be cytotoxic with IC_{50}values at 43.50 and 67.44 µg/ml. However, test compounds, JPC-5, JPC-7, JPC-11 and JPC-12 showed anticancer activity with IC_{50} values of 151.50, 132.49, 128.22 and 147.45µg/ml respectively, as shown in table 2.Further, test compounds, JPC-10 and JPC-13 were found as potent as anticancer compounds tested among the synthesized compounds on EAC cells.

Table 2:Antiglycation and anticancer activity*

Compound code	IC $_{50}$ (µg/ml)		
	BSA-Glucose assay	Hemoglobin-glucose assay	Anticancer activity
JPC-1	-	-	-
JPC-2	-	-	-
JPC-3	340	344.7	-
JPC-4	685	53.3	371.35
JPC-5	736	146	151.50
JPC-6	477	97.3	-
JPC-7	-	724.7	132.49
JPC-8	30	>1000	371.35
JPC-9	600	710.7	191.94
JPC-10	170	56.3	43.50
JPC-11	-	106.9	128.22
JPC-12	229	414.8	67.44
JPC-13	>1000	354.5	-
JPC-14	-	-	-
JPC-15	-	-	-
JPC-16	-	-	-
JPC-17	-	-	-
JPC-18	-	-	-
JPC-19	-	-	-
JPC-20	-	-	-
Quercetin	354.00	-	-
α-Tocopherol	-	12.7	-

** Data for the compounds showing activity are mentioned in the table*

CONCLUSION

The γ-benzopyronepharmacophore in the flavonoid ring could be further modified with other smaller hetero aryl substitution such asthiophenyl substitution at α-position.The 3-hydroxyflavones synthesized with thiophenyl ring at α-position showed moderate*in vitro* antiglycation and anticancer activity. Thus, the test compounds could be further studied for their biological activity in diseases where, the glycation process increase during the pathogenesis or progression.

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