AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Investigation of Flavones as Efficient anti–oxidant and anti-inflammatory Agents

Rajashree A. Markandewar*[1], M. H.Ghante[2], M. A. Baseer[3].

1Department of Applied Chemistry, Ballarpur Institute of Technology, Ballarpur-442701.
2Department of Pharmacognosy, Nanded Pharmacy College, Nanded-431601
3Department of Chemistry, Yeshwant Mahavidyalaya, Nanded-431601

ABSTRACT

Flavones, have been synthesized by the oxidative cyclization of 2'-hydroxychalcones using catalytic amount of iodine in presence of dimethyl sulfoxide (DMSO) in maximum yield. The synthesized compounds were evaluated for anti-oxidant and anti-inflammatory potential**.** Two compounds showed good anti-inflammatory activity as compared to their antioxidant activities. The synthesized compounds were characterized by IR, ^{1}HNMR and MS Spectroscopy.

Keywords: Flavones, anti-oxidant, anti-inflammatory, oxidative-cyclization, DMSO.

*Corresponding Author Email: rajashree.markandewar@gmail.com
Received 01 December 2013, Accepted 11 January 2014

Please cite this article in press as: Markandewar RA. *et al.*, Investigation of Flavones as Efficient anti–oxidant and anti-inflammatory Agents. American Journal of PharmTech Research 2014.

INTRODUCTION

Flavones (2-phenylchromen-4-one or (2-phenyl-1-benzopyran-4-one) represents one of the largest sub-groups within the flavonoids. Various biological activities of flavones in plants and in human nutrition and health make them valuable targets for metabolic engineering[1].

Flavones have been reported to possess many useful properties, including anti-inflammatory activity[2], oestrogenic activity[3], enzyme inhibition [4], antimicrobial activity [5], antiallergic activity[6], antioxidant activity [7], vascular activity [8]and cytotoxic antitumor activity [9].The members of this class synthesized, exhibit diverse biological activities like antiviral [10] and anti-hepatotoxic [11], depending upon their pattern of oxygenation. Flavones, an important and abundant group of flavonoids have been synthesized, by the oxidative cyclization of 2'-hydroxychalcones.DMSO is known to facilitate the reaction in different ways when used as reaction medium[12].

Interest in the biological activity of the flavonoids increases due to the potential health benefits of these polyphenolic components of foodstuff. Our research investigates biological properties of the flavonoids and their newly synthesized derivatives, focuses on the evaluation of their antioxidant potential and their anti inflammatory potential.

In the present study, novel substituted 2-4-(pyrrolidine-l-yl-phenyl) chroman-4-one, 2-4-(methyl-piperazine-1-yl-phenyl) chroman-4-one, 2-(4-piperidine-1-yl-phenyl) chroman-4-one and 2-4-(4-ethyl -piperazine-1-yl-phenyl) chroman-4-one are synthesized by oxidative cyclization of corresponding 2'-hydroxychalcones in presence of catalytic amount of Iodine in DMSO .

MATERIALS AND METHOD:

A solution of 1-(2'-hydroxy-substituted phenyl)-2-propen-1-ones in DMSO and few crystals of iodine was refluxed for 2 to $2^1/_2$ hours till the starting material had completely undergone conversion (monitored by TLC) The reaction mixture was cooled and to it was added sodium thiosulphate solution (10%). The solid so obtained was filtered and dried. The dry solid on recrystallization from alcohol afforded flavone. The M.P. and yields are listed in Table.1 The structures of flavones were confirmed by spectral analysis (IR, ^{1}H NMR and MS).

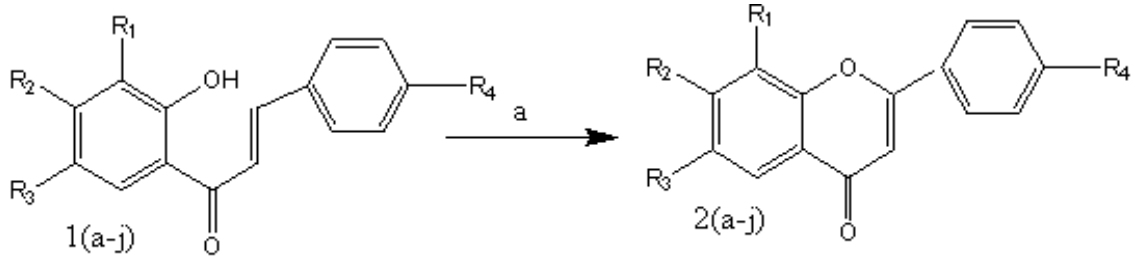
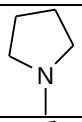
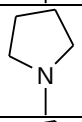
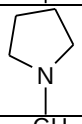
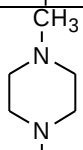
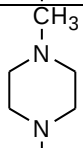
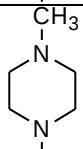
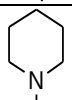
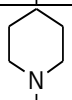
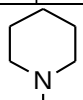
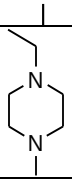


Figure -1 Cyclization of Chalcones to Flavones Reagents (a): Iodine,DMSO ,reflux, 2-2.5 h

Table-1- Physical data of synthesized Flavones.

Sr. No.	Entry	R_1	R_2	R_3	R_4	Molecular Formula	Yield (%)	M.P. ^{0}C
1	2a	H	H	H		$C_{19}H_{17}NO_2$	92	238
2	2b	H	H	Cl		$C_{19}H_{16}ClNO_2$	90	156
3	2c	I	H	CH_3		$C_{20}H_{18}INO_2$	89	173
4	2d	H	H	H		$C_{20}H_{20}N_2O_2$	88	118
5	2e	H	H	Cl		$C_{20}H_{19}ClN_2O_2$	90	125
6	2f	I	H	CH_3		$C_{21}H_{21}IN_2O_2$	86	178
7	2g	H	H	H		$C_{20}H_{21}NO_2$	90	98
8	2h	H	H	Cl		$C_{20}H_{18}ClNO_2$	86	196
9	2i	I	H	CH_3		$C_{21}H_{20}INO_2$	88	134
10	2j	H	H	H		$C_{21}H_{22}N_2O_2$	78	158

Spectral Analysis:

1)8-Iodo-6-methyl-2-(4-pyrrolidin-1-yl-phenyl)-chromen-4-one(2c)

I.R.:(cm^{-1}):1681(C=O); 1597 (C=C).

H^{1}NMR:: 1.55 (s, 3H, CH_3); 2.6(t, 4H, CH_2); 3.45 (t,4H, CH_2); 6.6(s, 1H ethylenic); 7.0-8.4(m, 6 H, Ar-H + CH=CH)

Mass: Mass (m/z) : 431 .

2)2[-4-(-4-Methyl-piperazin-1-yl)-phenyl)-chromen-4-one(2d)

I.R.:(cm^{-1}):1683(C=O); 1597 (C=C).

H^1NMR:: 1.8 (s, 3H, CH_3); 2.5(t, 4H, CH_2); 3.5 (t,4H, CH_2); 6.7(s, 1H ethylenic); 6.9-8.2(m, 8 H, Ar-H + CH=CH).

Mass: Mass (m/z) : 321(M+ion)

3)6-Chloro-2-(-4-piperidin-1-yl-phenyl)-chromen-4-one(2h)

I.R.:(cm^{-1}):1689(C=O); 1597 (C=C).

H^1NMR::3.3(t, 6H, CH_2); 3.6 (t,4H, CH_2); 6.8(s, 1H ethylenic); 7.0-8.0(m, 7 H, Ar-H + CH=CH)

Mass: Mass (m/z) : 340 (M+1).

4) 2[-4-(-4-Ethyl-piperazin-1-yl)-phenyl)-chromen-4-one(2j)

I.R.:(cm^{-1}):1682(C=O); 1564 (C=C).

H^1NMR:: 1.5-2.0 (m, 5H, CH_3,CH_2); 2.6(t, 4H, CH_2); 3.5 (t,4H, CH_2); 6.7(s, 1H, ethylenic); 6.9-8.3(m, 8 H,Ar-H + CH=CH);

Mass: Mass (m/z): 334.

Method :

In the present investigation, the synthesized flavonoids derivatives were screened for its anti oxidant as well as anti inflammatory potential by PASS software. The compounds showing greater activates (Pa> 70) were selected for screening. The selected flavonoid derivatives were evaluated for their *in vitro* free radical scavenging activity by the diphenyl picryl hydrazyl (DPPH) assay method[13], and for anti-inflammatory activity by membrane stabilization method[14].

Anti oxidant evaluation:

The aliquot of different concentrations (50 to 100 µg/ml) of the test sample was added to 0.5ml of 0.003M DPPH in methanol. Final volume was adjusted to 3ml.Quercetin was used as a positive control. Negative control was prepared by using the same amount of DPPH mixed with only methanol devoid of samples. Absorbance values were measured at 517nm using Ultraviolet –Visible spectrophotometer. Absorbance was converted into % Antioxidant activity using the following equation.

$$\text{Inhibition S } (\%) = 100\ (A_0 - A_S)/\ A_0$$

Where A_0 = Absorbance of control

(Containing all reagents except the test compound)

A_S= Absorbance of the test Compound

Table-2 Antioxidant activity of synthesized flavonoid derivatives and standard Quercetin represented as % DPPH inhibition

Products	Series	Concentration (µg/ml)		
		50	75	100
Flavones	2a	42.41	56.04	72.91
	2e	42.60	52.91	76.73
	2i	59.58	70.16	81.08
Querecetin (standard)		56.73	74.96	89.96
		5(µg/ml)	10(µg/ml)	20(µg/ml)

ANTI- INFLAMMATORY ACTIVITY EVALUATION.

i) Preparation of erythrocyte suspension

Blood was collected from the healthy Albino rats from the retro orbital plexus under light ether anesthesia using heparin as an anticoagulant and washed thrice with normal saline (NS). Total volume of saline was measured and reconstituted as a 40% v/v suspension with isotonic phosphate buffer solution (IPB), pH 7.4 (NaH_2PO_4. 2 H_2O, 0.26 g; Na_2HPO_4, 1.15 g; NaCl, 9 g,10 mM sodium phosphate buffer). The obtained suspension was considered as erythrocyte cell suspension.

ii) Effect on stabilization and lysis of erythrocytes

Test extracts (100 and 200 µg/ml) or standard drug acetyl salicylic acid (50 µg/ml) solution was prepared in IPB and taken in duplicate sets of centrifuge tubes. The test or standard solution (in 5 ml IPB) or vehicle (control group) was mixed with erythrocyte suspension. One set of tube was incubated for 20 min at 54^0C in a thermostat water bath, while another was maintained at 0-5^0C in an ice bath. The reaction mixture was centrifuged for 3 min at 3000 rpm, obtained supernatant was subjected for measurement of absorbance at 540 nm.

iii) Effect on Hypotonic solution-induced haemolysis

Test extracts (100 and 200 µg/ml) or standard drug acetyl salicylic acid (50 µg/ml) solution was prepared in hypotonic solution [56 mM NaCl in 10 mM sodium phosphate buffer (pH 7.4)] and mixed with erythrocyte stock suspension (30 µl). Whereas the control solution is test extract free solution. The mixtures were incubated at room temperature for 10 min and centrifuged at 3000 rpmfor 3 min and finally the absorbance of the obtained supernatant was measured at 540 nm. % Inhibition of haemolysis was calculated using formula .

$$\% \text{ Inhibition of haemolysis} = 100 \times [1-(OD_2-OD_1)/ (OD_3-OD_1)]$$

Where, OD_1- Absorbance of test sample unheated or in isotonic solution;

OD_2 - Absorbance of test sample heated or in hypotonic solution; and

OD_3 - Absorbance of control sample heated or in hypotonic solution.

Table.3-Anti inflammatory activity of synthesized flavones as compared to standard Acetyl salicylic acid.

Product Series	Concentration µg/ml	% Inhibition of haemolysis	
		Heat induced	Hypotonic solution induced
	50	42.36± 2.14	48.13± 1.09
2e	25	18.06± 2.14	22.27± 1.09
	50	58.92± 2.14	69.14± 1.09
2i	25	19.57± 2.14	23.11± 1.09
	50	38.14± 2.14	41.04 ± 1.09
Standard Acetyl salicylic acid	50	29.64 ± 2.14	28.20 ± 1.09*

In the present study, we have depicted the synthesis of ten substituted 3-phenyl flavone derivatives, also anti-inflammatory, and anti oxidant activity of selective compounds[based on the PASS software analysis]. The synthesized compounds were characterized by TLC, melting point, IR, NMR and Mass spectroscopy. These derivatives evaluated, *in-vitro* anti-inflammatory activity of the compounds using membrane stabilization method and anti-oxidant activity using hydrogen peroxide radical scavenging method.

CONCLUSION :

The compounds 2e and 2i showed good anti-inflammatory activity by reducing inflammation in carragenan induced paw edema in rat. The flavones showed poor anti oxidant potential. However, further *in-vivo* studies are needed to develop these compounds as potential pharmaceutical agents. The results obtained from this study can be used as guidelines for further development.

ACKNOWLEDGEMENT:

The authors are thankful to the Principal, Yeshwant Mahavidyalaya, Nanded for providing lab facilities and Director SAIF, Chandigarh for spectral analysis.

REFERENCES

1. Yao LH, Jiang YM, Shi J, ThomasFA-Barberian, Datta N, Singanusong R and ChenSS, Flavonoids in food and their health benefits, *Plant Foods for Human Nutrition*, 2004**;** 59: 113-117**.**
2. Ana Garcia- Lafuente, Eva Guillamen, Ana Villares, Maericio A Rostagno, Flavonoids as anti inflammatory agents; Inflamm Res, 2009;58:537-552.
3. Miksicek RJ, Commonly occurring plant flavonoids have estrogenic activity, Mol Pharmacol, 1993,44(1):37-43.

4. Valéria R. Moraes. de S; Daniela M. Tomazela; Ricardo J. Ferracin Cleverson F. Garcia; Míriam Sannomiya; M. del Pilar C. Soriano; M., Enzymatic inhibition studies of selected flavonoids and chemosystematic significance of polymethoxylated flavonoids**.** J. Braz. Chem. Soc;2003: 3:14-17.
5. Shohaib.T, M.Shafique, Dhanya.N, Madhu.C. Divakar, Importance of flavonoids in therapeutics, Hygeia. J. D. Med. 2011;3 (1):.1-18
6. Kawai M, Hirano T, Higa S, Arimitsu J, Maruta M, Kuwahara Y, Ohkawara T, Tanaka T. Flavonoids and related compounds as anti-allergic substances. Allergol Int. 2007;56(2):113-23.
7. Harborne, JB.; Baxter H. In The Handbook of Natural Flavonoids, Vol. 1. Manchester, UK: John Wiley and sons, 1999;211-17**.**
8. Middleton Jr. E.; Chithan K. The Flavonoids: Advances in Research since 1986,London, UK, Chapman and Hall, 1993;114-17**.**
9. Xu YC, Leung SW, Yeung DK, Hu LH, Chen GH, Che CM, Man RY. . Structure-activity relationships of flavonoids for vascular relaxation in porcine coronary artery.Phytochemistry. 2007 ;68(8):1179-88.
10. John A beutler, Ernst Hamel, Arnold J Mietinck, Achiel Haemens, Michael R Boyd, structure activity requirements for flavone cytotoxicity and Binding- to Tubulin, J. Med Chem, 1998, 41(13); 2333-2338.
11. Vrijsen R, Everaert L, Boeye A , Antiviral activity of flavones and potentiating by ascorbate.Journal of General Virology 1988; 69 (7):1749-51.
12. Sudha A, Sumati K, Manikandaselvi S, Prabhu NM and Srinivasan P, Anti hepatotoxic activity of crude flavonoid fraction of Lippa nodiflora L on ethanol induced liver injury in rats, Asian J Animal Sci.2013;7(1), 1-13.
13. Pradeep D. Lokhande, Sachin S. Sakate, Kiran N. Taksande, BeenaNavghare. Dimethylsulfoxide–iodine catalysed deprotection of 2′-allyloxychalcones: synthesis of flavones. Tetrahedron Letters;2005; 46(9):1573-1574.
14. Monika Majewska, Micha £ Skrzycki, Ma£Gorzta Podsiad And Hanna Czeczot. Evaluation of antioxidant potential of flavonoids: An in vitro study. Acta Poloniae Pharmaceutica ñ Drug Res 2011; 68(4); 611-615.
15. Umukoro S and Ashorobi RB. Evaluation of anti-inflammatory and membrane stabilizing property of aqueous leaf extract of *Momordicacharantia*in rats. African J Biomedical Res 2006; (9);119 -124