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SYNTHESIS AND BIOLOGICAL EVALUATION OF FLUORO-HYDROXY SUBSTITUTED PYRAZOLE CHALCONES AS ANTI-INFLAMMATORY, ANTIOXIDANT AND ANTIBACTERIAL AGENTS

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ABSTRACT

A series of fluoro-hydroxy pyrazole chalcones have been synthesized by using polyethylene glycol as a alternative reaction medium and evaluated for their in*vitro* anti-inflammatory (COX-1/COX-2), antioxidant (DPPH, SOR, and ·OH) and antibacterial activities. Among the synthesized compounds, the compounds 4b, 4c, 4d, 4f and 4g were found to be potent COX-2 inhibitors than COX-1. Compound 4f was found to be an excellent DPPH radical scavenger. Compounds 4b and 4f were found to be excellent SOR scavenger as well as compounds 4c, 4d and 4a were exhibited significant SOR scavenging activity. Compounds 4f, 4e, 4c and 4b were also found to be moderate hydroxyl radical scavengers. Compounds 4b, 4c, 4d, 4f and 4g have shown good antibacterial activity.

KEYWORDS:PEG-400,Pyrazolechalcones,Anti-inflammatory,Antioxidant,Antibacterial,Molecular docking.

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INTRODUCTION

Reducing or eliminating the use of volatile organic solvents can minimize the generation of waste, which is a requirement of one of the principles of green chemistry. Recently, poly ethylene glycol (PEG) has been found to be an interesting solvent system. PEG is an environmentally benign reaction solvent, it is inexpensive, potentially recyclable and water soluble, which facilitates its removal from the reaction product[1].Chalcones are the important precursors belonging to the flavonoid family, which have been reported to possess a wide range of biological activities, including antimicrobial, anti-inflammatory, antioxidant, anticancer and recently the hydroxyl chalcones are reported as potential anti-angiogenic agent[2]. On the other hand, pyrazoles are of interest as potent bioactive molecules. Pyrazole chalcones and their derivatives have been reported to possess anti-inflammatory, analgesic, antimicrobial, antitumor, antioxidant and xanthenes dehydrogenase[3].COX-1 and COX-2 are of particular interest because they are the major targets of nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin, indomethacin[4]. NSAIDs inhibit the activity of both COX-1 and COX-2 a property that accounts for their shared therapeutic and side effects[5]. The inhibition of COX-2 may well explain their therapeutic use as anti-inflammatory drugs, whereas inhibition of COX-1 may explain their unwanted side effects, such as gastric damage. Since COX-2 is involved in inflammation and pain, molecules that inhibit its enzymatic activity would be of therapeutic value. Many non-steroidal anti-inflammatory drugs (NSAIDs) were found to interact with these enzymes and inhibit their enzymatic activity. Reactive oxygen species (ROS) are formed and degraded by all aerobic organisms, leading to either physiological concentrations required for normal cell function, or excessive quantities, the state called oxidative stress. As the term implies, intracellular production of those oxygen intermediates threatens the integrity of various biomolecules including proteins[6], lipids as well as lipoproteins involved in atherosclerosis and DNA[7]. Oxidative stress is also proposed to be involved in the process of aging both by including damage to mitochondrial DNA and by other mechanisms[8]. Oxidative stress leads to the initiation or progression of various diseases such as cancer[9], atherosclerosis[10], cardiovascular[11], inflammation[12], and neurodegenerative disorders such as Alzheimer's and Parkinson[13]. Considering these reports, in the present study we have synthesized and characterized some fluoro-hydroxy substituted pyrazole chalcones by using PEG-400 as an alternative reaction medium[14] and evaluated for their *invitro* anti-inflammatory, antioxidant and antibacterial activities.

MATERIALS AND METHODS

Melting points were determined in open capillary tubes and were found uncorrected. IR spectra were recorded on FT-IR spectrometer (Perkin Elmer). ^{1}H NMR spectra were recorded on Varian-NMR-mercury 300 MHz spectrometer in $CDCl_3$ as a solvent. The mass spectra were obtained with a Shimadzu LCMS-2010 EV spectrophotometer. All the reagents and solvents were used of analytical grade and used as supplied unless otherwise stated. TLC was performed on silica gel coated plates for monitoring the reactions.

Synthesis of pyrazole Chalcones (4a-g)

A mixture of substituted 1,3-diphenyl-1*H*-pyrazole-4-carbaldehydes 3 (1 mmol) and 4-fluoro-2-hydroxy acetophenone (1 mmol) was dissolved in 15 ml PEG-400. To this mixture, sodium hydroxide (20%, 1ml) was added and the reaction mixture was stirred at 40-50^{o}C temperature for 1 hr. The reaction mixture was then poured into 100 ml ice cold water. The product was separated out, it was filtered and processed out. The obtained products were recrystallised from ethanol to afford pure compounds 4a-g.

Spectral data of some selected compounds

3-[3-(4-Bromo-phenyl)-1-phenyl-1H-pyrazol-4-yl]-1-(4-fluoro-2-hydroxy-phenyl)-propenone (4c) Yield 85%; m.p.156^{o}C, IR (cm^{-1}) : 3639, 2983, 2835, 1676, 1635, 1571, 1533, 1200, 1118, 832; ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm) : δ 8.40 (s, 1H, 5*H* of pyrazole), 7.36-7.41 (d, 1H, *J*=15 Hz, -CH=CH-), 7.93-7.98 (d, 1H, *J*=15 Hz, -CH=CH-), 6.5-8.0 (m, 12H, Ar-H); 10.4 (s, 1H, D_2O exchangeable, -OH); m/z = 464 (M^+) *1-(4-Fluoro-2-hydroxy-phenyl)-3-[3-(4-methoxy-phenyl)-1-phenyl-1H-pyrazol-4-yl]- propenone* (4e) Yield 85%; m.p. 208^{o}C, IR (cm^{-1}) : 3775, 2979, 2734, 1689, 1635, 1567, 1535, 1206,1110, 753; ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm) : δ 3.87 (s, 3H, -OCH_3); 8.25 (s, 1H, 5*H* of pyrazole), 6.83-6.88 (d, 1H, *J*=15 Hz, -CH=CH-), 7.74-7.79 (d, 1H, *J*=15 Hz, -CH=CH-), 7.0-8.0 (m, 12H, Ar-H); 10.06 (s, 1H, D_2O exchangeable, -OH); m/z = 415 (M+1) *3-(1,3-Diphenyl-1H-pyrazol-4-yl)-1-(4-fluoro-2-hydroxy-phenyl)-propenone* (4g) Yield 85%; m.p. 126^{o}C, IR (cm^{-1}) : 3183, 2833, 1665, 1593, 1517, 1216, 1117,750; ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): δ 8.52 (s, 1H, 5*H* of pyrazole), 7.37-7.42 (d, 1H, *J*=15 Hz, -CH=CH-), 7.93-7.98 (d, 1H, *J*=15 Hz, -CH=CH-), 6.5-8.0 (m, 13H, Ar-H); 10.06 (s, 1H, D_2O exchangeable, -OH); m/z = 384 (M+1)

COX INHIBITION ACTIVITY

The assay was performed by using colorimetric COX (human ovine) inhibitor screening assay kit[15]. Briefly, the reaction mixture contains, 150 µl of assay buffer, 10 µl of heme, 10 µl of enzyme (either COX-1 or COX-2), and 50 µl of sample (0.1 mmol). The assay utilizes the peroxidase component of the COX catalytic domain. The peroxidase activity can be assayed colorimetrically by monitoring the appearance of oxidized N, N, N, N'-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm.Indomethacin (0.1 mmol) was used as a standard drug[4]. The percent COX inhibition was calculated using the following equation,

$$\text{COX inhibition actvity (\%)} = 1 - \frac{T}{C} \times 100$$

Where T= Absorbance of the inhibitor well at 590 nm

C= Absorbance of the 100% initial activity without inhibitor well at 590 nm

DPPH RADICAL SCAVENGING ACTIVITY

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay was carried out as per reported method with slight modifications[16] . Briefly, 1 ml of test solution (0.1 mmol) was added to equal quantity of 0.1 mmol solution of DPPH in ethanol. After 20 min of incubation at room temperature, the DPPH reduction was measured by reading the absorbance at 517 nm. Ascorbic acid (1 mmol) was used as a reference compound. [4]

SUPEROXIDE RADICAL (SOR) SCAVENGING ACTIVITY

The superoxide anion scavenging assay was performed by the reported method[17]. Superoxide anion radicals were generated in a non-enzymatic Phenazine methosulphate - Nicotinamide Adenine Dinucleotide (PMS-NADH) system through the reaction of PMS, NADH and Oxygen. It was assayed by the reduction of Nitroblue tetrazolium (NBT). [4] In this experiment superoxide anion was generated in 3 ml of Tris HCL buffer (100 mmol, pH 7.4) containing 0.75 ml of NBT (300 µM), 0.75 ml of NADH (936 µM), and 0.3 ml of sample (0.1 mmol). The reaction was initiated by adding 0.75 ml of PMS (120 µM) to the mixture. After 5 min. of incubation at room temperature the absorbance at 560 nm was measured in a spectrophotometer. Ascorbic acid (1 mmol) was used as a reference compound.

HYDROXYL (˙OH) RADICAL SCAVENGING ACTIVITY

The OH radicals scavenging activity was demonstrated with Fenton reaction[18]. The

reaction mixture contained, 60 µl of $FeCl_2$ (1 mmol), 90 µl of 1-10 phenanthroline (1 mmol), 2.4 ml of phosphate buffer (0.2 M, pH 7.8), 150 µl of H_2O_2 (0.17 M) and 1.5 ml of individual samples (0.1 mmol). The reaction was started by adding H_2O_2. After 5 min. incubation at room temperature, the absorbance was recorded at 560 nm. Ascorbic acid (1 mmol) was used as a reference compound.

ANTIBACTERIAL ACTIVITY

The antibacterial activity was tested through agar diffusion method[19] against human pathogenic bacteria viz. *B. subtilis*, *S.aureus*, *E. coli*, and *P. species*. All the synthesized compounds were dissolved to prepare a stock solution of 1 mg/mL using DMSO. Stock solution was aseptically transferred and suitably diluted to have solutions of concentration ranging from 50 to 100 µg/mL. The test organism was inoculated on nutrient agar plates and spread uniformly using a sterile glass spreader. Wells of 6 mm in diameter were made on the nutrient agar using a sterile cork borer. In each well, the test compound (50 µg) was added. The plates were incubated at 37 ^{0}C for 24 hrs for bacteria. Each test was performed in duplicate and the results were shown as means. The zone of inhibition was measured in mm while standard antibiotic amphicilin were used as reference. The DMSO alone was used as control.

RESULTS AND DISCUSSION

CHEMISTRY

In the present investigation, the fluoro-hydroxy pyrazole chalcones (4a-g) have been prepared by the Claisen-Schmidt condensation of 4-fluoro-2-hydroxy acetophenone with various substituted 1,3-diphenyl-1*H*-pyrazole-4-carbaldehydes (3a-g) using sodium hydroxide in PEG-400 as a alternative reaction medium (Scheme 1). All the compounds were obtained in good to excellent yields (Table 1). The substituted 1,3-diphenyl-1*H*-pyrazole-4-carbaldehydes (3a-g) were prepared by the Vilsmeir-Haack reaction[20] of various substituted aryl hydrazones (2a-g) from various substituted acetophenones (1a-g). The completion of the reaction was monitored by TLC and some synthesized compounds were characterized by IR, ^{1}HNMR and Mass spectroscopy.

BIOLOGICAL EVALUATION

All the synthesized compounds were evaluated for their *invitro* COX-1 and COX-2 inhibitory activities[4] by using colorimetric COX (human ovine) inhibitor screening assay kit. Indomethacin was used as a reference standard. The results were shown in Table 2. The results showed that most of the synthesized compounds have shown selective inhibition against COX-2 enzyme and exhibited significant inhibitory profile against COX-2 enzyme in the range 22-53%. When their activities were compared with indomethacin, it was determined that the compounds 4b (43.69), 4c (44.72), 4d (46.58), 4f (53.83) and 4g (34.94) were found to be more active in this series and remaining two compounds were weak inhibitors of COX-2 enzyme as compared to indomethacin (28.24).1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide radical (SOR) and hydroxyl (OH) radical scavenging assays[4] are the well known methods for determining antioxidant activity of the compounds. All the synthesized compounds were also evaluated for their direct scavenging activity against the reactive oxygen and nitrogen species (DPPH, SOR and OH) and the results are summarized in Table 2. Most of the synthesized compounds have shown excellent to good inhibition against DPPH, SOR and OH radicals scavenging activity. Compounds 4f (96.49) was found to possess excellent inhibitor whereas compounds 4b (54.10) and 4c (48.95) were shown moderate inhibition against DPPH as compared to ascorbic acid (81.52) and remaining compounds were shown weak DPPH activity except 4g. Compounds 4b (62.14) and 4f (63.95) were found to possesses excellent inhibitors whereas the compounds 4a (35.47), 4c (49.26) and 4d (37.18) showed significant inhibition against SOR as compared to ascorbic acid (51.93) except 4g. Compounds 4b (36.47), 4c (40.56), 4e (42.36) and 4f (46.21) showed moderate inhibition against OH radical as compared to ascorbic acid (62.35) whereas

remaining compounds were shown weak OH radical scavenging activity. All the synthesized compounds (4a-g) were also evaluated for antibacterial activity against various bacterial strains such as *Bacillus subtilis*, *Pseudomonas species*, *Escherichia coli* and *Staphylococcus aureus*. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with amphicilin as standard and the results are summarized in Table 2. All the compounds were found to be comparable with the standard drug amphicilin. Compounds 4b, 4c, 4d, 4f and 4g showed significant inhibition against all the selected strains as compared to the standard drug amphicilin.

Table 1
Physical data of fluoro-hydroxy pyrazole chalcones (4a-g)

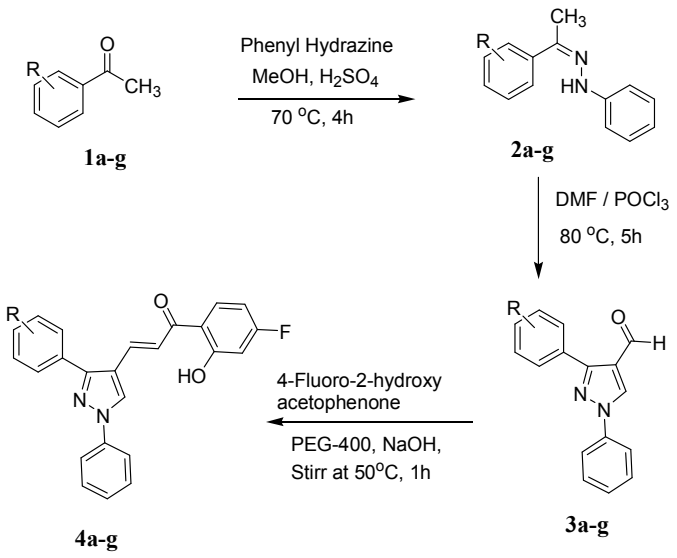
Entry	Product (R)	Molecular Formula	Molecular weight	Yield(%)	MP (^oC)
4a	4-F	$C_{24}H_{16}N_2O_2F_2$	402	84	142
4b	4-Cl	$C_{24}H_{16}N_2O_2FCl$	418	88	148
4c	4-Br	$C_{24}H_{16}N_2O_2FBr$	463	87	156
4d	4-CH_3	$C_{25}H_{19}N_2O_2F$	398	80	192
4e	4-OCH_3	$C_{25}H_{19}N_2O_3F$	414	82	208
4f	3-NO2	$C_{24}H_{16}N_4O_4F$	429	77	195
4g	4-H	$C_{24}H_{17}N_2O_2F$	384	85	126

Table 2
In vitro anti-inflammatory (COX-1 & COX-2), antioxidant and Antibacterial activites of fluoro-hydroxy pyrazole chalcones

Entry	% inhibition of COX (100 µM)[a]		Antioxidant activity % inhibition (100 µM)[a]			Antibacterial activity (MIC at 50µg/ml)[b]			
	COX-1	COX-2	DPPH	SOR	OH	BS	PS	EC	SA
4a	29.83	22.34	05.39	35.47	21.65	18	19	16	19
4b	37.47	43.69	54.10	62.14	36.47	17	13	11	13
4c	41.38	44.72	48.95	49.26	40.56	20	10	16	12
4d	28.29	53.83	14.76	37.18	25.47	15	12	12	13
4e	37.15	24.38	23.66	31.47	42.36	10	-	13	-
4f	14.46	46.58	96.49	63.95	46.21	17	13	16	13
4g	21.18	34.94	-	22.63	17.56	14	14	15	14
Standard 1	63.87	28.24							
Standard 2			81.52	51.93	62.35				
Standard 3						19	23	15	17

[a]The determination was performed in duplicate for two independent experiments.

[b]The data represent the mean of two experiments; "-" Not active; BS-Bacillus subtilis, PS-Pseudomonus species, EC-Escherichia coli, SA-Staphylococcus aureus. Standard 1- Indomethacine, Standard 2- Ascorbic acid, Standard 3- Amphicilin.



R(a-g) = 4-F, 4-Cl, 4-Br, 4-CH_3, 4-OCH_3, 3-NO_2, 4-H

Scheme 1
Protocol for the synthesis of fluoro-hydroxy pyrazole chalcones

CONCLUSION

In conclusion, various fluoro-hydroxy substituted pyrazole chalcones have synthesized by using PEG-400 as a alternative reaction medium and evaluated for their *invitro* anti-inflammatory, antioxidant, antibacterial activities. The reaction was clean and the products were obtained in excellent yields without formation of any detectable side products. Most of the synthesized compounds were found to be the most active anti-inflammatory agents since they inhibit COX-2 enzyme more selectively than COX-1 enzyme except compounds 4a and 4e. Compound 4f was found to be excellent DPPH radical scavenger. Compound 4b and 4f were excellent SOR scavenger as well as compounds 4c, 4d and 4a were exhibited significant SOR scavenging activity. Compounds 4f, 4e, 4c and 4b were also found to be moderate hydroxyl radical scavengers. Compounds 4b, 4c, 4d, 4f and 4g have shown good antibacterial activity. Hence, the compounds 4b, 4d, 4c and 4f can be considered as lead molecules for novel selective COX-2 inhibitors, antioxidants and antibacterial agents. Overall, these compounds further need to be screened for their *invivo* study and anticancer activities.

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