Synthesis, characterization and antinociceptive activities of Novel 2-(2,4-dichlorophenyl)-4H-chromen-4-one

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Abstract: A novel flavone derivative has been synthesized in good yield from ketone and aldehyde. The structure has been established by different spectroscopic techniques like ^{1}H NMR, ^{13}C NMR, IR and elemental analysis. The compound was then screened for its acute toxicity and antinociceptive activity studies on animal model. The novel compound was safe upto a maximum dose of 500mg/kg body weight oral dose in mice and showed 65.92 and 82.18% pheriperal analgesic activity at 15 and 30mg/kg body weight doses. Central antinociceptive activity of the compound was 53.13 and 64.44% at 15 and 30mg/kg body weight respectively.

Keywords: Flavonoids, chalcone, flavone, acute toxicity, antinociceptive activity.

INTRODUCTION

Flavonoids are natural products and important part of human diet that are abundantly present in vegetables, fruits, nuts, seeds, wine, tea and flowers. Flavonoids are bioactive polyphenols of low molecular weight that play a key role in photosynthesizing cells of plants (Sandhar *et al*., 2011). On the basis of chemical structures, flavonoids are mainly classified into six classes, i.e., flavones, flavanones, flavans (flavanols), isoflavonoids, anthocyanins and flavonols. All these groups mainly differ by the structure of the C-ring and functional groups attached to C-3 and C-4 of the main structure (Peterson and Dwyer, 1998).Pharmacological role of flavonoids in human is a subject of extensive research and they have been known for a longtime to possess numerous biological activities both in animals and humans (Fernández *et al*., 2006; Maikai, 2011).

Flavonoids exhibit several biological and pharmacological effects such as antihepatotoxic, anti-ulcer actions (Bors *et al*., 1990; Smith *et al*., 1988), strongradical scavenging and antioxidant activity (Zhang *et al*., 2006; Chan *et al*., 2000; Rice-Evans *et al*., 1996). They also appears to be associated with reduction of certain chronic ailments (Kris-Etherton *et al*., 2004), like cardiovascular complications (Gross, 2004) and various kind of cancers (Moon *et al*., 2006). Flavonoids exhibit antimicrobial (Cushnie and Lamb, 2005), antiviral (Asres *et al*., 2005), antiulcer (Wightman, 2004), anti-inflammatory activities (Kim *et al*., 2004, Dao *et al*., 2004), antiallergenic (Middleton and Kandaswami, 1992; Berghe *et al*., 1993), beneficial effects on capillary fragility (Benavente-García *et al*., 1997), an ability to inhibit human platelet aggregation (Tijburg *et al*., 1997), antipyretic (Ray *et al*., 2006), analgesic (Ahmed *et al*., 2007) and antidepressant (Fan *et al*., 2012) properties.

Chalcones are an important class of compounds which are good intermediates for the synthesis of various heterocyclic compounds like flavones, flavanols, flavanones, isoxazolines and aurones (Shah and Goswami, 2013). Different methods have been reported for flavone synthesis like Allan-Robinson synthesis, synthesis from chalcones, Wettig method, from o-benzoyl acetophenone and synthesis under catalytic microwave irradiation (Mostahar *et al.,* 2006). These flavoneshave antioxidant (Gabrielska *et al*., 1997), antimicrobial (Husain *et al*., 2013), anticancer (Zhang *et al*., 2008), anti-inflammatory (Carvalho *et al*., 1999), analgesic (Thirugnanasambantham *et al*., 1993) and antidepressant (Fan *et al*., 2012) activities.

Based on the reported literature regarding the synthesis of flavones from chalcones and their potentials to have biological and pharmacological activities, we have synthesized and explored antinociceptive effects of a novel flavone on scientific grounds.

MATERIALS AND METHODS

General

Chemicals/ reagents used for the synthesis of the flavone were of Sigma Chemical Company (Aldrich, Germany). Solvents like ethanol, *n*-hexane, ethyl acetate etc were synthesis grade purchased from E. Merck and used as such without prior distillation.

^{1}H-NMR and ^{13}C NMR spectra were recorded in deutrated chloroform ($CDCl_3$) on Bruker SF spectrometers operating at 300 and 75 megahertz (MHz) frequencies, respectively. Chemical shifts values are

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expressed in δ (ppm) downfield relative to TMS which was used as an internal standard. Infrared spectra were recorded on Thermo scientific USA (Nickolet 6700), Infrared spectrometer on KBr disk mathod. All melting points are uncorrected and were taken in open capillary tubes using Electrothermal 9100 apparatus (Barnstead UK). Reaction extents and final products purities were checked on TLC plates (Merck 60 F_{254}, Darmstadt Germany) and spots were visualized under UV Lamp (180-365 nm) and with subsequent staining with iodine vapours.

Synthesis of 2-(2,4-dichlorophenyl)-4H-chromen-4-one derivative (F5)

1.80mL of 2-hydroxy acetophenone (15 mmol) was added to absolute ethanol (20mL) in a round bottom flask and then sodium hydroxide solution 10mL (40% ethanolic) was added drop wise to this solution at room temperature. Then 2.62gm of 2,4-dichlorobenzaldehyde (15mmol) in ethanol was added dropwise to this mixture and stirred for 24 hours at room temperature (25±2ºC). The reaction was monitored by TLC using ethyla cetate: *n*-hexane (2:8 v/v). Upon completion of the reaction, it was poured into crushed ice and neutralized with 1N HCl solution resulting in yellow precipitates of chalcone. The chalcone was filtered and washed with water to remove the impurities.

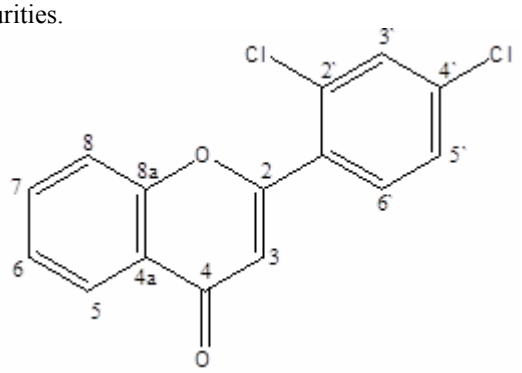

Fig. 1: Structure of the synthesized flavone F5 (2-(2,4-dichlorophenyl)-4H-chromen-4-one)

In next step, chalcone was cyclized to flavone in 15mL DMSO in the presence of iodine (375mg) at 140ºC for 1 hour and the extent of reaction was monitored by TLC. Upon completion of reaction, mixture was cooled to room temperature and was poured into water followed by extraction with ethyl acetate (25mL×3), treated with sodium thiosulphate solution (20%), brine solution and dried over sodium sulphate. The final product (mixture of flavones and chalcone) was subjected to gravity column chromatography. The flavone (fig. 1) was obtained as white solid on elution with *n*-hexane: ethyl acetate (9:1).

Pharmacological activities

Drugs and animals

Tween-80 was purchased from Daejung Chemicals, Korea. Diclofenac sodium and Morphine sulphate were purchased from local market. Swiss Albino mice of either sex weighing 30-35g were purchased from National Institute of Health (NIH) Islamabad. The animals were housed in individual cages at the animal house of University of Malakand with free access to water and standard diet and starved for 12-18 hours before experimentation. Ethical Committee of the Department of Pharmacy, University of Malakand approved the experimental protocols and ensured its compliance with provisions of the "Animal Bye-Laws 2008, Scientific Procedures Issue-I of the University of Malakand".

Acute toxicity

The synthesized compound (F5) was subjected to acute toxicity study on mice. In first phase, three groups of overnight fasted mice (n=6) were given an oral dose of 50, 75 and 150 mg/kg body weight of synthesized novel flavone. In second phase, further 3 groups were given an oral dose of 300, 400 and 500 mg/kg body weight. All the treated animals were kept under observation for 24 hours. Finally, LD_{50} was calculated in all groups by recording the number of deaths in each group within 24 hours (Lorke, 1983; Ali *et al*., 2011).

Acetic acid-induced writhing test (peripheral activity)

Antinociceptive response of the novel synthesized compound (F5) was assessed by using mice as an animal model with slight modifications (Koster *et al*., 1959). The synthesized compound at different dose concentrations in 2% tween-80and 2% tween-80 in normal saline were dispensed to respective groups of animals (n=6) orally, and Diclofenac sodium was administered intraperitonially. After 30 minutes of time interval, 0.1 mL of acetic acid at a concentration of 1% (v/v) was injected intraperitonially. Data was recorded in the form of number of writhes (constriction of abdomen, turning of trunk and extension of hind legs) during 30 minutes of test period, beginning 3 minutes after the injection of acetic acid (Razmi *et al*., 2013).

Tail immersion test (central activity)

Albino mice were used to assess the central antinociceptive response of the synthesized novel compound. Briefly, different dose concentrations of the synthesized compound and vehicle (2% tween-80) were administered orally 30 minutes and morphine (standard) intraperitonially 15 minutes before the screening to respective groups. The latency period (time taken by the mice to deflect the tail) was determined by immersing 1-2 cm of mice tail in water that was kept warm at 53±1ºC (Imam and Sumi, 2014).

STATISTICAL ANALYSIS

Data are presented as mean ±SEM. Analysis of variance and Dunnett's test is statistically manipulated with Graph Phad In Stat Version 3.10 software.

Table 1: Acetic acid induced analgesic activity data of the compound

Treatment	Dose (mg/kg)	Number of writhing	% inhibition
Control	2 % w/v of Tween 80	74.83±1.01	-------
Standard	Diclofenac sodium 100 mg	10.16±0.70**	86.42
F5	15 mg	25.50±0.84**	65.92
	30 mg	13.33±0.80**	82.18

All the values were expressed as mean ±SEM (n=6). **P<0.01 when compared to control group.

Table 2: Percent analgesic activity (Tail flick) data of the compound

Treatment	Dose	Time in Sec/% response					
		15min	30min	45min	60min	75min	90min
Control	2% w/v of Tween 80	0.628±0.04	0.698±0.01	0.686±0.02	0.633±0.01	0.623±0.01	0.641±0.01
Standard	Morphine sulphate 5mg	0.77±0.01*** (18.92)	1.13±0.04*** (38.38)	1.61±0.021*** (57.52)	2.241±0.02*** (71.74)	3.53±0.03*** (82.35)	2.33±0.05*** (72.50)
F5	15mg	0.685±0.07* (8.27)	0.79±0.06* (11.6)	0.958±0.02*** (28.34)	1.041±0.02*** (39.2)	1.33±0.01*** (53.13)	1.21±0.25* (47.26)
	30mg	0.73±0.01** (14.31)	0.928±0.10** (24.77)	1.05±0.04*** (34.70)	1.316±0.02*** (51.89)	1.75±0.02*** (64.44)	1.325±0.03*** (51.57)

All the values were expressed as mean ±SEM. *P<0.05, **P<0.01 and ***P<0.001 when compared to control group.

RESULTS

Chemistry

Proton NMR of F5 shows singlet at δ 6.68ppm referring to the methene proton, ^{13}C NMR of this compound show fifteen different peaks at respective chemical shifts. Signal at δ 177.9 ppm show characteristic peak for ketonic carbon in ^{13}C NMR. IR spectrum of the compound show peaks at 3066.5, 2920.6, 1734.1, 1645.4, 1221.1 and 748.2cm^{-1} which confirms the presence of C-H Aromatic, C-H, methene, C=C, C=O,C-O and C-Cl bonds. CHN data calculated for compound $C_{15}H_8Cl_2O_2$is: C, 61.88; H, 2.77. and Found is: C, 60.99; H,2.28. This confirms the synthesis of the novel flavone.

Yield: 87%, m.p: 90.5ºC, R_f: 0.57 (ethyl acetate: *n*-hexane (3:7)), ^{1}H NMR (300 MHz, Chloroform-*d*,ppm) δ 8.27 (dd, *J*=8.0, 1.7 Hz, 1H, H-3`), 7.74 (ddd, *J*=8.7, 7.1, 1.7 Hz, 1H, 5`-H), 7.64-7.56 (m, 2H, 5-H, 6`-H), 7.55-7.40 (m, 3H, 6-H, 7-H, 8-H), 6.68 (s, 1H, H-3).$^{13}$C NMR (75 MHz, $CDCl_3$, ppm) δ 177.98 (C-4), 161.51 (C-2), 156.54 (C-8a), 137.43(C-2`), 134.06 (C-4`), 133.81 (C-6`), 131.42 (C-5`), 130.77 (C-3`), 130.40 (C-1`), 127.58 (C-7), 125.82 (C-6), 125.50 (C-5), 123.81 (C-4a), 118.18 (C-8), 113.16(C-3). IR (KBr) $V_{max}cm^{-1}$: 3066.5, 2920.6, 1734.1, 1645.4, 1221.1, 748.2.Anal.Calcd. ForC_{15} $H_8Cl_2O_2$: C, 61.88; H, 2.77. Found: C, 60.99; H, 2.28.

Acute toxicity

In our study, we evaluated the acute toxicity study in mice model. The animals were given different doses of the synthesized compound in different stages of the study. To a highest dose of 500mg/kg body weight, the compound did not show any mortality.

Analgesic activity (pheripheral activity)

In our study for evaluation of peripheral analgesic activity of the synthesized flavone, we used the acetic acid induced mice model. Results of the activity are summarized in table 1. From the results it is evident that the synthesized compound showed 65.92 and 82.18% analgesic activity at 15 and 30mg/kg body weight, respectively in comparison with standard diclofenac sodium i.e 86.42%. From table 1, it is clear that the flavone at 30mg showed almost the same activity like that of standard.

Tail immersion test (central activity)

The latency time of mice to thermal stimuli in this study induced by hot water was significant in both doses at 15 and 30mg/kg body weight however the time for 50% of analgesic response in both doses is different (table 2). At 15mg/kg body weight, the compound showed 50% response in 75 minute while it was observed in 60 minute for 30mg/kg body weight. The standard drug morphine showed 50% response at 45 minute. Both doses showed highly significant activity at 45-75 minutes (P<0.001). Maximum effect was recorded for both doses of the compound at 75min. Based upon above findings, it may be assumed that the novel flavone possess good peripheral and central antinociceptive activities.

DISCUSSION

To evaluate pharmacological screening of a compound whether from plant or synthetic sources, its acute toxicity study should be carried out first. Acute toxicity screening in animal model is important preliminary test that provides basis for the dose estimation and predicts

potential toxicity of the drug. From this study, nature of acute response in humans can be anticipated and also provide rough idea about of the organ system involvement (Bhardwaj *et al*., 2009). To a highest dose of 500 mg/kg body weight, the compound did not show any mortality. So up to this dose the compound is safe (Bala *et al*., 2013).

Analgesic activity (pheripheral activity)

Analgesic drugs alleviate pain symptoms without terminating its etiologic cause (Tripathi, 2013). Available analgesics classes like NSAIDs and opiates has adverse effects and are not considered useful in all respects. For this purpose, to improve the pain relieving quality of analgesics and to produce analgesics with less side effects, new agents are constantly sought out (Akter *et al*., (2008). Writhing induced by acetic acid assay is a rapid and sensitive way of measuring pheripheral analgesic potentials of compounds. Mechanism of the writhing induction is increased concentration of PGE2 and PGF2 produced by acetic acid (Collier *et al*., 1968;Bentley *et al*., 1983). For evaluating central analgesic effect of compounds, tail flick or hot plate methods are adopted. These methods were for the first time successfully described by Woolfe and MacDonald (Woolfe and MacDonald, 1944). Sensory nerves sensitize nociceptors and the involvement of substances like prostaglandins (PGs) are minimized in this activity (Mustaffa *et al*., 2010). Analgesia of spinal origin and pain sensation to thermal stimulus is well demonstrated by the tail immersion in hot water and hot plate studies using mice for various opoid derivatives and other drugs (Subhan *et al*., 2008).

As discussed earlier that acetic acid increases the concentration of prostaglandins (PGs) that cause the peripheral pain response (Bentley *et al*., 1983) and NSAIDs like diclofenac inhibits the productions of PGs (Simmons *et al*., 2004). So it is assumed that the analgesic activity of the flavone might be due to such mechanisms like that of the standard.

Tail immersion test (central activity)

Response of tail withdrawal in mice is considered to be centrally mediated (Srinivasan *et al*., 2003). The latency time of mice to thermal stimulus is measured in both tail flick and hot plate tests. Hot plate method measures supraspinal reflexes mediated through μ1- and μ2-opioid receptors while tail immersion measures spinal reflexes of μ2- and δ-opioid receptors (Imam and Sumi, 2014). Based upon above findings, it may be assumed that the novel flavone possess good peripheral and central antinociceptive activities.

CONCLUSION

Results of this study indicates that the synthesized compound possess both central and peripheral analgesic activities. The peripheral activity is more significant than the central analgesic activity. Further elucidation of the compound for evaluation of its exact mechanisms of analgesic response is necessary.

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