Evaluation of Antinociceptive Profile of Chalcone Derivative (3-(2,5-dimethoxyphenyl)-1-(5-methylfuran-2-yl) prop-2-en-1-one (DMPF-1) in vivo

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INTRODUCTION

Pain is a major global health issue, where its pharmacotherapy prompts unwanted side effects; hence, the development of effective alternative compounds from natural derivatives with lesser side effects is clinically needed. Chalcone, the precursors of flavonoid, and its derivatives have been widely investigated due to its pharmacological properties. Objective: This study addressed the therapeutic effect of 3-(2,5-dimethoxyphenyl)-1-(5-methyl furan-2-yl) prop-2-en-1-one (DMPF-1); synthetic chalcone derivative, on antinociceptive activity in vivo. Materials and Methods: The antinociceptive profile was evaluated using acetic-acid-induced abdominal writhing, hot plate, and formalin-induced paw licking test. Capsaicin, phorbol 12-myristate 12 acetate (PMA), and glutamate-induced paw licking test were carried out to evaluate their potential effects toward different targets. Results: It was shown that the doses of 0.1, 0.5, 1, and 5 mg/kg of DMPF-1 given via intraperitoneal injection showed significant reduction in writhing responses and increased the latency time in hot-plate test where reduced time spent on licking the injected paw in formalin and dose contingency inhibition was observed. The similar results were observed in capsaicin, PMA, and glutamate-induced paw licking test. In addition, the challenge with nonselective opioid receptor antagonist (naloxone) aimed to evaluate the involvement of the opioidergic system, which showed no reversion in analgesic profile in formalin and hot-plate test. Conclusion: Collectively, this study showed that DMPF-1 markedly inhibits both peripheral and central nociception through the mechanism involving an interaction with vanilloid and glutamatergic system regardless of the activation of the opioidergic system.

KEYWORDS: 3-(2,5-dimethoxyphenyl)-1-(5-methylfuran-2-yl) prop-2-en-1-one, abdominal writhing, antinociceptive, chalcone, glutamatergic, hot plate, opioidergic, transient protein vanilloid-1

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great opportunity to reestablish natural products in drug discovery.

Chalcones are simple but contain highly biological active forms of naturally or synthetic class of compounds, which comprise two aromatic rings linked by three carbon α,β-unsaturated system. These compounds possess several biological activities including anti-inflammatory, antileishmanial, anti-invasive, antituberculosis, antifungal, antimalarial, antitumor, antimicrobial, antinociceptives, and anticancer. Our group is working on chalcone and its derivatives since last decade to find a reasonable compound against various diseases, especially cancer on nociception.[3-5]

**Materials and Methods**

**Drugs and reagents**

Glacial acetic acid (Scharlau Chemie S.A., Barcelona, Spain), formalin (HmbG Chemical, Germany), naloxone, acetylsalicylic acid (ASA), morphine, capsazepine, capsaicin, and glutamate (Sigma Chemical, St. Louis, MO, USA) were used. All drugs were dissolved in saline (0.9%; NaCl); DMPF-1 compound was dissolved in a vehicle (ethanol, Tween 20 [Sigma Chemical] and distilled water; 5:5:90 [v/v] fraction). The DMPF-1 doses used (0.1, 0.5, 1, and 5 mg/kg) were chosen based on a pilot experiment, and volume administered was 10 mL/kg body weight.

**Animals**

Adult male ICR albino mice (20–25 g, n = 6 for each group) were used. These animals were kept in constant temperature 24 ± 1°C, 12h with free access to food and water ad libitum. The experiments followed the rules of ethical guideline for evaluation of pain on conscious animals[6] with approval by Animal Care and Use Committee, Universiti Putra Malaysia, Serdang, Selangor.

**Synthesis of (E)-3-(2,5 dimethoxyphenyl)-1-(5-methyl furan-2-yl) prop-2-en-1-one**

DMPF-1 was synthesized by Claisen–Schmidt condensation reaction as discussed in our previous publication [Figure 1A].[8] The compound was purified by column chromatography using silica gel mesh size (200–400 mesh, Merck) and elution with petroleum ether and ethyl acetate. Yield: 58%; yellow crystals, infrared (CHC{l}_i),\text{v:} 2946 (\text{C–H stretch}), 1652 (\text{C=O}), 1600 (\text{C=C}), 1562 (\text{C–O}) aromatic, 1074 cm\textsuperscript{-1}; proton nuclear magnetic resonance (1H nuclear magnetic resonance (NMR)) (500 MHz, CDC{l}_3): \delta 2.38 (s, 3H, CH\text{3}), 3.78 (s, 3H, OCH\text{3}), 3.80 (s, 3H, OCH\text{3}), 6.38 (d, J = 3.0 Hz, 1H, H-4 furanyl), 6.97 (br, s, 2H, H-3 and H-4 phenyl), 7.42 (s, 1H, H-6 phenyl), 7.60 (d, J = 16.0 Hz, 1H, H-\alpha), 7.70 (d, J = 3.0 Hz, 1H, H-3 furanyl), and 8.01 (d, J = 16.0 Hz, 1H, H-\beta). 13C NMR (125 MHz, dimethyl sulfoxide [DMSO]): \delta: 176.7, 159.3, 154.0, 153.3, 152.6, 137.0, 124.2, 122.6, 121.9, 118.5, 113.7, 113.4, 109.9, 56.7, and 14.5. electron ionization mass spectral (m/z (rel. int.) calcd for C\textsubscript{16}H\textsubscript{16}O\textsubscript{4}: 272 (M\textsuperscript{+}, 43), 257, 241, 191, 137, and 81.

**Acetic-acid-induced abdominal writhing test**

The acetic-acid-induced abdominal writhing test was performed as described previously.[9] The groups of mice (n = 6) were pretreated with DMPF-1 (0.1–5 mg/kg), vehicle (10 mL/kg), and ASA (100 mg/kg) intraperitoneally 30 min before 0.6% intraperitoneal acetic acid injection and they were placed individually in glass observation chambers. The number of writhing episodes was recorded cumulatively between 5 and 30 min postinjection.

**Conclusion**

This study was performed to evaluate the pharmacological activity of DMPF-1 compound on its antinociceptive action on induction with inflammatory mediators and to assess its potential mechanisms on different biological receptors involved using acute pain animal models.
Formalin-induced paw licking test
The method used similarly as described previously.[10] Briefly 20 μL of 2.5% formaldehyde solution was administered intraplantar (i.p.) to the left hind paw of a pretreated group of mice (n = 6) 30 min with DMPF-1 (0.1–5 mg/kg, respectively), vehicle (10 mL/kg), ASA (100 mg/kg i.p.), and morphine (5 mg/kg subcutaneous (s.c.)). Time spent on licking or biting the injected paws was recorded in two phases: neurogenic phase (0–5 min) and inflammatory phase (15–30 min).

Hot-plate test
This test was performed using the method described.[11] The groups of preselected mice (n = 6) with latency of 6–8 s were pretreated 30 min with DMPF-1 (0.1–5 mg/kg, respectively), vehicle (10 mL/kg i.p.), and morphine (5 mg/kg i.p.) before placing the animals on the hot-plate apparatus (Ugo Basile, model- 7189, Gemonio (VA), Italy), 52.5°C ± 0.2°C. The latency time between placing and observed thermonocifensive reactions such as paw licking or shaking, jumping, or an attempt to escape was recorded every 30-min interval for 210 min. The cutoff time on the heated surface was 20 s to avoid further tissue injuries.

Evaluation of opioid receptor’s involvement
In evaluation of opioidergic system involvement, separated groups of mice were pretreated with naloxone (5 mg/kg i.p.) 15 min before administering of DMPF-1 (1 mg/kg i.p.) 30 min. Hot-plate test was applied. Morphine (5 mg/kg s.c.) was used as a reference drug.[10]

Antinociceptive evaluation against capsaicin-, phorbol 12-myristate 12 acetate-, and glutamate-induced nociception
The evaluation of the DMPF-1 antinociceptive mechanism was carried out according to the procedures described.[12] The capability of DMPF-1 to antagonize the effect of the phlogistic agents 30 min after assessing the administration of DMPF-1 (0.1–5 mg/kg i.p., respectively). Vehicle (10 mL/kg), capsazepine (0.17 μmol/kg i.p.; capsaicin positive control), and ASA (100 mg/kg i.p.; PMA and glutamate positive control) were used as control group.

Statistical analysis
The statistical significance of differences between groups was assessed using one-way analysis of variance (ANOVA) with Tukey’s, Dunnet’s, and Bonferroni’s post hoc tests using Prism 4.0 software (GraphPad Software, San Diego, California); a value of P < 0.05 was considered significant for all cases. The percentage of inhibition was calculated using the following formula: ([mean of control group – mean of the test group]/[mean of control group]) × 100.

RESULT
Acetic-acid-induced abdominal writhing test
DMPF-1 compound at doses 0.1, 0.5, 1, and 5 mg/kg i.p. caused a significant abdominal writhing reduction as compared to control group [Figure 1B] with percentage of reduction 36.27%, 59.72%, 93.68%, and 98.15%, respectively. DMPF-1 treatment at dose of 0.5 mg/kg produced comparable effect with ASA at dose 100 mg/kg.

Formalin-induced paw licking test
DMPF-1 produced a significant inhibition in formalin-induced nociception in both phases with 61.6% and 88.2%, respectively, at dose 5 mg/kg [Figure 1C and D].

Hot-plate test
Mice treated with DMPF-1 (1 and 5 mg/kg) and morphine (5 mg/kg) significantly decreased the withdrawal latencies in hot-plate test starting at 30 min onward as compared to the control group as depicted in Table 1.

Evaluation of opioid receptor’s involvement
Pretreatment of mice with naloxone (5 mg/kg i.p.) showed no significant reduction in the antinociceptive profile of DMPF-1 in the hot-plate test [Table 1].

Antinociceptive effect of DMPF-1 against capsaicin-, phorbol 12-myristate 12 acetate-, and glutamate-induced nociception
DMPF-1 pretreatment caused a significant inhibition in capsaicin-induced nociception. The compound at a dose of 5 mg/kg produced 78.8% inhibition, respectively, as compared to its competitive antagonist, capsazepine with 36.4% inhibition [Figure 1E]. A similar reduction response was seen in PMA- and glutamate-induced nociception with 91.04% and 53%, respectively, at a similar dose [Figure 1F and G]. DMPF-1 at a dose of 5 mg/kg produces a higher reduction in nocifensive response than the standard drug ASA.

DISCUSSION
This study has shown the peripheral and central antinociceptive profile of DMPF-1 as this compound attenuated the chemical and thermal-induced nociception.[13] From the finding, it is postulated that DMPF-1 inhibit the release of various mediators that is capable of influencing nociception including COX and lipoxgenase (LOX) upon acetic acid administration.[13] Local i.p. induction of the formalin produced a biphasic nociceptive response. The first phase indicates neurogenic phase due to the direct activation of the nociceptor by the chemical; meanwhile, the second phase is an inflammatory phase due to the release...
of tissue by-products and various inflammatory mediators including PG, histamine, bradykinin, cytokines, substance P, and 5-hydroxytryptamine on stimulation with the noxious stimuli.\(^{[14]}\) Taking this into account, the formalin test is proficient at distinguishing the possible antinociceptive mechanism of DMPF-1. Meanwhile, the hot-plate test is used in screening centrally acting drugs. From the experiment, DMPF-1 supplementation had increased the latency response, which suggests the involvement of the central system.

Naloxone antagonism assay was conducted to evaluate the possible participation of the opioidergic system of this compound in modulating nociception.\(^{[13]}\) Various analgesics exert their effects via an opioid system such as morphine, which is often associated with sedative and tolerance. Naloxone is a well-known antagonist that occupies opioid receptors to perform its action. In this study, the antinociceptive activity of DMPF-1 was not altered by naloxone pretreatment in the hot-plate test, which indicates that DMPF-1 is not likely to mediate its analgesic activity via opioid receptors antagonism.

A recent report stated the role of transient protein vanilloid-1 (TRPV1) receptor in nociception,\(^{[15]}\) which can be activated by capsaicin, heat >42°C, and pH <6.5.\(^{[16]}\) The binding of capsaicin to the TRPV1 receptor triggers the release of various mediators such as neuropeptides, pro-inflammatory mediators, NO, and excitatory amino acids at the free nerves ending.\(^{[15]}\) Studies have shown that capsaicin also induces nociception via the reduction of the threshold level and increases the sensitivity of certain receptors.\(^{[17]}\) In this study, a capsaicin-induced paw licking test was conducted to determine the involvement of TRPV1 receptor in DMPF-1-induced antinociception. In this experiment, the introduction of DMPF-1 produced a significant reduction in nociception caused by capsaicin i.p. injection. Thus, these data proposed that DMPF-1 elicited neurogenic antinociception through the binding with the TRPV1 receptor, which then inhibited the activation of this receptor on stimulation with capsaicin.

In sequence, protein kinase C (PKC) is another mediator that directly phosphorylates the activation of TRPV1 receptor where challenging the TRPV1 receptor agonist with PKC inhibitor had abolished its phosphorylation.\(^{[18]}\) Because of that, the study on the participation of this mediator by DMPF-1 was conveyed. As the pretreatment of DMPF-1 had attenuated the behavioral response of the mice injected with PMA, it is suggested that DMPF-1 mediates its action on PKC with subsequence blocking of

### Table 1: The effect of 3-(2,5-dimethoxyphenyl)-1-(5-methyl furan-2-yl) prop-2-en-1-one (DMPF-1) compound on opioid receptor involvement using hot plate test

| Treatment       | Dose (mg/kg) | 0 min | 30 min | 60 min | 90 min | 120 min | 150 min | 180 min | 210 min |
|-----------------|--------------|-------|--------|--------|--------|---------|---------|---------|---------|
| Vehicle (i.p.)  |              | 6.8 ± 0.40 | 7.0 ± 0.26 | 6.7 ± 0.21 | 7.0 ± 0.37 | 6.3 ± 0.21 | 6.6 ± 0.21 | 6.6 ± 0.21 | 6.0 ± 0.26 |
| DMPF-1 (i.p.)   | 0.1          | 6.7 ± 0.21 | 6.6 ± 0.40 | 6.3 ± 0.57 | 6.3 ± 0.42 | 6.3 ± 0.67 | 7.8 ± 0.40 | 8.0 ± 0.89 | 6.6 ± 0.65 |
|                | 0.5          | 6.7 ± 0.33 | 8.0 ± 0.73 | 7.2 ± 0.75 | 7.5 ± 0.92 | 8.2 ± 0.50 | 9.7 ± 0.42 | 8.0 ± 0.48 | 9.9 ± 0.56 |
|                | 1            | 7.0 ± 0.53 | 10.5 ± 0.85 | 10.5 ± 0.96* | 10.8 ± 1.49 | 15.7 ± 1.48* | 15.7 ± 1.48* | 15.7 ± 1.48* | 9.2 ± 1.79* |
|                | 5            | 7.3 ± 0.21 | 12.0 ± 1.61* | 12.0 ± 1.61* | 15.0 ± 1.44 | 14.8 ± 1.44 | 14.8 ± 1.44 | 14.8 ± 1.44 | 12.2 ± 0.79* |
| DMPF-1 (i.p.) + Nal (i.p.) | 0.1 | 7.4 ± 0.21 | 10.0 ± 1.03 | 10.0 ± 1.03* | 15.7 ± 1.48 | 15.7 ± 1.48 | 15.7 ± 1.48 | 15.7 ± 1.48 | 12.4 ± 0.79* |
|                | 0.5          | 7.2 ± 0.21 | 11.8 ± 0.62 | 11.8 ± 0.62 | 14.8 ± 0.62 | 14.8 ± 0.62 | 14.8 ± 0.62 | 14.8 ± 0.62 | 11.0 ± 1.03* |
|                | 1            | 7.2 ± 0.21 | 11.8 ± 0.62 | 11.8 ± 0.62 | 14.8 ± 0.62 | 14.8 ± 0.62 | 14.8 ± 0.62 | 14.8 ± 0.62 | 11.0 ± 1.03* |
|                | 5            | 7.3 ± 0.21 | 11.8 ± 0.62 | 11.8 ± 0.62 | 14.8 ± 0.62 | 14.8 ± 0.62 | 14.8 ± 0.62 | 14.8 ± 0.62 | 11.0 ± 1.03* |
| Morphine (s.c.)| 5            | 6.87 ± 0.33 | 6.67 ± 0.33 | 6.67 ± 0.33 | 6.67 ± 0.33 | 6.67 ± 0.33 | 6.67 ± 0.33 | 6.67 ± 0.33 | 6.67 ± 0.33 |
| Morphine (s.c.) + Nal (i.p.) | 5 | 6.33 ± 0.21 | 6.67 ± 0.33 | 6.67 ± 0.33 | 6.67 ± 0.33 | 6.67 ± 0.33 | 6.67 ± 0.33 | 6.67 ± 0.33 | 6.67 ± 0.33 |

Each data in the column represent mean ± standard error mean (SEM). Two way analysis of variance (ANOVA) followed by repeated measure and Bonferroni post hoc test used. Asterisks (*) indicate significant different (\(P < 0.05\)) as compared to DMPF-1 (1 mg/kg; i.p.), whereas hashes (#) indicate significant different (\(P < 0.05\)) as compared to morphine (3 mg/kg; i.p.).
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the TRPV1 receptor. It also postulated to increase the threshold level of certain receptors together in decreasing the membrane permeability followed by inhibition of impulse transmission.

Previous reports stated that PKC was also a phosphorylated glutamatergic receptor: AMPA directly and N-methyl-aspartate (NMDA) receptor indirectly.[19] Glutamate mediates its action on

Figure 1: (A) Chemical structure of 3-(2,5 dimethoxyphenyl)-1-(5-methyl furan-2-yl) prop-2-en-1-one (DMPF-1), effect of DMPF-1 compound in (B) acetic-acid-induced abdominal writhing test, (C) phase 1 formalin-, (D) phase 2 formalin-, (E) capsaicin- (F) phorbol 12-myristate 12 acetate (PMA)-, and (G) glutamate-induced paw licking test. Every column signifies mean ± standard error mean (SEM). Asterisks (*) represent the significant difference at $P < 0.05$ as compared to the vehicle-treated group.
ionotropic receptors at three different levels that are peripheral, spinal, and supraspinal and activates the metabotropic receptors via second messenger system. As glutamate receptors give a big contribution to the pain pathway, modulation of its receptors becomes a therapeutic target in neuropathic pain, inflammation, and joint pain. Therefore, the involvement of the glutamatergic system in DMPF-1 action was evaluated. Our finding verified that DMPF-1 effectively attenuates glutamate-induced nociception, thus occupying this receptor in mediating its analgesic activity.

Moreover, glutamate-induced nociception and paw edema were greatly influenced by the presence of NO where activation of the NMDA receptor was shown to increase calcium influx, thus rising the intracellular concentration. This situation indirectly influences NO synthase to convert -arginine to NO and -citrulline. Our finding was in parallel with the study, which showed that chalcone inhibits the production of NO in murine macrophage cell line RAW 264.7. Thus, this suggests that DMPF-1 reduces NO production through interaction with the glutamatergic system.

**Conclusion**

In summary, this study revealed that DMPF-1 is capable as an antinociceptive and anti-inflammatory agent. Various complex pathways took part in DMPF-lantinociception such as TRPV-1, glutamatergic, and PKC system, excluding the opioid system.

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**Conflicts of interest**

There are no conflicts of interest.

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