Trop J Nat Prod Res, August 2023; 7(8):3735-3740 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Evidence of Nitric Oxide-Cyclic GMP-Potassium Channels Involvement in Antinociceptive Activity of Chalcone Derivative; 3-(2,5-dimethoxy phenyl)-1-(5-methyl furan-2-yl) prop-2-en-1 (DMPF-1) Using Behaviour-Induced Nociception

Noor A. A. Bakar[1], Noor A. Suliman[1], Mohd R. Sulaiman[2], Muhammad N. Akhtar[3]

[1]*Faculty of Medicine, Universiti Sultan Zainal Abidin, Jalan Sultan Mahmud, 20400 Kuala Terengganu, Malaysia.*
[2]*Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*
[3]*Department of Chemistry, Ghazi University, Dera Ghazi Khan, Pakistan*

ARTICLE INFO

Article history:
Received 12 May 2023
Revised 08 August 2023
Accepted 20 August 2023
Published online 01 September 2023

ABSTRACT

Chalcones are interesting and versatile compounds due to their various pharmacological properties. Important biological activities of chalcones have been reported for decades using different experimented models. The 3-(2,5-dimethoxy phenyl)-1-(5-methyl furan-2-yl) prop-2-en-1 (DMPF-1) is one of the chalcone analogues that has reported to have analgesic properties despite of it published safety profile. The present study examined the possible anti nociceptive modulatory activity exerted by the DMPF-1 compound in behavioural-induced nociception in animal model using Institute of Cancer Research (ICR) mice. Administration of DMPF-1 compound at a dose of 1 mg/kg intraperitoneally exerted a pronounced antinociceptive effect against acetic acid-induced nociception. The antinociceptive effect of the DMPF-1 compound significantly (P<0.001) was reversed by the pre-treatment of the animals with the nitric oxide precursor; L-arginine (100 mg/kg; i.p), and the soluble guanylyl cyclase inhibitor; oxadiazole (4,3-a) quinoxaline-1-one (ODQ) (2.0 mg/kg; i.p.). A similar inhibitory pattern was observed upon the challenge of DMPF-1 against two potassium channel inhibitors, glibenclamide (10 mg/kg; i.p.) and tetraethylammonium (4 mg/kg; i.p.). Overall, these findings suggest the possible contribution of nitric oxide-cyclic GMP-potassium signalling pathway in the antinociceptive activity exhibited by the DMPF-1 compound. This chalcone analogue and its molecular structure might be further investigated as a model that could be used to obtain more potent analgesic agents.

***Keywords*:** 3-(2,5-dimethoxy phenyl)-1-(5-methyl furan-2-yl) prop-2-en-1, DMPF-1, nitric oxide, L-arginine, ATP dependent potassium channel, Voltage gated potassium channel, soluble guanylyl cyclase, guanosine monophosphate, abdominal writhing

Introduction

Many naturally active compounds with potential pharmacological properties shared a similar structural backbone of chalcones.[1,2] Due to its unique scaffold, chalcones have become the template for the discovery of many drugs.[3,4] Various chalcone derivatives have been synthesised and studied for their medicinal properties. One of these derivatives is 3-(2,5-dimethoxy phenyl)-1-(5-methyl furan-2-yl) prop-2-en-1 (DMPF-1). Previous evidence on the anti-inflammatory activity of DMPF-1 towards airway inflammation in the asthmatic model showed that this chalcone analogue was capable of inhibiting β-chemokines, including Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted (RANTES), eotaxin-1, Monocyte chemoattractant protein-1 (MCP-1) and T helper 2 (Th2) cytokines which contribute to the pathogenesis of inflammatory reaction in asthma.[5] Further analysis from a similar study reported the specific action of this compound on the NF-κB pathway without affecting the MAPK pathway.

*Corresponding author. E mail: noorazlina@unisza.edu.my
Tel: +60174700395

Citation: Bakar NAA, Suliman NA, Sulaiman MR, Akhtar MN. Evidence of Nitric Oxide-Cyclic GMP-Potassium Channels Involvement in Antinociceptive Activity of Chalcone Derivative; 3-(2,5-dimethoxy phenyl)-1-(5-methyl furan-2-yl) prop-2-en-1 (DMPF-1) Using Behaviour-Induced Nociception. Trop J Nat Prod Res. 2023; 7(8):3735-3740 http://www.doi.org/10.26538/tjnpr/v7i8.25

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Being aware that inflammation may regulate nociception, a study on the effect of DMPF-1 in modulating nociception was done in 2020. The study revealed that this chalcone analogue could attenuate peripheral and central nociception using animal models.[6] As the toxicity evaluation indicated its safety profile, thus, further study on its possible mechanism of action was carried out, and the findings showed that antinociceptive activity DMPF-1 was by the interaction of this chalcone analogue with several nociceptive signalling pathways inclusive of vanilloid and glutamatergic system with the exclusion of opioidergic system. [6,7]

In addition to the previously studied signalling pathway, there are other pathways that play a role in the mechanism of pain perception, and one such pathway involves the presence of nitric oxide (NO). Nitric oxide is a gaseous substance that can pass through the cell membrane. The production of NO is controlled by an enzyme called nitric oxide synthase (NOS), which converts L-arginine into NO and L-citrulline. There are three different forms of NOS that have been identified based on their specific functions and where they are found in the body. However, only the nNOS and iNOS isoforms are involved in pain signalling, while eNOS is not.[8] Both nNOS and eNOS are similar in that they depend on calcium and calmodulin for their activity, and they are present in both the spinal cord and brain. On the other hand, iNOS does not require calcium (Ca^{2+}) and is expressed in macrophages and inflammatory cells in response to cytokines or endotoxin.[9] The existence of NO in the cell stimulates an enzyme called soluble guanylate cyclase, which converts guanosine triphosphate (GTP) into cyclic guanylate monophosphate (cGMP). The cGMP then acts as a secondary messenger that regulates various processes inside the cell, such as the activation of kinases, ion channels, and phosphodiesterase.

Additionally, the activation of the cyclooxygenase (COX) enzyme and the S-nitrosylation of proteins may also occur.[10]

The role of chalcones and its derivatives in scavenging NO is well established. In 2018, Kim and colleagues reported that isolated prenylated chalcone from *Psoralea Corylifolia* modulates the expression of iNOS in microglial and attenuate the inhibitory-κBα (I-κBα) degradation and nuclear factor κB (NF-κB) thus preventing neuroinflammation.[11] This finding was aligned with a study using 24 different pyrrolylated-chalcones whose derivatives not only inhibited the production of NO but also attenuated the release of prostaglandins E2 (PGE2) using RAW 264.7 macrophage cell line.[12] The carrageenan-induced paw oedema model further confirmed the findings, in which a marked reduction in oedema formation was indicated by Rojas and colleagues in 2002 and supported by other findings in using different chalcone analogue.[13,14] In addition to its activity towards NO, the computational review on various chalcones structure was done to obtain the most effective NO scavenging molecular structure by positioning the hydroxyl as well as methoxy group of chalcone backbone. [15]

Due to chalcone's versatility, research on drug discovery using chalcone keeps ongoing. Many chalcone derivatives were synthesised to search for most efficacious and potent analgesic compound. Various study models were conducted to verify the specific target and mechanistic event exerted by numerous chalcone derivative.[16-18] A review written in 2020 provided a comprehensive view of the medicinal chemists for the rationale in designing novel and potent iNOS inhibitors using chalcone scaffold.[19]

Further evaluation of the antinociceptive activities of DMPF-1 was thus carried out. Given the broad nociceptive signalling pathway and potential analgesic activity shown by this compound, the present study sought to scrutinise the antinociceptive mechanism of the compound via a specific nociceptive signalling pathway. Noticing the role of NO in the pain signalling pathway, the possible participation of this pathway was carried out and further streamed down into the possible involvement of the cGMP pathway.

Further evaluation of the antinociceptive activities of DMPF-1 was thus carries out. Given the broad nociceptive signalling pathway and potential analgesic activity shown by this compound, the present study sought to scrutinise the antinociceptive mechanism of the compound via a specific nociceptive signalling pathway. Noticing the role of NO in the pain signalling pathway, the possible participation of this pathway in antinociceptive activity of DMPF-1 was carried out and further streamed down into the possible involvement of the cGMP pathway.

Reaching the cGMP level is not the end of the nociceptive quest. There are further downstream nociceptive modulatory events following its stimulation. Sensitisation of cGMP capable to modulates the family of K^+ channels in which the principle of these channel involvement in pain signalling are to regulate the firing threshold with subsequent neuronal excitation, thus contributing to the perception of pain sensation.[20] Considering the downstream regulation that cGMP activation may exert, by which its stimulation may modulate several K^+ channel family activities, thus the potential engagement of DMPF-1 onto this family channel was also explored.

Accordingly, the result from this study may provide a better understanding of the nociceptive signalling pathway and offer insight into the capability of these chalcone derivatives as potential analgesic agents.

Materials and Methods

Animal

Animal used for the study were 4 to 6-week-old male ICR mice with weights ranging between 20-25 g. The mice were maintained in a room with a standard constant temperature of 24±1°C with 12:12 hours dark-light cycle and were allowed free access to food and water ad libitum. The rules of the ethical guidelines to assess the pain in conscious animals were strictly adhered and the study was conducted upon approval by Animal Care and Use Committee (Approval number: UPM/FPSK/PADS/BR-UUH/00425), Universiti Putra Malaysia, Serdang, Selangor. The least number of animals (n=6) and stimulus (chemical) enough to present the effect of the compound was given. The experimental animals were sacrificed at the end of the study.[7]

Preparation of DMPF-1 compound

The chalcone derivative DMPF-1 was synthesized using the Claisen-Schmidt condensation reaction. The DMPF-1 treatment doses applied throughout this study were chosen based on the IC_{50} result obtained from the preliminary antinociceptive study, and the volume administered was 10 ml/kg; i.p.[6]

Drugs and reagent

L-arginine (L-Arg) and its enantiomer; L-NG-nitro arginine (L-NOARG), the soluble guanylyl cyclase inhibitor; 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), Potassium channel blockers; tetraethylammonium (TEA), and glibenclamide were purchased from Sigma Aldrich Chemical, USA, Glacial acetic acid from Scharlau Chemie, SA, Normal saline (NaCl) of 0.9% was used to dissolve all the chemicals except L-NOARG, ODQ and glibenclamide, which were diluted using 5% dimethyl sulfoxide (DMSO). The DMPF-1 compound was diluted using three different solvents which were absolute ethanol, Tween 20 (Sigma, Aldrich), and distilled water in fractions of 5:5:90 (v/v).

Involvement of Nitric Oxide-cGMP signalling

To investigate the hypothesis that Nitric Oxide-cGMP signalling might have participated in the antinociceptive profile induced by DMPF-1, its analgesic response was evaluated using the acetic acid-induced abdominal writhing test, described formerly by Koster and colleagues with minor modification.[21] Mice were divided into six subgroups (n=6). Two groups of mice were pre-treated with nitric oxide precursor, L-arginine (100 mg/kg; i.p. for 15 minutes prior to the challenge with either DMPF-1 (1 mg/kg; i.p.) or nitric oxide synthase (NOS) inhibitor, L-NOARG (20 mg/kg; i.p.) for the next 30 minutes. Later, acetic acid of 0.6% (v/v) with a volume of 10 ml/kg body weight, which acts as a nociceptive inducer, was administered with a volume of 10 ml/kg body weight, i.p.. The animals were then placed individually in a clear glass observation chamber 9 inches in diameter and 11 inches in height for observation. Writhing episodes, which are characterised by internal rotation of the feet, elongation of the body, and arching of the back together with a stretching of the hind limbs, indicative of pain, were recorded. The rest of the subgroups which were treated alone with either vehicle (10 ml/kg; i.p), DMFP-1 (1 mg/kg; i.p.), L-Arg (100 mg/kg; i.p.) or L-NOARG (20 mg/kg; i.p.) served as control. On the other sets, using a similar protocol, a group of animals was challenged with ODQ (2 mg/kg; i.p.) to scrutinise its possible regulation on the cGMP pathway. The vehicle (10 ml/kg; i.p), DMFP-1 (1 mg/kg; i.p.) and ODQ (2 mg/kg; i.p.) treatments alone were used as control.[22]

Participation of Potassium Channels

To evaluate the involvement of the ATP-dependent potassium channel in the antinociceptive mechanism of DMPF-1, the first two out of four groups of animals were pre-treated with glibenclamide, an ATP-dependent potassium channel blocker at a dose of 10 mg/kg, i.p. After 15 minutes, the animal received an intraperitoneal injection of either vehicle (10 ml/kg) or DMPF-1 (1 mg/kg), while the other two remaining groups of animals that served a control received only vehicle (10 ml/kg) or DMPF-1 (1 mg/kg) for the next 30 minutes followed by injection with acetic acid (0.6% (v/v)). The nociceptive response indicated by writhing episodes as described above, was recorded post-5 minutes of acetic acid injection for 30 minutes.[23]

To determine the involvement of the other type of potassium channel, which is the voltage-gated potassium channel, in DMPF-1 antinociception, another separate set of the similar experiment was implemented by which the DMPF-1 treatment was challenged with TEA, non-selective K^+ channel blocker (4 mg/kg; i.p). The remaining protocol was similar to that described in the above procedure.

Statistical analysis

The results were presented as the mean ± standard error mean (SEM) involving six animals per group. The statistical significance comparison between treated groups was evaluated using a one-way analysis of variance (ANOVA) with Dunnett's posthoc test. All procedures were carried out using Prism 4.0 software (GraphPad Software, San Diego, CA, USA), by which $P<0.001$ was considered significant.

Results and Discussion

Involvement of Nitric Oxide-cGMP signalling

This study aimed to scrutinise the possible anti-nociceptive activity that DMFP-1 would exert using acetic acid algogenic behavioural model. Back to the basis of nociceptive signalling, activation of NMDA receptor by glutamate upon algogenic stimulation is known to increase intracellular calcium concentration. Thus, resulting the activation of nNOS and subsequent conversion of L-arginine to nitric oxide and L-citrulline by which this happens directly or via indirect activation of secondary messenger, cGMP.[24] Concerning on the role of nitric oxide in nociception, the involvement of this mechanism in the antinociceptive activity of DMPF-1 was evaluated.

As shown in Figure 1, pre-treatment of the mice with L-arginine at a dose of 100 mg/kg;i.p., 15 minutes before administration of DMPF-1 compound (1 mg/kg;i.p.) and L-NOARG (20 mg/kg;i.p.) had significantly reversed (P<0.001) the antinociceptive effect of both compound (DMPF-1 and L-NOARG). Additionally, a similar result was observed in the second set of experiments by which pre-treatment of ODQ significantly inhibited (P<0.001) the effect of DMPF-1 activity, as shown in Figure 1B.

Our result demonstrated that systemic administration of the DMPF-1 compound attenuated the nociceptive behaviour demonstrated by the mice. However, the antinociceptive response was reverted by the pre-treatment with L-arginine using an optimal dose with no influence on the nociceptive response exerted by acetic acid. To validate, the activity of the DMPF-1 compound was compared with the effect of nitric oxide synthase (NOS) inhibitor, L-NOARG. Pre-treatment of L-arginine also attenuated the activity of L-NOARG when given together. The nociceptive behaviour reduction was significant compared to the group that received its inhibitor alone. In addition, there was no significant difference in the DMPF-1 inhibition pattern when compared with L-NOARG, showing the on-par effect of both treatments. This result revealed at least part of the DMPF-1 mechanism of antinociception, and it is postulated that this compound uses a similar inhibitory mechanism as L-NOARG. Even though the role of NO in pain remains ambiguous due to its dual effect, as a pro and antinociceptive effect, studies have shown that L-arginine at low dose concentration stimulates pro-nociceptive effect and vice versa.[25]

This finding supports the previous findings reported by Liew and colleagues (2010), where the study demonstrated that chalcone derivative attenuates NO production in RAW 264.7 murine macrophage cell lines.[26] Based on the evidence reported, this result suggests that DMPF-1 exhibits anti-nociceptive properties by interacting with the glutamatergic system, as published in previous data, with subsequent reduction in NO production.[6]

Fundamentally, in nociceptive signalling, NO mediates its action via downstream pathway regulation of cyclic GMP (cGMP). Stimulation of soluble guanylyl cyclase (sGC) by the presence of NO plays a role in converting guanosine triphosphate (GTP) into cGMP, thus leading to the phosphorylation of various receptors and increased influx of calcium ions.[27]

To investigate the involvement of this signalling pathway inclusive of the involvement of sGC enzymatic system and subsequent cGMP signalling in antinociceptive activity of DMFP-1, this compound was further challenged with ODQ. The result showed that ODQ pre-treatment had reversed this compound's antinociceptive profile, thus proposing the utilisation of this mechanism in their activity. Conclusively, DMPF-1 does not only participate in inhibiting said nociceptive receptors, as published previously, but also the downstream line until the cGMP level.[6] This finding was parallel with another study using cardamonin, the naturally occurring chalcone as well as nitric oxide-hydrogen sulphide donor chalcone.[28,29] These compound's backbone structure was suggested to be responsible for the activity observed.

Involvement of Potassium Channels

Apart from terminating at the cGMP level, there is downstream regulation in this signalling pathway. Stimulation of cGMP potentiates several K^+ channel family activation with subsequent modification of the nociceptive threshold. Theoretically, the K^+ channel is responsible for coordinating the resting membrane potential. Upregulation or downregulation of these channels modifies the nociceptive threshold, thus neuronal excitability. In 2013, Ooi and colleagues demonstrated that inhibiting NO-mediated M channels or non-inactivating voltage-gated potassium channels might lead to hyperexcitability and over-secretion of CGRP, which participates in vasodilation. This mechanism has been reported in migraine pain.[30]

The most frequently studied potassium channel includes ATP-sensitive, voltage-gated, small, and large calcium-activated channels. Numerous antinociceptive drugs pose similar characteristics and act as K^+ channel openers in mediating their effect. Thus, this channel has been listed as a therapeutic target in developing new pain-relieving agents.[31]

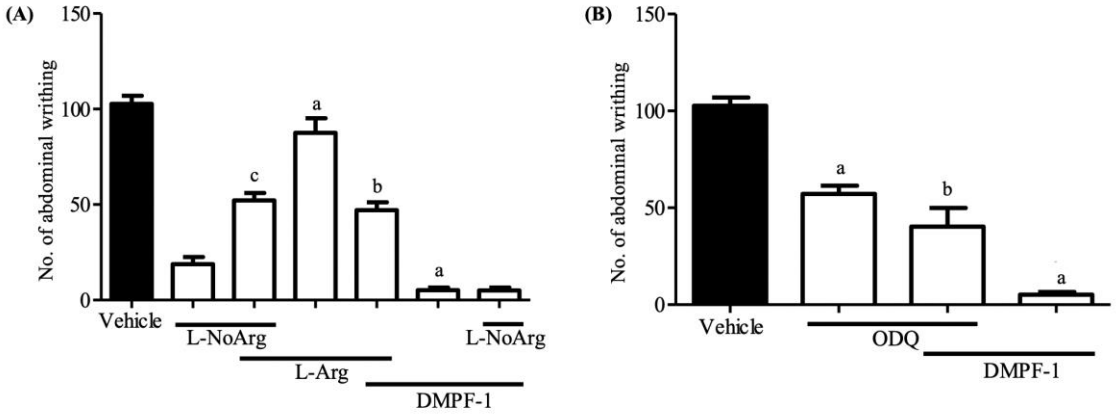

Figure 1: The effect of pre-treatment of the animal with (A) L-arginine (L-Arg; 100 mg/kg, i.p.) and (B) 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) (22 mg/kg; i.p.) on the antinociceptive profile of DMPF-1 compound (1 mg/kg, i.p.) against acetic acid-induced abdominal writhing response. L-NOARG (20 mg/kg; i.p.) was a control positive for L-arginine. Each column represents mean ± Standard Error Mean (SEM) with n=6 using one-way ANOVA followed by Tukey's post hoc test. A denotes a significant difference (P <0.001) compared to the vehicle-treated group. b Denotes a significant difference (P<0.001) compared to DMPF-1 treated group, and c denotes a significant difference (P<0.001) compared L-NOARG treated group.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

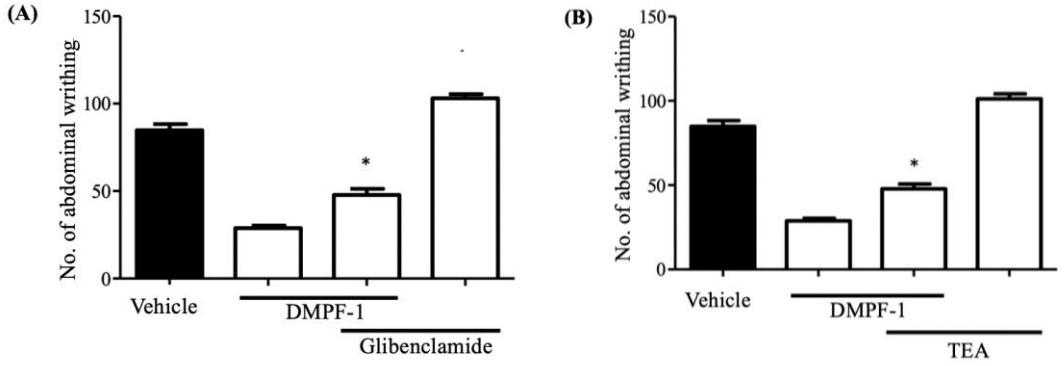

Figure 2: The effect of (A) glibenclamide (10 mg/kg, i.p.) and (B) tetraethylammonium (TEA) (4 mg/kg; i.p.) pre-treatment on the antinociceptive profile of DMPF-1 compound (1 mg/kg; i.p.) using acetic acid-induced abdominal writhing response. Each column represents mean ± Standard Error Mean (SEM) with n=6 using one-way ANOVA followed by Tukey's post hoc test. * Denotes significant different (P<0.001) compared to DMPF-1 (1 mg/kg; i.p.) treated group.

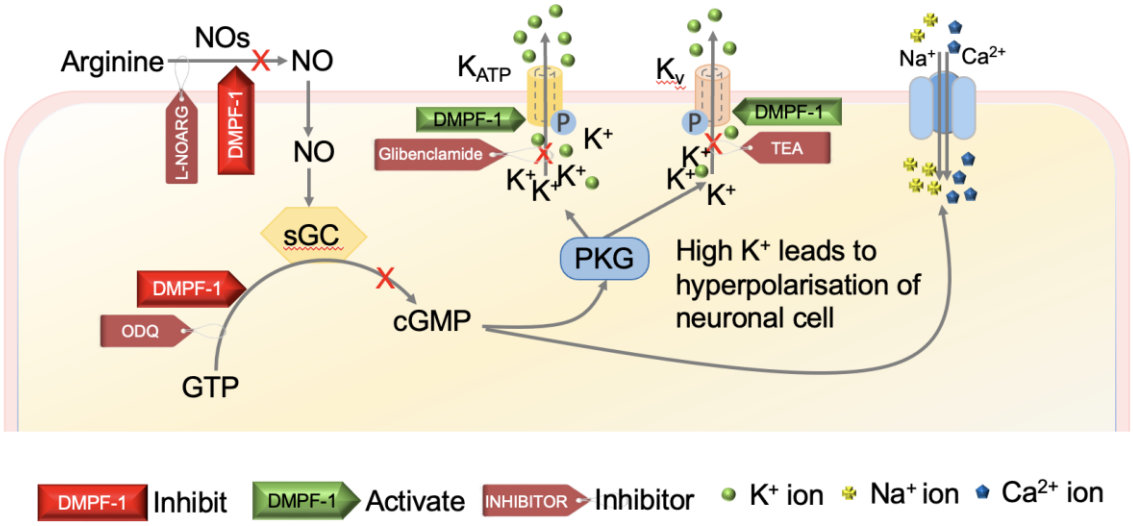

Figure 3: Schematic presentation on the possible interaction of the DMPF-1 compound to the potential analgesic targets. The rectangular labelled DMPF-1 indicates the inhibitory effect of this compound; meanwhile, the arrow indicates its activation. NO: Nitric oxide; NOs: Nitric oxide synthase, sGC: Soluble guanylyl cyclase., CGMP: cyclic guanosine monophosphate, P: Phosphate, PKG: Protein kinase G, TEA: tetraethylammonium, ODQ: 1H-[1,2,4] Oxadiazole[4,3-a]quinoxalin-1-one.

In evaluating the contribution of this channel in the antinociceptive action of DMPF-1, the method of challenging the action of DMPF-1 with a specific potassium channel blocker was implemented. Figure 2 demonstrates a significant difference (P< 0.001) in the effect of DMPF-1 upon pre-treatment of the mice with glibenclamide. There was attenuation in the antinociceptive activity of DMPF-1 instead of its treatment alone at an equivalent dose. A similar result was observed in the group being pre-treated with TEA, as shown in Figure 2B, by which pre-treatment with this voltage-dependent potassium channel blocker had significantly (P<0.001) attenuated the antinociceptive activity of DMPF-1 as if it is being treated alone with this compound.

Blockage of ATP-stimulated potassium channel and voltage-gated potassium channel by glibenclamide and TEA, respectively, prevents the efflux of potassium ions from the neuronal intracellular, thus permitting extensive neuronal firing. Glibenclamide blocked only ATP-sensitive K^+ channels with no influence on Ca^{2+} or voltage-dependent K^+ Channels.[32] Our study revealed that pre-treatment of subjects with both potassium channel blockers prior to treatment of DMPF-1 had reversed the antinociceptive activity of this compound. With this finding, it is stipulated that DMPF-1 partially mediated its effect by activating these channels. Back to the roles of the ATP-sensitive K^+ channel, it serves as a cellular metabolic sensor and simultaneously protects the neuronal cells under pathological conditions as intracellular ATP inhibits it. These channel enhancers, such as pinacidil and diazoxide, have been shown to attenuate bradykinin-induced pain by causing hyperpolarisation of the neurons.[33]

With regards to the voltage-gated potassium channel, 12 subtypes have been reported to be found in mammalian bodies and they have been differentiated based on the gene coding, its channel kinetics, its location on the neuronal membrane and sensitivity to its inhibitory agents.[34] The fast and slow kinetics were the most significant in the axonal ion channel function. Slow kinetics channel was found to be less sensitive to the TEA compared to the fast voltage-gated potassium channel.[35] Reversion of the DMPF-1 activity by TEA postulated the involvement of fast voltage-gated K^+ channel. The Voltage-gated K^+ channel, which was originally identified as an M channel; due to their sensitivity towards the muscarinic acetylcholine receptor activation, was found to be regulated by G protein-coupled receptors acting through Gq/11 type of Gα subunit.[36] Stimulation of nociceptive activity by acetic acid in this study triggered the release of various inflammatory mediators such as bradykinin, prostaglandins, and substance P by which it activates the Gq/11 receptors. This activation causes subsequent alteration in K^+ channel coordination, leading to neuron hyperexcitability and lowering the pain threshold.[37]

Besides these two potassium channels, the DMPF-1 antinociceptive activity also involves a large Ca^{2+}-activated potassium channel but not a small Ca^{2+}-activated potassium channel, as published in our previous report.[38] Activation of this type of channel contributes to the repolarisation of membrane potential in certain cell types. In addition, it also limits the neuronal firing frequency due to the excessive efflux of potassium, which leads to a hyperpolarisation state. Therefore, pain signals will be reduced or fired limitedly to the higher brain centre. This result further justifies the active participation of this channel in its mechanism of action. The mode of action of this compound found to be comparable to the existing NSAID such as diclofenac and ketorolac and currently search endocannabinoids as being reported by many studies.[39-41] It is thus proposed that this compound having the potential to be developed as a new analgesic agent. Figure 3 summarises the target of the DMPF-1 compound in mediating its antinociceptive effect.

Conclusion

The result obtained from this study revealed the broad spectrum analgesic activity of DMPF-1 analgesic activity against the explored nociceptive signalling. The data obtained can further be used to evaluate the other possible analgesic targets exerted by the compound. The background structure of this compound can further be evaluated, making it one of the potential analgesic candidates.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

We gratefully thank Universiti Putra Malaysia for providing facilities in conducting this research work. Special thanks to the laboratory staff of pain and inflammation team from Universiti Putra Malaysia for their assistance and guidance in conducting this study.

References

1. Chavan BB, Gadekar AS, Mehta PP, Vawhal PK, Kolsure A K, Chabukswar AR. Synthesis & Medicinal Significance of Chalcones-A Review. Asian J. Biomed. Pharm. Sci. 2016; 1 ;6(56):01-07.
2. Atukuri D, Vijayalaxmi S, Sanjeevamurthy R, Vidya L, Pras annakumar R, Raghavendra MM. Identification of quinoline -chalcones and heterocyclic chalcone-appended quinolines a s broad-spectrum pharmacological agents. Bioorg. Chem. 20 20; 1;105:104419.
3. Gao F, Huang G, Xiao J. Chalcone hybrids as potential antic ancer agents: Current development, mechanism of action, an d structure-activity relationship. Med. Res. Rev. 2020; 40(5) :2049-84.
4. Pasricha S, Gahlot P. Synthetic Strategies and Biological Po tential of Coumarin-Chalcone Hybrids: A New Dimension t o Drug Design. Curr. Org. Chem. 2020; 1;24(4):402-38.
5. Rajajendram R, Tham CL, Akhtar MN, Sulaiman MR, Israf DA. Inhibition of epithelial CC-family chemokine synthesis by the synthetic chalcone DMPF-1 via disruption of NF-κB nuclear translocation and suppression of experimental asthm a in mice. Mediators inflamm. 2015; 2;2015.
6. Bakar NA, Sulaiman MR, Lajis N, Akhtar MN, Mohamad A S. Evaluation of Antinociceptive Profile of Chalcone Deriva tive (3-(2, 5-dimethoxyphenyl)-1-(5-methylfuran-2-yl) prop -2-en-1-one (DMPF-1) in vivo. J Pharm Bioallied Sci. 2020; 12(Suppl 2):S711.
7. Bakar NA, Suliman NA, Sulaiman MR, Akhtar N. Acute An d Subacute Toxicity Profile Of (3-(2, 5-Dimethoxyphenyl)-1 -(5-Methylfuran-2-Yl) Prop-2-En-1-One, Chalcone Derivati ve In Experimental Animal Model. Malays Appl Biol. 2021; 30;50(2):9-18.
8. Gühring H, Tegeder I, Lötsch J, Pahl A, Werner U, Reeh P W, Rehse K, Brune K, Geisslinger G. Role of nitric oxide in zymosan induced paw inflammation and thermal hyperalges ia. J. Inflamm. Res. 2001; 50:83-8.
9. Ali EM, Soha HM, Mohamed TM. Nitric oxide synthase an d oxidative stress: Regulation of nitric oxide synthase. Oxid ative stress-molecular mechanisms and biological effects. In Tech. 2012; 25:61-72.
10. Bredt DS, Snyder SH. Nitric oxide: a physiologic messenger molecule. Annu. Rev. Biochem. 1994; 63(1):175-95.
11. Kim DH, Li H, Han YE, Jeong JH, Lee HJ, Ryu JH. Modula tion of inducible nitric oxide synthase expression in LPS-Sti mulated BV-2 microglia by prenylated chalcones from *Culle n corylifolium* (L.) Medik. through inhibition of I-κBα degra dation. Molecules. 2018 Jan 4;23(1):109.
12. Faudzi SM, Abdullah MA, Manap MR, Ismail AZ, Rullah K , Aluwi MF, Ramli AN, Abas F, Lajis NH. Inhibition of nitr ic oxide and prostaglandin E2 production by pyrrolylated-ch alcones: Synthesis, biological activity, crystal structure anal ysis, and molecular docking studies. Bioorg. Chem. 2020 ;9 4:103376.
13. Rojas J, Domínguez JN, Charris JE, Lobo G, Payá M, Ferrá ndiz ML. Synthesis and inhibitory activity of dimethylamino -chalcone derivatives on the induction of nitric oxide syntha se. Eur. J. Med. Chem. 2002 ;37(8):699-705.
14. Rojas J, Paya M, Dominguez JN, Ferrandiz ML. The synthe sis and effect of fluorinated chalcone derivatives on nitric ox ide production. Bioorganic Med. Chem. Lett. 2002; 5;12(15):1951-4.
15. Mittal A, Vashistha VK, Das DK. Recent advances in the an tioxidant activity and mechanisms of chalcone derivatives: A computational review. Free Radic. Res. 2022; 3;56(5-6):378 -97.
16. El-Sherief HA, Abuo-Rahma GE, Shoman ME, Beshr EA, A bdel-baky RM. Design and synthesis of new coumarin–chal cone/NO hybrids of potential biological activity. Med Chem Res. 2017; 26:3077-90.
17. Yang Y, Wei Z, Teichmann AT, Wieland FH, Wang A, Lei X, Zhu Y, Yin J, Fan T, Zhou L, Wang C. Development of a novel nitric oxide (NO) production inhibitor with potential t herapeutic effect on chronic inflammation. Eur. J. Med. Che m. 2020 ;193:112216.
18. Emam SH, Sonousi A, Osman EO, Hwang D, Kim GD, Has san RA. Design and synthesis of methoxyphenyl-and couma rin-based chalcone derivatives as anti-inflammatory agents b y inhibition of NO production and down-regulation of NF-κ B in LPS-induced RAW264. 7 macrophage cells. Bioorg Ch em. 2021; 107:104630.
19. Minhas R, Bansal Y, Bansal G. Inducible nitric oxide syntha se inhibitors: A comprehensive update. Med. Res. Rev. 2020 ; 40(3):823-55.
20. Liu L, Yang T, Bruno MJ, Andersen OS, Simon SA. Voltag e-gated ion channels in nociceptors: modulation by cGMP. J . Neurophysiol. 2004; 92(4):2323-32.
21. Koster R, Anderson M, De Beer E. Acetic acid-induced anal gesic screening. Fred. Proc. 1959.
22. Zakaria ZA, Roosli RA, Marmaya NH, Omar MH, Basir R, Somchit MN. Methanol extract of Dicranopteris linearis lea ves attenuate pain via the modulation of opioid/NO-mediate d pathway. Biomolecules. 2020 Feb; 10(2):280.
23. Ferdous A, Janta RA, Arpa RN, Afroze M, Khan M, Moniru zzaman M. The leaves of Bougainvillea spectabilis suppress ed inflammation and nociception in vivo through the modul ation of glutamatergic, cGMP, and ATP-sensitive K^+ channe l pathways. J. Ethnopharmacol. 2020; 261:113148.
24. Collado-Alsina A, Rampérez A, Sánchez-Prieto J, Torres M . Nitric Oxide and Synaptic Transmission in the Cerebellum

. In Handbook of the Cerebellum and Cerebellar Disorders 2021; (pp. 1025-1046). Cham: Springer International Publishing.
25. Cury Y, Picolo G, Gutierrez VP, Ferreira SH. Pain and analgesia: The dual effect of nitric oxide in the nociceptive system. Nitric oxide. 2011 Oct 30;25(3):243-54.
26. Liew CY, Lam KW, Kim MK, Harith HH, Tham CL, Cheah YK, Sulaiman MR, Lajis NH, Israf DA. Effects of 3-(2-Hydroxyphenyl)-1-(5-methyl-furan-2-yl) propenone (HMP) upon signalling pathways of lipopolysaccharide-induced iNOS synthesis in RAW 264.7 cells. Int. Immunopharmacol. 2011; 11(1):85-95.
27. Ishikawa Y, Iida H, Ishida H. The muscarinic acetylcholine receptor-stimulated increase in aquaporin-5 levels in the apical plasma membrane in rat parotid acinar cells is coupled with activation of nitric oxide/cGMP signal transduction. Mol. pharmacol. 2002; 61(6):1423-34.
28. Pui Ping C, Akhtar MN, Israf DA, Perimal EK, Sulaiman MR. Possible participation of ionotropic glutamate receptors and l-arginine-nitric oxide-cyclic guanosine monophosphate-ATP-sensitive K^+ channel pathway in the antinociceptive activity of cardamonin in acute pain animal models. Molecules. 2020; 25(22):5385.
29. Sherikar A, Dhavale R, Bhatia M. Vasorelaxant Effect of Novel Nitric Oxide-Hydrogen Sulfide Donor Chalcone in Isolated Rat Aorta: Involvement of cGMP Mediated sGC and Potassium Channel Activation. Curr. Mol. Pharmacol. 2020; 13(2):126-36.
30. Ooi L, Gigout S, Pettinger L, Gamper N. Triple cysteine module within M-type K^+ channels mediates reciprocal channel modulation by nitric oxide and reactive oxygen species. J. Neurosci. 2013; 33(14):6041-6.
31. Ocaña M, Cendán CM, Cobos EJ, Entrena JM, Baeyens JM. Potassium channels and pain: present realities and future opportunities. Eur. J. Pharmacol. 2004; 500(1-3):203-19.
32. Ortiz MI, Cariño-Cortés R, Castañeda-Hernández G. Participation of the opioid receptor–nitric oxide–cGMP–K^+ channel pathway in the peripheral antinociceptive effect of nalbuphine and buprenorphine in rats. Can. J. Physiol. Pharmacol. 2020; 98(11):753-62.
33. Du XN, Zhang X, Qi JL, An HL, Li JW, Wan YM, Fu Y, Gao HX, Gao ZB, Zhan Y, Zhang HL. Characteristics and molecular basis of celecoxib modulation on Kv7 potassium channels. Br. J. Pharmacol. 2011; 164(6):1722-37.
34. Judge SI, Bever Jr CT. Potassium channel blockers in multiple sclerosis: neuronal Kv channels and effects of symptomatic treatment. Pharmacol. Ther. 2006; 111(1):224-59.
35. Krishnan AV, Lin CS, Park SB, Kiernan MC. Axonal ion channels from bench to bedside: a translational neuroscience perspective. Prog. Neurobiol. 2009; 89(3):288-313.
36. Geng X, Du XN, Rusinova R, Liu BY, Li F, Zhang X, Chen XJ, Logothetis DE, Zhang HL. Specificity of Gβγ signaling depends on Gα subunit coupling with G-protein-sensitive K^+ channels. Pharmacology. 2009; 84(2):82-90.
37. Du X, Gamper N. Potassium channels in peripheral pain pathways: expression, function and therapeutic potential. Curr. Neuropharmacol. 2013; 11(6):621-40.
38. Abu Bakar NA. Antinociceptive and anti-inflammatory activities of 3-(2,5 dimethoxyphenyl)-1-(5 methylfuran-2-YL) prop-2-en-1 in mice. 2016.
39. Ortiz MI, Granados-Soto V, Castañeda-Hernández G. The NO–cGMP–K^+ channel pathway participates in the antinociceptive effect of diclofenac, but not of indomethacin. Pharmacol. Biochem. Behav. 2003; 76(1):187-95.
40. Lázaro-Ibáñez GG, Torres-López JE, Granados-Soto V. Participation of the nitric oxide–cyclic GMP–ATP-sensitive K^+ channel pathway in the antinociceptive action of ketorolac. Eur. J. Pharmacol. 2001; 426(1-2):39-44.
41. Lin YF. Potassium channels as molecular targets of endocannabinoids. Channels. 2021; 15(1):408-23.