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Original Article

In vivo anti-inflammatory, analgesic and antipyretic activities of novel thiazolidine-2,4-dione analogs derived from some classical NSAIDs

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Abstract

In an attempt to develop selective inhibitors for the cyclooxygenase-2 enzymes as candidates for drugs to treat inflammation and pain, two thiazolidine-2,4-dione analogs were designed and synthesized, (*E*)-5-(4-isobutylbenzylidene) thiazolidine-2,4-dione (AW 01) and (*E*)-5-(2-hydroxybenzylidene)thiazolidine-2,4-dione (AW 05). They were synthesized by refluxing 4-isobutylbenzaldehyde or 2-hydroxybenzaldehyde, respectively in dried toluene, with thiazolidine-2,4-dione, glacial acetic acid and piperidine. The anti-inflammatory activities of AW 01 and AW 05 were investigated in a rat model of a carrageenan-induced paw edema and a croton oil-induced mouse ear edema. The analgesic and antipyretic activities of AW 01 and AW 05 were evaluated using the mouse model of acetic acid-induced writhing and in a rat model using yeast-induced fever, respectively. Oral administration of AW 01 and AW 05 at a dose of 20 mg/kg significantly inhibited the carrageenan-induced rat paw edema. Administration of AW 01 and AW 05 at the higher dose of 40 and 80 mg/kg, also exhibited a significant reduction of paw edema that was similar to the lower dose of 20 mg/kg. When AW 01 or AW 05 was applied topically at doses of 0.5, 1.0 and 2.0 mg/ear, it had no significant effect on the mouse ear edema induced by croton oil. An analgesic activity of AW 01 and AW 05 was observed at a lower dose (10 mg/kg, p.o.) in the acetic acid-induced writhing model. After administration of AW 01 at a dose of 20, 40 and 80 mg/kg, it significantly reduced writhing, compared with the control group. A similar result was also observed after administration of AW 05 at the same dosage range as for AW 01. For the antipyretic activity, AW 01 and AW 05 at the dose of 10 mg/kg, p.o. reduced rat rectal temperature at all time intervals (1-5 h) in the same way as did the standard drugs. When using AW 01 and AW 05 at the higher dose of 20, 40 and 80 mg/kg, both compounds significantly reduced pyrexia that was similar to the lower administered dose. Both AW 01 and AW 05 demonstrated a similar magnitude of anti-inflammatory, analgesic and antipyretic effects. The oral LD50 values of AW 01 and AW 05 were 1631 mg/kg and greater than 2000 mg/kg, respectively in mice. These results indicate that AW 01 and AW 05, thiazolidine-2,4-dione analogs, possess systemic anti-inflammatory, analgesic as well as antipyretic potentials but have no topical anti-inflammatory activity in experimental animal models.

Keywords: thiazolidine-2,4-dione analogs, inflammation, pain, fever, experimental animal models

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used therapeutically, primarily for the treatment of pain and inflammatory diseases especially osteoarthritis or rheumatoid arthritis (Dannhardt and Kiefer, 2001). The main action of classical NSAIDs such as aspirin, indomethacin, and other NSAIDs is inhibition of the biosynthesis of pro-inflammatory prostaglandins (PGs) by inhibiting the cyclo-oxygenase (COX) enzyme that contributes to a variety of physiological and pathophysiological functions (Vane, 1971).

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There are three isoenzymes of COX, COX-1, COX-2 and COX-3. Each performs essentially the same catalytic reaction while they differ in expression, function and structure. COX-3 has been reported to be a slice variant of COX-1 and is hypothesized to represent part of a CNS mechanism by which acetaminophen and phenacetin exert their analgesic and antipyretic effects (Chandrasekharan *et al.*, 2002). COX-1 is constitutively expressed in most tissues and is involved in the regulation of physiological functions such as platelet aggregation and the integrity of the GI tract and renal functions (Smith *et al.*, 1996). The prostanoids produced via COX-1 ensure the protection of the gastric mucosa by lowering acid secretion, enhancing mucosal blood flow and stimulating mucus formation and bicarbonate secretion. In the compromised kidney, PGE_2 and PGI_2 produced by COX-1 stimulate renal blood flow and diuresis (Vane *et al.*, 1998). COX-2, in contrast, is rapidly induced in inflammatory cells in response to proinflammatory stimuli such as cytokines, growth factors, tumor-promoting agents and bacterial endotoxin (Charlier and Michaux, 2003). It has been hypothesized that inhibitors of COX-2 would share the beneficial anti-inflammatory properties of traditional NSAIDs but have less or do not interfere at all with the GI-protective functions of COX-1.

In the our previous study (Wongmayura and Songkram, 2008), we have attempted to develop COX-2 selective inhibitors from non-selective NSAIDs with the methodology of substituting the carboxylic acid group or the center of acidity. The only available data have indicated that exchanging the carboxylate moiety in aspirin with an alkyl sulfide functionality might produce a specific COX-2 inhibitor (Kalgutkar *et al.*, 1998). Because many NSAIDs contain a carboxylic acid group, this methodology could represent a general strategy for the conversion of a non-selective NSAIDs into a selective COX-2 inhibitor. Thiazolidine-2,4-dione is the mother nucleus of thiazolidinediones. Thiazolidinediones, also known as glitazones, are a class of medications used in the treatment of diabetes mellitus type 2. By binding to and agonizing peroxisome proliferator-activated receptors gamma (PPARγ), thiazolidinediones improve insulin sensitivity and lower blood glucose, free fatty acids and triglyceride levels. Thiazolidine-2,4-dione consists of a five-membered ring containing one sulfur atom, one nitrogen atom and two carbonyl groups on the 2 and 4 positions. It possesses acidity comparable to that of the carboxyl moiety. The pKa value of thiazolidine-2,4-dione is about 6.8 and the pKa value of the carboxyl moiety is in the range of 4-6 depending on the whole structure of the molecule (Sleevi, 2003). Thiazolidine-2,4-dione has been used as a bioisostere of the carboxyl group in the development of several classes of nuclear receptor ligands such as retinoids and thyroid hormone receptor ligands (Kagechika, 2002; Komatsu *et al.*, 2007). Because of its lower acidity and more bulky volume compared to the carboxyl group, we hypothesize that the replacement of the carboxyl group in non-selective NSAIDs with this thiazolidine-2,4-dione will result in a series of compounds with larger sizes that can then take advantage of the much larger NSAID binding site on the COX-2 compared to the NSAID binding site on COX-1, as well as having a lower acidity that would reduce the adverse GI effects of a traditional NSAID. According to the data obtained in an *in vitro* study of the COX inhibitory activity test by thiazolidine-2,4-dione analogs, compound (*E*)-5-(4-isobutylbenzylidene)thiazolidine-2,4-dione (AW 01) showed a non-selective COX inhibitory activity while compound (*E*)-5-(2-hydroxybenzylidene)thiazolidine-2,4-dione (AW 05) exhibited a selective COX-2 inhibition (Wongmayura and Songkram, 2008). Both AW 01 and AW 05 had anti-inflammatory potential, so were selected and synthesized for investigating their pharmacological activities. These included their anti-inflammatory, analgesic as well as their antipyretic activities in experimental animals. In addition, the acute toxicity (oral LD50) of these compounds was also assessed.

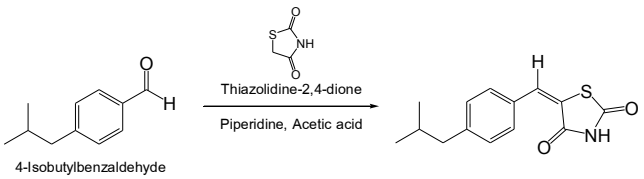
2. Materials and Methods

2.1 Chemistry

Thiazolidine-2,4-diones AW 01 and AW 05 were synthesized according to our previous reported methods (Wongmayura and Songkram, 2008). The obtained compounds were compared of their physical properties and spectroscopic data with the reference AW 01 and AW 05, respectively. All chemicals used in the synthesis and characterization are listed as follows, 2-hydroxybenzaldehyde (Sigma-aldrich, U.S.A.), 4-isobutylacetophenone (TCI, Japan), thiazolidine-2,4-dione (TCI, Japan), piperidine (Merck, U.S.A.) and glacial acetic acid (Mallinckrodt Baker, Inc., U.S.A.)

The melting points of the synthesized compounds were determined using a melting point apparatus (Mel-Templ, Laboratory devices, USA). Spectroscopic experiments were performed with a Fourier Transform-Infared spectrophotometer (FT-IR Model Spectrum One Perkin Elmer™), Fourier Transform Nuclear Magnetic Resonance spectrophotometer (FT-NMR 500 MHz; Model UNITY INOVA, Varin and a JEOL GSX400 instrument operated at 400 MHz) was performed in DMSO-d_6 solutions.

2.2 Synthesis of (*E*)-5-(4-isobutylbenzylidene)thiazolidine-2,4-dione (AW 01)

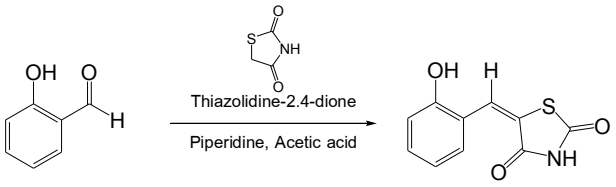


A solution of 4-isobutylbenzaldehyde (5.80 g, 35.8 mmol) in 275 mL of dried toluene was refluxed with thiazolidine-2,4-dione (4.50 g, 38.4 mmol), glacial acetic acid (250 mg, 4.2 mmol) and piperidine (150 mg, 1.8 mmol) for 24 h. The reaction was monitored by TLC using 1:4 ethyl acetate: *n*-

hexane as the mobile phase. The yellow precipitate finally obtained was filtered, washed well with water and *n*-hexane to give a crude (*E*)-5-(4-isobutylbenzylidene)thiazolidine-2,4-dione (AW 01) as a yellow solid. The reactions were conducted three times with the same procedure to provide AW 01; 7.41, 7.32 and 7.50 g, respectively (the average yield was 79.3%). The obtained AW 01 samples were combined and recrystallized from a mixture of ethyl acetate and *n*-hexane to give (*E*)-5-(4-isobutylbenzylidene)thiazolidine-2,4-dione (AW 01) 20.4 g as a yellow solid.

mp (ethyl acetate-*n*-hexane): 165-167ºC. IR (KBr), νmax, cm^{-1}: 3144, 2949, 2862, 1754, 1692. ^{1}H-NMR (DMSO, d_6), δ, ppm : 0.84 (*d*, *J* = 6.5 Hz, 6H), 1.84 (*m*, 1H), 2.49 (*d*, *J* = 7.2 Hz, 2H), 7.31 (*d*, *J* = 8.2 Hz, 2H), 7.34 (*d*, *J* = 8.2 Hz, 2H), 7.5 (*s*, 1H, ArC*H*=C), 12.6 (*s*, 1H, N-*H*). ^{13}C-NMR (DMSO, d_6), δ, ppm : 22.34, 29.76, 44.58, 122.58, 130.15, 130.22, 130.79, 132.06, 144.49, 167.65, 168.18.

2.3 Synthesis of (*E*)-5-(2-hydroxybenzylidene)thiazolidine-2,4-dione (AW 05)



2-Hydroxybenzaldehyde

(AW 05)

A solution of 2-hydroxybenzaldehyde (5.45 g, 44.6 mmol) in 380 mL of dried toluene was refluxed with thiazolidine-2,4-dione (5.75 g, 49.1 mmol), glacial acetic acid (950 mg, 15.8 mmol) and piperidine (1.50 g, 17.6 mmol) for 24 h. The reaction was monitored by TLC using 1:4 ethyl acetate: *n*-hexane as the mobile phase. The yellow precipitate obtained was filtered, then washed well with water and *n*-hexane to give a crude (*E*)-5-(2-hydroxybenzylidene)thiazolidine-2,4-dione (AW 05) as a yellow solid. The reactions were conducted four times using the same procedure to provide AW 05; 5.00, 5.15, 5.25 and 5.20 g, respectively (the average yield was 52.2%). The obtained AW 05 lots were combined, then recrystallized from a mixture of methanol and *n*-hexane to give (*E*)-5-(2-hydroxybenzylidene)thiazolidine-2,4-dione (AW 05) 20.0 g as a yellow solid.

mp (methanol-*n*-hexane): 253-254ºC (from previous studies, mp (tetrahydrofuran-*n*-hexane): 252-254 ºC). IR (KBr), νmax, cm^{-1} : 3417, 3137, 3036, 1734-1672. ^{1}H-NMR (DMSO, d_6), δ, ppm : 6.93 (*d*, *J* = 7.8 Hz, 1H), 6.94 (*t*, *J* = 6.8 Hz, 1H), 7.29 (*t*, *J* = 8.0, 1.4 Hz, 1H), 7.31 (*d*, *J* = 7.2 Hz, 1H), 8.0 (*s*, 1H), 10.50 (*s*, 1H), 12.50 (*s*, 1H). ^{13}C-NMR (DMSO, d_6), δ, ppm : 116.36, 119.94, 120.17, 122.12, 127.23, 128.55, 132.51, 157.53, 167.79, 168.45.

2.4 Chemicals used on experimental animals

The following chemicals were used: carrageenan lambda, aspirin, croton oil and brewer's yeast (Sigma Chem. Co., St. Louis, U.S.A.), tween 80 (Srichand United Dispensary Co., Ltd., Bangkok, Thailand), indomethacin (Fluka BioChemika, Japan), sodium chloride (Carlo Erba, Germanny), acetic acid (J.T, Baker Inc., Phillipsburg, U.S.A.), propylene glycol and acetone (Vidhyasom Co., Ltd., Bangkok, Thailand), ether (Labscan Asia Co., Ltd., Bangkok, Thailand). Celecoxib and ibuprofen were kindly donated by the Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand and Medica Innova Ltd., Thailand, respectively.

2.5 Animals

ICR mice (28-35 g) and Wistar rats (150-230 g) were obtained from the Southern Laboratory Animal Facility, Prince of Songkla University, Thailand. The animals were housed in standard environmental conditions. All experimental protocols were approved by the Animal Ethics Committee, Prince of Songkla University (MOE 0521.11/560, Ref. 13/2010).

2.6 Anti-inflammatory activities

2.6.1 Carrageenan-induced rat paw edema

The test vehicle (solution of propylene glycol, tween 80 and water; 5 mL/kg), standard drugs e.g., indomethacin (5 mg/kg), celecoxib (10 mg/kg), aspirin (200 mg/kg), ibuprofen (100 mg/kg) or the synthesized thiazolidine-2,4-diones (10, 20, 40 and 80 mg/kg) were orally administered 30 min before injection of a 1% carrageenan suspension into the subplantar area of the rats' right hind paw (n = 6). The paw thickness (mm) was measured at 0, 1, 2, 3, 4 and 5 h after injection of the carrageenan using an electronic caliper (Series 1137, Insize CO., LTD., China). This method followed that of previously described by Winter *et al.* (1962), with a slight modification.

2.6.2 Croton oil-induced mouse ear edema

The method described by Tubaro *et al.* (1985) was used. Cutaneous inflammation was induced by application of 5% croton oil (10 µl) in acetone to the inner surface of the mouse right ear. The left ear was used as a blank. Test samples (0.5, 1.0 and 2.0 mg/ear), of each standard drug (0.5 mg/ear) or vehicle was applied topically to the right ear 1 h before the application of croton oil. Four h after the application of croton oil, the mice were sacrificed and a plug (7 mm diameter) was removed from both the treated and untreated ears (n = 10). The oedematous response was measured as the weight difference between the two plugs.

2.7 Antinociceptive activity

Writhing was induced in mice (n = 10) by the intraperitoneal injection (10 mL/kg) of 0.6% acetic acid. The number of writhings was counted over a 20 minutes period as previously described (Koster *et al.*, 1959). Thiazolidine-2,4-diones (10, 20 40 and 80 mg/kg AW 01 or AW 05), indomethacin (5 mg/kg), celecoxib (10 mg/kg), aspirin (200 mg/kg), ibuprofen (100 mg/kg) or vehicle (10 mL/kg) was administered orally to mice 30 min before injection of acetic acid.

2.8 Antipyretic activity

The antipyretic activity of the compounds was measured by a slightly modified method of Adam *et al.* (1968). Male Wistar rats were fasted overnight except for *ad libitum* water before the experiments. Pyrexia was induced by injecting a 20% (w/v) brewer's yeast suspension (10 mL/kg) subcutaneously into the animals' dorsum region. Seventeen h after the injection, the rectal temperature of each rat was measured using a digital thermometer (SK-1250 MC, Sato Keiryoki Mfg. Co., Ltd., Japan). Only rats that showed an increase in temperature of at least 0.7°C were used for the experiments. The test vehicle (5 mL/kg), indomethacin (5 mg/kg), celecoxib (10 mg/kg), aspirin (200 mg/kg), ibuprofen (100 mg/kg) or test agents, AW 01 or AW 05 (10, 20, 40 and 80 mg/kg), were administered orally and the temperature was measured at 1, 2, 3, 4 and 5 h after administration of the compounds.

2.9 Acute oral toxicity in mice

An acute oral toxicity test of the thiazolidine-2,4-dione analogs was performed in mice according to OECD 423 guidelines (OECD, 2001). ICR mice (n=5) were used in this study. The animals were fasted for 4 h with free access to water only. AW 01 or AW 05 was administered orally at a dose of 2000 mg/kg initially and mortality was observed within 14 days. The LD50 was measured by the up and down method (Bruce, 1985).

2.10 Statistical analysis

The results were expressed as a mean ± S.E.M. and statistically analysed using one way analysis of variance (ANOVA) followed by the Bonferroni's test. A significant difference was considered at $P<0.05$.

3. Results

3.1 Effects of synthesized thiazolidine-2,4-diones on inflammatory responses

3.1.1 Carrageenan-induced paw edema in rats

Both AW 01 and AW 05 at a dose of 20 mg/kg produced a significant inhibition of paw edema induced by carrageenan at 5 h with a 20.43 and 24.23 % inhibition, respectively, while at the lower dose of 10 mg/kg neither compound had any significant effects on the rat paw edema, compared to the control group (Figure 1A). All standard drugs e.g., indomethacin, celecoxib, aspirin, ibuprofen showed a significant decrease of hind paw edema at 5 h with a percentage inhibition of 38.72, 27.32, 31.83 and 41.33 %, respectively. Administration of AW 01 and AW 05 at the higher dose of 40 and 80 mg/kg, produced a significant reduction of paw edema that was similar to that produced by the lower dose of 20 mg/kg (Figure 1B).

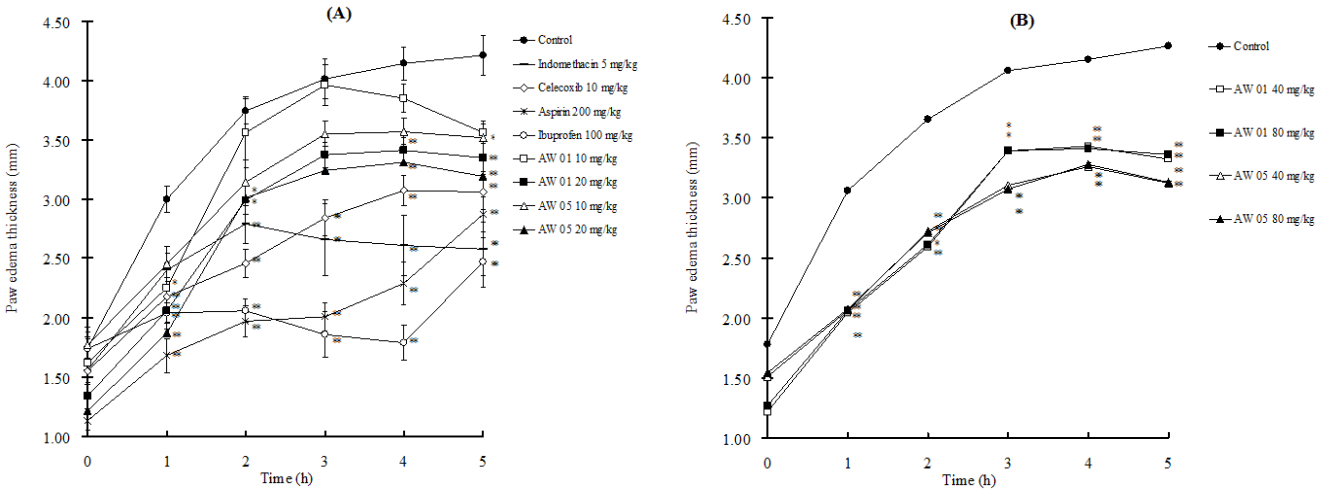


Figure 1. Effects of AW 01, AW 05 (10, 20 mg/kg) and standard drugs (A) and AW 01, AW 05 at the dose of 40 and 80 mg/kg (B) on carrageenan-induced rat paw edema. Each value is presented as the mean ± S.E.M. (n = 6).
* $P<0.05$; ** $P<0.01$ compared to the control group (Bonferroni's test).

3.1.2 Croton oil-induced ear edema in mice

When AW 01 or AW 05 was applied topically at a dose range of 0.5, 1.0 and 2.0 mg/ear, they had no significant effect on ear edema induced by croton oil while indomethacin, celecoxib and aspirin exhibited a significant inhibition of ear edema (34.38%, 33.63% and 31.98%, respectively, Table 1).

3.2 Effects of synthesized thiazolidine-2,4-diones on the nociceptive responses in mice

Both AW 01 and AW 05 (10 mg/kg, p.o.) significantly reduced the number of writhings induced by acetic acid in mice. The AW 01 showed a slightly greater effect than AW 05 with 48.3 % and 39.6 % inhibition, respectively. The standard drugs, celecoxib and ibuprofen produced a 44.4% and a 38.3% inhibition, respectively, while indomethacin and aspirin exhibited a strong suppression of writhing in mice of more than 75%. After administration of AW 01 at a dose of 20, 40 and 80 mg/kg, it significantly reduced writhing by 50.9%, 52.5% and 54.1%, respectively, compared to the control group. A similar result was also observed after administration of AW 05 at the same dosage range as AW 01, with a 52.3%, 51.9% and 52.3% inhibition, respectively (Table 2).

3.3 Effects of synthesized thiazolidine-2,4-diones on yeast-induced fever

The reference drugs, indomethacin, celecoxib, aspirin and ibuprofen significantly reduced the rat rectal temperature at all interval times used in this study. Similar results were also observed using AW 01 and AW 05 (10 mg/kg, p.o.) with comparable effects to ibuprofen and aspirin (Figure 2A). When using AW 01 and AW 05 at the higher dose of 20, 40 and 80 mg/kg, both compounds caused significant reductions in pyrexia that were similar to the lower administered dose (Figure 2B).

3.4 Acute oral toxicity

In the acute toxicity test in mice, the signs and symptoms of toxicity included lethargy, dyspnea, coma and death after oral administration of 2000 mg/kg AW 01. The median lethal dose (LD50) value of AW 01 in mice was 1631 mg/kg after oral administration. There were no toxic symptoms or mortality observed during the 14 days after oral administration of AW 05 to mice at the high dose of 2000 mg/kg.

4. Discussion

The results have demonstrated that the thiazolidine-2,4-diones (AW 01 and AW 05) possess anti-inflammatory, antinociceptive as well as antipyretic activities in rodent models. Both AW 01 and AW 05 exhibited a similar magnitude of inhibition of these responses. The standard drugs, used for comparison of the effects of the new thiazolidine-2,4-diones, were indomethacin that represented a classical non-selective COX inhibitor and celecoxib, a selective COX-2 inhibitor (Grosser *et al.*, 2011). As AW 01 and AW 05 were synthesized from 4-isobutylbenzaldehyde and 2-hydroxybenzaldehyde and their structures are related to ibuprofen and aspirin, respectively, therefore, ibuprofen and aspirin were also used for comparing their activities.

No lethality was observed after oral administration of AW 05 even at the high dose of 2000 mg/kg in mice. Hence,

Table 1. Effects of AW 01, AW 05 and standard drugs on mouse ear edema induced by croton oil.

Drug	Dose (mg/ear)	Ear weight (mg)	Inhibition (%)
Control	-	19.98 ± 0.95	-
Indomethacin	0.5	13.11 ± 1.40*	34.38
Celecoxib	0.5	13.26 ± 0.83*	33.63
Aspirin	0.5	13.59 ± 1.44*	31.98
Ibuprofen	0.5	15.84 ± 1.28	20.72
AW 01	0.5	17.74 ± 0.75	11.21
	1.0	16.20 ± 1.36	18.92
	2.0	16.13 ± 1.10	19.27
AW 05	0.5	18.75 ± 0.87	6.16
	1.0	16.73 ± 0.90	16.27
	2.0	16.64 ± 1.28	16.72

Each value is presented as the mean ± S.E.M. (n = 10).
* $P<0.05$ compared to the control group (Bonferroni's test)

Table 2. Effects of AW 01, AW 05 and standard drugs on the writhing response in mice induced by acetic acid.

Drug	Dose (mg/kg, p.o.)	Number of writhings (counts/ 20 min)	Inhibition (%)
Control	-	60.4 ± 4.4	-
Indomethacin	5	13.7 ± 2.4*	77.3
Celecoxib	10	33.6 ± 5.4*	44.4
Aspirin	200	12.9 ± 2.3*	78.6
Ibuprofen	100	37.3 ± 1.8*	38.3
AW 01	10	31.2 ± 4.0*	48.3
AW 05	10	36.5 ± 6.6*	39.6
Control	-	56.6 ± 1.0	-
AW 01	20	27.8 ± 0.3*	50.9
	40	26.9 ± 0.4*	52.5
	80	26.0 ± 0.4*	54.1
AW 05	20	27.0 ± 0.3*	52.3
	40	27.2 ± 0.3*	51.9
	80	27.0 ± 0.4*	52.3

Each value is presented as the mean ± S.E.M. (n = 10).
* $P<0.01$ compared to the control group (Bonferroni's test)

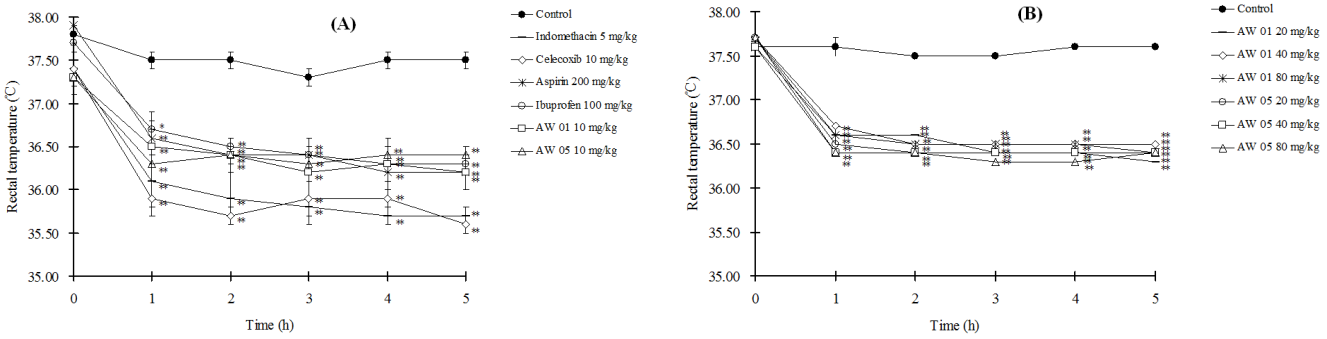


Figure 2. Effects of AW 01, AW 05 (10 mg/kg) and standard drugs (A) and AW 01, AW 05 at the dose of 20, 40 and 80 mg/kg (B) on brewer's yeast-induced fever in rats. Each value is presented as the mean rectal temperature (°C) ± S.E.M. (n = 6). *$P < 0.05$, **$P < 0.01$ compared to the control group (Bonferroni's test).

this compound was considered to be safe. But toxicity was observed e.g., lethargy, dyspnea, coma and death after oral administration of AW 01 at the dose of 2000 mg/kg with an LD50 of 1631 mg/kg, p.o. in mice. Nevertheless, AW 01 still has a wide margin of safety when compared to its pharmacologically effective dose used in this study (10, 20, 40 and 80 mg/kg, p.o.).

The carrageenan-induced paw inflammation is a useful acute inflammatory model for investigation of systemic anti-inflammatory agents. This test is sensitive to most clinically effective anti-inflammatory drugs. It is composed of two phases. The initial phase occurs within 1-2 h after carrageenan injection, due to the release of serotonin and the increase of prostaglandin, histamine and bradykinin in the inflammatory area. The second phase occurs 3-5 h after carrageenan injection, which is correlated with the result from the production and release of kinins and prostaglandins in the inflamed area (Garcia *et al.*, 1973; Vineger *et al.*, 1987; Crunkhon and Meacock, 1971). Both synthesized thiazolidine-2,4-diones showed inhibition of the rat paw edema in both phases. The obtained results indicated that they each possessed an anti-inflammatory property that might be interacting at least by inhibition of COX associated with the inflammatory cascade induced by carrageenan. These results are also supported by investigating them in an *in vitro* study. It was found that at the concentration of 10 mg/mL, AW 01 inhibited COX-1 and COX-2 on COX-2 null or COX-1 null murine fibroblast cell line derived from lung tissue with a 39.03±7.07% and 37.79 ±14.35% inhibition, respectively while AW 05 produced COX-1 and COX-2 inhibitions of 27.79±18.75% and 62.40 ± 6.31% (IC_{50} = 2.99 mg/mL), respectively. The COX-2 selectivity of AW 01 and AW 05 was 0.81 and 2.24, respectively. Therefore, AW 05 was more selective for inhibiting COX-2 than AW 01 (Wongmayura and Songkram, 2008). In the present study, when using AW 01 and AW 05 at the higher dose (40 and 80 mg/kg), although they significantly reduced rat paw edema, there was not a strong suppression. There is a need to further develop thiazolidine-2,4-dione analogs that possess more potent anti-inflammatory activities.

Croton oil-induced ear edema is a useful model for testing topical anti-inflammatory activity (Tonelli *et al.*, 1965). 12-*O*-tetradecanoylphorbol-13-acetate (TPA), a kind of phorbol ester contained in croton oil, has been reported to stimulate phospholipid-dependent protein kinase C and is involved in the release of arachidonic acid and its metabolism as well as inducing an overexpression of cyclooxygenase-2, that results in local inflammation with edema formation (Castagna *et al.*, 1982; Nakadate, 1989; Sanchez and Moreno, 1999; Wang *et al.*, 2001). However, when topically applied both thiazolidine-2,4-diones produced no significant reduction of ear edema. It is possible that these compounds may not be easily absorbed or have minimal dermal absorption when applied topically to inflamed skin. Thus, it will require further developments to produce topical formulations with increased skin permeation.

Acetic acid-induced writhing, a visceral pain model, is a chemical stimulus widely used for the evaluation of a general analgesic activity (Koster *et al.*, 1959). In this model, pain is generated indirectly via endogenous mediators like bradykinin, serotonin, histamine, substance P and prostaglandins, that act by stimulating peripheral nociceptive neurons, that are sensitive to NSAIDs and narcotic analgesics. NSAIDs can inhibit cyclooxygenase in peripheral tissues, thus, interfering with the mechanism of transduction in primary afferent nociceptors via inhibition of the synthesis of prostaglandins (Collier *et al.*, 1968; Derardt *et al.*, 1980). From the result presented here, the suppression of acetic acid-induced writhing by these synthesized compounds was comparable to those of the standard drugs, ibuprofen and celecoxib, although they produced less suppression than indomethacin and aspirin. These results indicated that the thiazolidine-2,4-dione analogs may possess antinociceptive activity through reducing the synthesis of mediators involved in the nociceptive response, especially prostaglandins by inhibition of COX (Wongmayura and Songkram, 2008).

In addition to their anti-inflammatory and analgesic activities, the synthesized thiazolidine-2,4-dione analogs also exhibited antipyretic action by reducing the pyrexia induced by yeast in rats with a similar activity to the NSAIDs e.g., ibuprofen and aspirin. NSAIDs suppress fever by inhibiting the synthesis of prostaglandin E_2 (Grosser *et al.*, 2011). It was observed that the dose for demonstrating their

antipyrexia was comparable to that of their analgesic activity (10 mg/kg) but needed a higher dose for their anti-inflammatory activity (20 mg/kg) that was similar to those of general NSAIDs e.g., aspirin which is known to be anti-inflammatory at a higher dose than is required for its analgesic and antipyretic activities (Grosser *et al.*, 2011).

In conclusion, AW 01 and AW 05, thiazolidine-2,4-dione analogs derived from classical NSAIDs possess systemic anti-inflammatory and analgesic as well as antipyretic potentials but have no topical anti-inflammatory activity in experimental animal models.

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