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ORIGINAL ARTICLE

Synthesis and pharmacological evaluation of aminoacetylenic isoindoline-1,3-dione derivatives as anti-inflammatory agents

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KEYWORDS

Aminoacetylenic derivatives;
Isoindoline derivatives;
Anti-inflammatory COX-1 and COX-2 inhibitors

Abstract Aminoacetylenic isoindoline-1,3-dione derivatives were synthesized from the reaction of potassium phthalimide with propargyl bromide to generate 2-(prop-2-yn-1-yl)isoindoline-1,3-dione (ZM1). Treatment of 2-(prop-2-yn-1-yl)isoindoline-1,3-dione with appropriate cyclic amines through Mannich reaction yielded five desired aminoacetylenic isoindoline-1,3-diones called, ZM2–ZM6. The IR, NMR and elemental analysis were consistent with the assigned structures. These synthetic compounds, except ZM6, produced significant ($p < 0.05$–0.01) dose-related inhibition of carrageenan-induced edema in rats following 3 and 5 h post-oral administration of 5, 10, and 20 mg/kg doses. The percent inhibition of edema varied between the compounds at 10 mg/kg dose being ZM3 > ZM5 > ZM4 > ZM2. These percent inhibitions for ZM3 and ZM5 were not significantly different than those of induced by Ibuprofen, Diclofenac and Celecoxib. At 20 mg/kg dose, ZM4 produced a statistically significant reduction of inflammation ($p < 0.01$) 1 h following administration and persisted for 5 h. Furthermore, all the compounds showed inhibition of COX-1 and COX-2 with maximum inhibition at 5 μM. However, the inhibition values were less than Diclofenac

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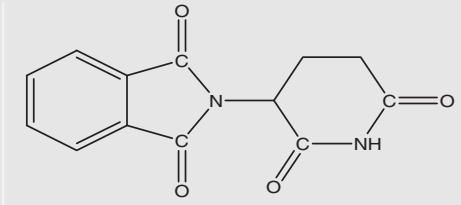
and Celecoxib. The best response was by ZM4 for COX-2 inhibition ranging from 28%, 91%, and 44%, for 2, 5, and 10 μM, respectively. Other ZM compounds such as ZM2, ZM3, and ZM5 exhibited inhibitory responses for COX-2 more than COX-1 at 5 μM. These results indicate that these ZM compounds have the potential to become anti-inflammatory drugs following further pharmacological and toxicological evaluations.

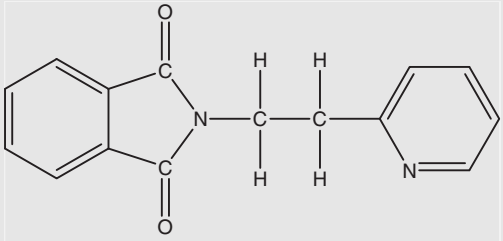
1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat acute or chronic inflammation and offer symptomatic pain relief (Bhati and Kumar, 2008; Lombardino, 1985). Conventional NSAIDs act by non-selective inhibition of cyclooxygenase (COX) enzymes, which are involved in prostaglandins (PGs) biosynthesis from arachidonic acid (Dannhardi and Kiefer, 2001; Carter, 2000; Farooqui et al., 2009). There are at least two main mammalian COX isoforms, COX-1 and COX-2. Constitutive COX-1 has a housekeeping function; including gastro-protective and kidney function regulation PGs, whereas COX-2 is induced in inflammatory cells and generate PGs that help mediate the inflammatory response (Dannhardi and Kiefer, 2001; Carter, 2000; Farooqui et al., 2009). Classical NSAIDs such as Aspirin and Ibuprofen are selective inhibitors of COX-1 isoenzyme and cause gastric failure like bleeding and ulcer. In contrast, selective COX-2 inhibitors such as Celecoxib, Rofecoxib, and Valdecoxib exert anti-inflammatory and analgesic activity with markedly less gastrointestinal toxicity than the traditional NSAIDs (Xie et al., 1991; Ranatunge et al., 2004). However, the worldwide withdrawal of Rofecoxib (Vioxx®) is because of evidence of increased risk to cardiovascular events in patients with heart disease. The latter patients are more prone to myocardial infarction. This may be due to the thromboxane A2/PGI2 imbalance created by selective COX-2 inhibitors (Orjales et al., 2008; Sharma and Ray, 2008; Reddy et al., 2008). In order to abolish or decrease these clinical side effects, a current strategy consists of designing COX inhibitors with different chemical structure from the already known COX inhibitors. We are interested in phthalimide derivative, since some studies in 2005 revealed that thalidomide was effective in treatment of many inflammatory processes (Sano et al., 2005). More recently oxadiazolo-phthalimides showed a significant analgesic and anti-inflammatory properties (Kuogsgaard-Larsen and Ulfmadsen, 2002; Desteven's, 1965; Chen et al., 2005). This indicates that the incorporation of phthalimide group in our designed compounds is safe and could contribute to anti-inflammatory activities. Furthermore, N-2-(2-pyridylethyl) phthalimide showed a significant analgesic activity (Desteven's, 1965). The insertion of acetylenic group in aspirin analogue resulted in higher potency and selectivity toward COX-2 inhibition (Table 1). These structural observations in regard to the pharmacological effect of acetylenic groups and phthalimide promoted our interest to synthesize a novel series of N-[4-(t-amino-yl)-but-2-yn-1-yl]isoindoline-2,3-diones (Table 2) and investigating the anti-inflammatory activity and selectivity of these compounds to COX-1 and COX-2 enzymes. This unique combination represents a new series of compounds as anti-inflammatory agents; differ from the generally used drugs on the market with acidic, enolic, sulfonamide or sulfon groups in their structures.

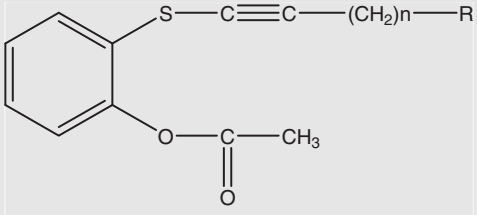
Table 1 Compounds with analgesic and anti-inflammatory activities.



2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione



2-(2-(pyridin-2-yl)ethyl)isoindoline-1,3-dione



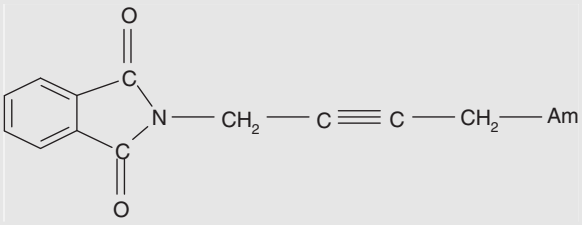
Acetylenic aspirin analogue

2. Experimental

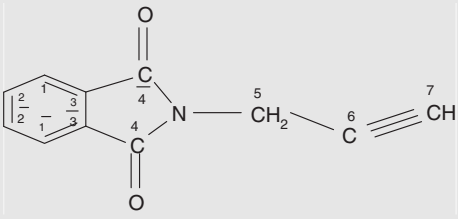
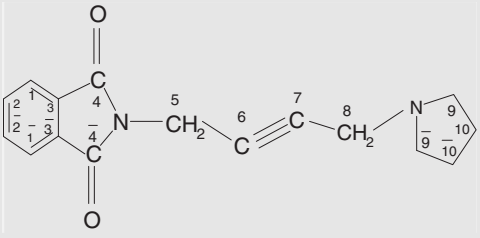
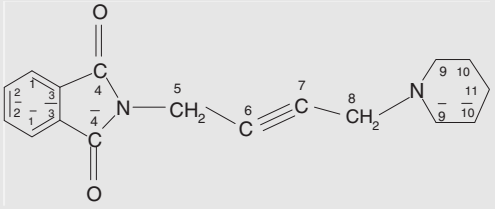
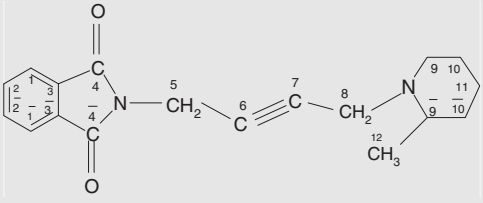
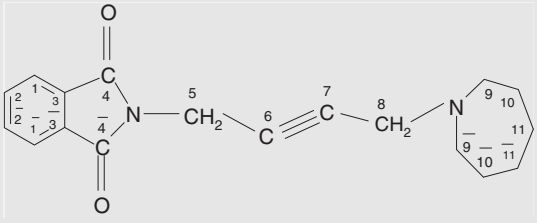
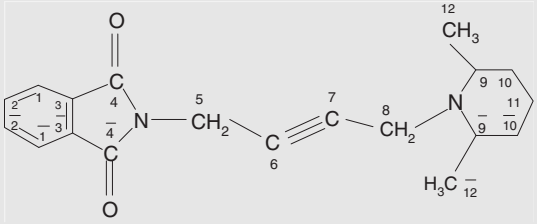
2.1. Chemistry

Melting points were measured by Fischer–Johns melting Point Apparatus and DSC measured were carried out by using DSC-50 (Shimadzu, Japan). Infrared spectra (IR) were recorded, as potassium bromide (KBr) discs on a Nicolet Impact-400 FT-IR spectrophotometer. ^{1}H and ^{13}C NMR were acquired with the aid of Bruker-DPX 300 MHz spectrometers with DMSO-d_6 as solvents and TMS as an internal standard. Elemental analysis was obtained using EU Elemental Analyzer.

Table 2 The synthesized aminoacetylenic isoindoline-1,3-dione compounds.



N-{4-(t-amino-l-yl)but-2-yn-1-yl}isoindoline-1,3-dione

Compound	Am	Formula	Structure
ZM1		$C_{11}H_7NO_2$	
ZM2	Pyrrolidine	$C_{16}H_{16}N_2O_2$	
ZM3	Piperidine	$C_{17}H_{18}N_2O_2$	
ZM4	2-Methylpiperidine	$C_{18}H_{20}N_2O_2$	
ZM5	Hexamethyleneimine	$C_{18}H_{20}N_2O_2$	
ZM6	2,6-Dimethylpiperidine	$C_{19}H_{22}N_2O_2$	

2.2. Synthesis of 2-[prop-2-yn-1-yl]isoindoline-1,3-dione (ZM1)

A solution of potassium phthalimide (1.87 g, 0.01 mol) in 30 ml benzene was refluxed to 40 °C, propargyl bromide (1.4 g, 0.01 mol) was added drop wise to the solution during 30 min. The mixture was stirred for 2 h and then filtered. The solvent was removed under reduced pressure to afford the desired compound (1.2 g, 66.6%) as a white crystalline powder, m.p. (152–153) °C. IR (KBr, cm^{-1}): 3294 (≡CH, stretch), 3049 (ArH, stretch), 2245 (C≡C, stretch), 1766, 1720 (C═O, stretch), 1612, 1553, 1396 (Ar, C═C, stretch), 1000–900 (C═C, bending), 802, 694, 632, (ArH, bending). ^{1}H NMR (DMSO-d_6): δ, 3.24 (t, 1H, J = 2.01 Hz, C≡CH), 4.32 (d, 2H, J = 2.01 Hz, N–CH_2–C≡), 7.76–7.8 (m, 4H, ArH). ^{13}C NMR (DMSO-d_6): δ, 27 (C^5), 74 (C^7), 78 (C^6), 123 ($C^{1,1'}$), 131 ($C^{3,3'}$), 135 ($C^{2,2'}$), 167 ($C^{4,4'}$). Anal. Calcd. ($C_{11}H_7NO_2$): C, 73.50; H, 3.78; N, 7.56. Found: C, 73.4; H, 3.75; N, 7.52.

2.3. Synthesis of N-[4-(pyrrolidino-1-yl)-but-2-yn-1-yl]isoindoline-1,3-dione (ZM2)

A mixture of 2-[prop-2-yn-1-yl]isoindoline-1,3-dione (0.9 g, 0.1 mol), paraformaldehyde (0.15 g, 0.12 mol), pyrrolidine (0.78 g, 0.11 mol) and cuprous chloride catalytic amount (0.03 g) in peroxide-free dioxane 20 ml was refluxed for 1 h. After cooling, 100 ml of water was added and the crude product recrystallized from ethanol (5–10 ml) afforded the desired compound (1.6 g, yield 61.9%), m.p. (112–113) °C. IR (KBr, cm^{-1}): 2931, 2845 (ArH, stretch), 2260 (C≡C, stretch), 1720, 1755 (C═O, stretch), 1612.5, 1458 (Ar, C═C, stretch), 1080, 9412 (Ar, C═C, bending), 895, 725, 640 (ArH, bending). ^{1}H NMR (DMSO-d_6): δ, 1.9 (m, 4H, $^{10}CH_2$–$^{10'}CH_2$), 1.6 (m, 4H, $^9CH_2$–$^9CH_2$), 3.05 (t, 2H, J = 2.4 Hz, ≡C–$^8CH_2$–N), 3.66 (t, 2H, J = 2.4 Hz, $^5CH_2$), 7.7–8 (m, 4H, ArH). ^{13}C NMR (DMSO-d_6): δ, 20 (C^{12}), 24 (C^{11}), 26.2 ($C^{10'}$), 27.4 (C^{10}), 54.4 ($C^{9'}$), 43.8 (C^9), 52.9 (C^8), 54.5 (C^5), 78.2 (C^6), 79.5 (C^7), 123.8 ($C^{1,1'}$), 131.9 ($C^{3,3'}$), 135.2 ($C^{2,2'}$), 167.2 ($C^{4,4'}$). Anal. Calcd. ($C_{16}H_{16}N_2O_2$): C, 72.97; H, 6.75; N, 9.41. Found: C, 72.14; H, 6.72; N, 9.43.

2.4. Synthesis of N-[4-piperidino-1-yl]-but-2-yn-1-yl]isoindoline-1,3-dione (ZM3)

ZM3 was prepared following the same procedure for the synthesis of ZM2 afforded (1.7 g, 63%) as a white crystalline compound, m.p. (83–84) °C. IR (KBr, cm^{-1}): 2931 (ArH, stretch), 2252 (C≡C, stretch), 1720, 1755 (C═O, stretch), 1612, 1458 (Ar, C═C, stretch), 995, 941.2 (C═C, bending), 864, 786, 717, (Ar, H, bending). ^{1}H NMR (DMSO-d_6): δ, 1.53 (m, 2H, $^{11}CH_2$–C), 1.59 (m, 4H, C–$^{10}CH_2$–$^{10'}CH_2$), 1.64 (m, 4H, $^9CH_2$–$^{9'}CH_2$), 3.39 (t, 2H, J = 2.4 Hz, $^8CH_2$–N), 3.66 (t, 2H, J = 2.4 Hz, $^5CH_2$), 7.7–8 (m, 4H, ArH). ^{13}C NMR (DMSO-d_6): δ, 24.03 (C^{11}), 25.8 ($C^{10,10'}$), 27.1 ($C^{9,9'}$), 52.9 (C^8), 66.6 (C^5), 74.2 (C^6), 79.3 (C^7), 123.8 ($C^{1,1'}$), 131.9 ($C^{3,3'}$), 135.2 ($C^{2,2'}$), 167 ($C^{4,4'}$). Anal. Calcd. ($C_{17}H_{18}N_2O_2$): C, 72.34; H, 6.30; N, 9.90. Found: C, 72.31; H, 6.27; N, 9.92.

2.5. Synthesis of N-[4-(2-methyl piperidino-1-yl)-but-2-yn-1-yl]isoindoline-1,3-dione (ZM4)

ZM4 was prepared following the same procedure described for the synthesis of ZM2 afforded (1.8 g, 63.1%) as a white crystalline compound, m.p. (105–107) °C. IR (KBr, cm^{-1}): 2931, 2845 (ArH, stretch), 2260 (C≡C, stretch), 1720, 1755 (C═O, stretch), 1612.5, 1458 (Ar, C═C, stretch), 1080, 9412 (Ar, C═C, bending) 895, 725, 640 (ArH, bending). ^{1}H NMR (DMSO-d_6): δ, 1.12 (d, 3H, J = 4.2 Hz, $^{12}CH_3$), 1.5 (m, 6H, $^{10}CH_2$–$^{10'}CH_2$–$^{11}CH_2$), 1.5 (m, 2H, $^9CH_2$), 1.64 (m, 1H, $^{9'}CH$), 3.05 (t, $^5CH_2$, J = 2.4 Hz), 3.66 (t, 2H, $^5CH_2$, J = 2.4 Hz), 7.7–8.0 (m, 4H, ArH). ^{13}C NMR (DMSO-d_6): δ, 20.0 (C^{12}), 24 (C^{11}), 26.2 ($C^{10'}$), 27.4 (C^{10}), 43.8 ($C^{9'}$), 52.9 (C^8), 54.5 (C^5), 78.2 (C^6), 79.5 (C^7), 123.8 ($C^{1,1'}$), 131.9 ($C^{3,3'}$), 135.2 ($C^{2,2'}$), 167.2 ($C^{4,4'}$). Anal. Calcd. ($C_{18}H_{20}N_2O_2$): C, 72.97; H, 6.75; N, 9.41. Found: C, 72.92; H, 6.73; N, 9.42.

2.6. Synthesis of N-[4-(2-azepan-1-yl)-but-2-yn-1-yl]isoindoline-1,3-dione (ZM5)

The title compound was prepared to according to synthetic procedure described for ZM2, affording (1.8 g, 62.5%) as a white crystalline product, m.p. (72–75) °C. IR (KBr, cm^{-1}): 2924, 2831 (ArH, stretch), 2225 (C≡C, stretch), 1712, 1755 (C═O, stretch), 1612, 1465, 1319 (Ar, C═C, stretch), 1111, 1087, 941 (Ar, C═C, bending), 840, 794, 717 (ArH, bending). ^{1}H NMR (DMSO-d_6): δ, 1.2–1.4 (m, 8H, $^{10}CH_2$–$^{10'}CH_2$–$^{11}CH_2$–$^{11'}CH_2$), 1.6 (m, 4H, $^9CH_2$–$^{9'}CH_2$), 3.03 (t, 2H, J = 2.4 Hz, –$^8CH_2$), 3.46 (t, 2H, J = 2.4 Hz, –$^6CH_2$–), 3.66 (t, 2H, J = 4.04 Hz, N–CH_2–C≡), 7.7–8 (m, 4H, ArH). ^{13}C NMR (DMSO-d_6): δ, 26.8 ($C^{11,11'}$), 27.4 ($C^{10,10'}$), 28.15 ($C^{9,9'}$), 47.7 (C^8), 54.7 (C^5), 78.5 (C^7), 80.8 (C^6), 123.8 ($C^{1,1'}$), 131.9 ($C^{3,3'}$), 135.1 ($C^{2,2'}$), 167.2 ($C^{4,4'}$). Anal. Calcd. ($C_{18}H_{20}N_2O_2$): C, 72.97; H, 6.75; N, 9.45; O, 10.81. Found: C, 72.94; H, 6.79; N, 9.4.

2.7. Synthesis of N-[4-(2,6-dimethylpiperidino-1-yl)-but-2-yn-1-yl]isoindoline-1,3-dione (ZM6)

ZM6 was prepared following the same procedure described for the synthesis of ZM2 afforded (1.6 g, 52%) as a white crystalline compound, m.p. (132–134) °C. IR (KBr, cm^{-1}): 3030, 2931 (ArH, stretch), 2250 (C≡C, stretch), 1750, 1720 (C═O, stretch), 1612, 1465, 1390 (Ar, C═C, stretch), 1100, 1085, 942 (Ar, C═C, bending), 840, 794, 717 (ArH, bending). ^{1}H NMR (DMSO-d_6): δ, 1.14 (d, 3H, J = 4.4 Hz, $^{12}CH_3$), 1.14 (d, 3H, J = 4.2 Hz, $^{12'}CH_3$), 1.5 (m, 6H, $^{10}CH_2$–$^{11}CH_2$–$^{10'}$ CH_2), 1.80 (m, 2H, ^9CH–$^{9'}CH$), 3.4 (t, 2H, J = 2.4 Hz, $^8CH_2$), 3.68 (t, 2H, J = 2.4 Hz, $^5CH_2$), 7.6–7.8 (m, 4H, ArH). ^{13}C NMR (DMSO-d_6): δ, 22.02 (C^{12}), 24.03 (C^{11}), 25.8 ($C^{10,10'}$), 27.1 ($C^{9,9'}$), 52.9 (C^8), 66.6 (C^5), 74.2 (C^6), 79.3 (C^7), 128.8 ($C^{2,2'}$), 131.9 ($C^{3,3'}$), 135.2 ($C^{1,1'}$), 167.0 ($C^{4,4'}$). Anal. Calcd. ($C_{19}H_{22}N_2O_2$): C, 73.54; H, 7.09; N, 9.03. Found: C, 73.52; H, 7.07; N, 9.06.

3. Pharmacology

3.1. Animals

Male Sprague–Dawley rats (7–9 weeks old) were obtained from Yarmouk University animal house unit (Irbid, Jordan).

Animals were housed at the Petra University animal facility in a 12 h light/dark cycle and a constant temperature of 22 °C. All animals were acclimatized for 10 days prior to experiments with free access to standard diet and drinking water. All animal experiments were performed in compliance with relevant laws and institution guidelines.

3.2. Anti-inflammatory activity

The paw edema was induced by subcutaneous injection of 0.1 ml of 1% carrageenan solution into the plantar region of the left hind paw of rats. The thickness of edema was measured and recorded for each rat at 1, 3 and 5 h intervals, using an electronic caliper (Mitutouo Corp., Japan). Different doses of the tested compounds (0, 5, 10 and 20 mg/kg) in comparison with Ibuprofen (5 and 10 mg/kg), Diclofenac (5 and 10 mg/kg), and Celecoxib (3, 6 and 10 mg/kg) were given to the rats by oral gavages 1 h prior to the administration of carrageenan. The percent inhibition of paw edema thickness was calculated using the following formula:

$$\text{Percent inhibition} = 100 \times [1 - (x_2 - x_1)/(y_2 - y_1)]$$

where x_1 is the thickness of paw of rats before administration of carrageenan and test or reference compounds, x_2 is the thickness of paw of rats after administration of carrageenan in the test group, y_1 is the thickness of paw of rats before the administration of carrageenan in the control group and y_2 is the thickness of paw of rats after administration of carrageenan in the control group.

3.3. COX-1 and COX-2 inhibition assay

This assay measures directly prostaglandin ($PGF_{2\alpha}$) produced by $SnCl_2$ reduction of Cox-derived prostaglandin H synthase (Caynman, Chemical Co., MI, USA). Briefly, the $PGF_{2\alpha}$ are produced using ovine Cox 1 and human recombinant Cox 2, arachidonic acid and heme in a reaction buffer (0.1 M Tris–HCl, pH 8.0 containing 5 mM EDTA and 2 mM phenol). The reaction is stopped by adding 1 M HCl followed by adding saturated stannous chloride solution which is used to reduce PGH_2 produced in the COX reaction to more stable PGF_2. The produced $PGF_{2\alpha}$ was then assayed by an enzyme immunoassay using a capture assay mouse anti-rabbit IgG (captured antibody) for rabbit anti-prostaglandin antiserum. Prostaglandin standards (15.6–2000 pg/ml) were used to construct a standard curve, and prostaglandin tracer was used to establish a competitive type of assay. After 18 h of incubation, plates were washed and Ellman's Reagent was added. The intensity of color was measured at a 405 nm using SCO GmbH (Dingelstadt, Germany) ELISA Plate Reader. The absorbance was transformed to pg/ml of $PGF_{2\alpha}$ using standard curve computed on excel after transforming values to % binding or (B/Bo) and present them on log–log graph paper.

3.4. Data analysis

The overall differences between the treated groups were analyzed using one way ANOVA with Dunnett's post hoc test. The level of significant difference was defined as $p < 0.05$.

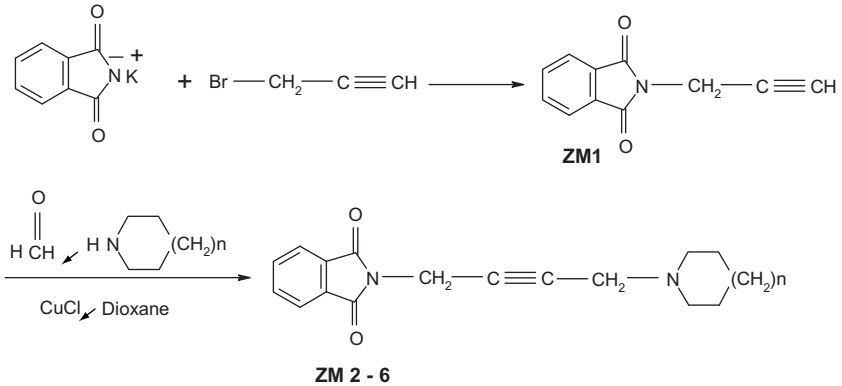
4. Results

4.1. Chemistry

The desired compounds listed in Table 2 were synthesized through the following steps: potassium phthalimide was treated with propargyl bromide in benzene under reflux yielded the appropriate 2-(prop-2-yn-1-yl)isoindoline-1,3-dione. The Mannich reaction of 2-(prop-2-yn-1-yl)isoindoline-1,3-dione (ZM1) with paraformaldehyde and appropriate amines in peroxide-free dioxane with catalytic amount of cuprous chloride generated the designed aminoacetylenic isoindoline derivatives: ZM2, ZM3, ZM4, ZM5 and ZM6 (Table 2). The IR, ^{1}H NMR, ^{13}C NMR spectra, DEPT 135, DEPT 90, and elemental analyses were consistent with the all assigned structures as shown in the experimental part (see Scheme 1).

4.2. Pharmacology

The acute anti-inflammatory activity of ZM2, ZM3, ZM4 and ZM5 showed to be effective, with varying potencies. ZM6 was inactive in reducing any inflammatory caused by carrageenan-induced edema, for this reason it was excluded from testing its inhibitory activity against COX-1 and COX-2. ZM2 at doses of 10 and 20 mg/kg produced significant dose-dependent inhibition of inflammation (in the range from 22% to 57%) after 3 and 5 h post-carrageenan administration ($p < 0.05$). This compound at 20 mg/kg, showed equipotent activity ($p > 0.05$) to that of Ibuprofen (10 mg/kg), Diclofenac (10 mg/kg) and Celecoxib (12 mg/kg) at 3 and 5 h post-administration (Table 3). On the other hand, ZM3 exhibited more inhibitory activity



Scheme 1

Table 3 The percent of inhibition of carrageenan-induced inflammation produced by ZM compounds after oral administration.

Compound	Dose (mg/kg)	Percent of inhibition of carrageenan-induced inflammation after time of administration of ZM compounds[a]		
		1 h	3 h	5 h
ZM2	5	−17.0 ± 0.1	22.0 ± 1.3	22.0 ± 1.2
	10	−17.0 ± 0.1	23.0 ± 1.5	38.0 ± 1.5[b]
	20	2.3 ± 0.3	42.0 ± 3.1[b]	57.0 ± 4.8[b]
ZM3	5	4.0 ± 1.0	44.0 ± 4.2[b]	60.5 ± 4.8[b,c]
	10	21.0 ± 1.2	58.0 ± 4.8[b]	61.2 ± 8.2[b,c]
	20	19.5 ± 1.1	53.0 ± 4.1[b]	72.3 ± 5.1[b,c]
ZM4	5	−11.0 ± 0.1	21.0 ± 1.1	27.0 ± 1.4
	10	−21.1 ± 0.3	−3.5 ± 0.5	40.0 ± 1.6[b]
	20	73.0 ± 5.1[b]	58.0 ± 4.9[b]	76.0 ± 5.2[b]
ZM5	5	23.0 ± 1.2	45.0 ± 10[b]	48.0 ± 4.6[b]
	10	19.5 ± 1.1	23.0 ± 1.2	49.5 ± 4.8[b]
	20	31.0 ± 1.5[b]	68.0 ± 5.2[b]	69.0 ± 5.4[b]
Ibuprofen	5	19.5 ± 1.1	37.5 ± 3.5[b]	27.0 ± 3.9
	10	40.6 ± 2.3[b]	38.0 ± 3.4[b]	40.6 ± 5.6[b]
Diclofenac	5	5.0 ± 1.2	40.5 ± 3.9[b]	56.0 ± 3.9[b]
	10	0.5 ± 0.1	38.0 ± 4.6[b]	40.6 ± 3.5[b]
Celecoxib	3	11.5 ± 1.5	35.0 ± 3.3[b]	38.0 ± 4.1[b]
	6	−29.4 ± 3.8	33.0 ± 3.1[b]	47.0 ± 4.6[b]
	12	55.0 ± 3.6[b]	47.5 ± 3.7[b]	65.0 ± 4.8[b]

[a] Mean ± SD, all points of mean percent inhibition were calculated as mentioned in the text with 8–11 rats per data point except for 20 mg/kg dose groups for ZM4 and ZM5 that had 4 rats per inhibition point.
[b] $p < 0.05$ when compared to control rats.
[c] $p < 0.05$ when compared to Ibuprofen and Diclofenac (10 mg/kg) and Celecoxib (6 mg/kg).

than ZM2 since the inhibition of inflammation induced by carrageenan is more pronounced and independent to dose. Its activity was comparable to Ibuprofen and Diclofenac (5 and 10 mg/kg), and Celecoxib (12 mg/kg) ($p < 0.05$).

The anti-inflammatory activity of ZM4 was dose- and time-dependent. A significant reduction of carrageenan-induced inflammation was detected only at 20 mg/kg dose of ZM4 at 1, 3 and 5 h post-administration ($p < 0.01$). On the other hand, ZM5 produced a varying degree of inhibition of inflammation at 1, 3 and 5 h intervals post-carrageenan-induced inflammation. The maximal inhibition of inflammation was observed with 20 mg/kg after 3 and 5 h intervals, which was equipotent to Diclofenac and Celecoxib (Table 3).

The COX-1 and COX-2 inhibition assay (IC_{50} values) for the tested compounds are presented in Table 4. The results showed that all the compounds ZM2, ZM3, ZM4, and ZM5 showed inhibition of COX-1 and COX-2 with a maximum inhibition at 5 μM (see Table 5).

However, the inhibition values were less than Diclofenac and Celecoxib, as COX-1 and COX-2 inhibitors. All of the ZM compounds exhibited a bell-shaped inhibition curve being the maximum at 5 μM and to a lesser extent at 2 and 10 μM. The best response was by ZM4 for COX-2 inhibition ranging from 27.5%, 90.5%, and 44.0%, for 2, 5, and 10 μM, respectively. Other ZM compounds such as ZM2, ZM3 and ZM5 exhibited inhibitory responses for COX-2 more than COX-1 at 10 μM.

Table 4 The percent inhibition of COX-1 activity by different concentration of ZM compounds using COX inhibition immunoassay (EIA) as compared with Diclofenac. Each value represents the mean ± SD.

Compound	Concentration		
	2 μM	5 μM	10 μM
ZM2	12.5 ± 7.5	73.7 ± 12.6	25.5 ± 3.5
ZM3	28.10 ± 1.0	72.5 ± 20.5	29.0 ± 1.0
ZM4	30.0 ± 1.0	72.0 ± 18.0	8.00 ± 2.0
ZM5	20.5 ± 0.5	74.5 ± 14.3	20.5 ± 0.5
Diclofenac	73.1 ± 3.1	99.44 ± 0.2	98.0 ± 0.2

5. Discussion

The tested aminoacetylenic isoindoline-1,3-diones (ZM2–5), except ZM6, were effective in reducing the inflammation induced by carrageenan in the paw male rats. When the anti-inflammatory activities of some newly synthesized compounds (ZM2–ZM5) were compared with Ibuprofen, Diclofenac and Celecoxib at a dose of 10 mg/kg at 3 and 5 h intervals post-oral administration, it was evident that ZM3 was more effective ($p < 0.05$) than Ibuprofen, Diclofenac, and equal to or slightly more effective than Celecoxib. The order of activity for ZM compounds at 10 mg/kg dose at 3 and 5 h intervals was as follow: ZM3 ⩾ ZM5 ⩾ ZM4 ⩾ ZM2. This variation in activity may be attributed to the nature of the cyclic amino groups; piperidine is preferred over hexamethyleneimine, pyrrolidine, and the least with the 2-methyl piperidine. Such difference may be rationalized on differences in ring size, lipophilicity and conformational stability of the cyclic amine. Steric factor neighboring the basic nitrogen may be the reason in decreasing the potency and/or absorption as seen in ZM4 relative to ZM3

Table 5 The percent inhibition of COX-2 activity by different concentration of ZM compounds using COX inhibition immunoassay (EIA) as compared with Celecoxib. Each value represents the mean ± SD.

Compound	Concentration		
	2 μM	5 μM	10 μM
ZM2	4.0 ± 1.5	79.0 ± 8.0	34.0 ± 5.0
ZM3	8.5 ± 1.0	85.0 ± 11.0	41.0 ± 6.0
ZM4	27.5 ± 1.5	90.5 ± 6.1	44.0 ± 4.0
ZM5	21.5 ± 1.5	85.5 ± 9.0	32.5 ± 2.5
Celecoxib	70.0 ± 0.0	97.5 ± 1.5	93.5 ± 4.5

and inactivity of ZM6. However, higher doses of ZM4 (20 mg/kg) exhibited significant inhibition at 1 h post-administration which persisted up to 5 h in addition to its low IC_{50} against COX-1 and COX-2 activities. This latter phenomenon needs further investigation.

The inhibitory activities of ZM compounds to COX-1 and COX-2 enzyme but were maximal at 5 μM concentration for all ZM compounds and Diclofenac and Celecoxib. However, all tested ZM compounds (ZM2–5) showed lower inhibitory activities than Diclofenac and Celecoxib as selective Cox-1 and COX-2 inhibitors, respectively. The basic aminoacetylenic isoindolines-1,3-diones showed slightly higher inhibitory activity COX-2 as compared to COX-1. The relative orders of inhibitory activities of ZM compounds to COX-1 and COX-2 were ZM2 ⩾ ZM5 ⩾ ZM3 ⩾ ZM4 and ZM4 ⩾ ZM5 ⩾ ZM3 ⩾ ZM2, respectively. The differences in the order of inhibitory activity of ZM compounds to COX-1 or COX-2 were varied from the % of inflammation inhibition. Such variation may be attributed to different factors such as absorption and metabolism in vivo in addition to their ability to induce anti-inflammatory cytokines that would enhance the anti-inflammatory activities.

6. Conclusion

In conclusion, the new series of basic aminoacetylenic isoindolines showed significant activity as anti-inflammatory agents and as COX-1 and COX-2 inhibitors. These observed anti-inflammatory effects should open the door for compounds other than those acidic or enolic drugs currently available on the market as anti-inflammatory agents. Furthermore, recent investigations preferred compounds that block COX-1, COX-2 and LOX enzymes (Ranatunge et al., 2004). Some of these properties are shown in this series of compounds which necessitate further investigation to test their possible inhibitory activity toward lipoxygenase enzyme.

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