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ORIGINAL ARTICLE

3 Synthesis and pharmacological evaluation
4 of aminoacetylenic isoindoline-1,3-dione derivatives
5 as anti-inflammatory agents6 Jinan A. Al-Qaisi ^a, Tawfik M. Alhussainy ^a, Nidal A. Qinna ^a, Khalid Z. Matalaka ^a,
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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 μ M. However, the inhibition values were less than Diclofenac

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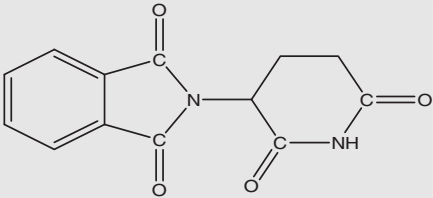
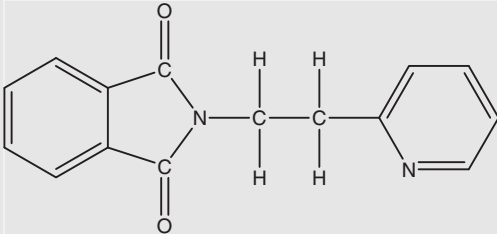
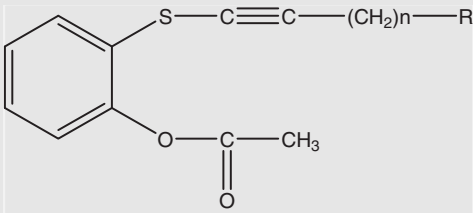
and Celecoxib. The best response was by ZM4 for COX-2 inhibition ranging from 28%, 91%, and 44%, for 2, 5, and 10 μM , respectively. Other ZM compounds such as ZM2, ZM3, and ZM5 exhibited inhibitory responses for COX-2 more than COX-1 at 5 μM . These results indicate that these ZM compounds have the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

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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.

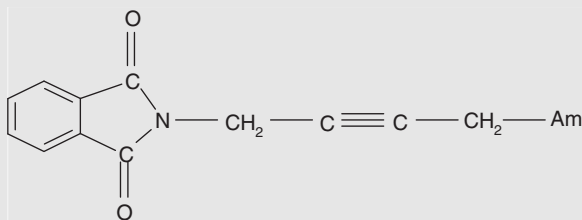
Table 1 Compounds with analgesic and anti-inflammatory activities.


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. ¹H and ¹³C NMR were acquired with the aid of Bruker-DPX 300 MHz spectrometers with DMSO-d₆ 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-1-yl)but-2-yn-1-yl}isoindoline-1,3-dione

Compound	Am	Formula	Structure
ZM1		C ₁₁ H ₇ NO ₂	
ZM2	Pyrrolidine	C ₁₆ H ₁₆ N ₂ O ₂	
ZM3	Piperidine	C ₁₇ H ₁₈ N ₂ O ₂	
ZM4	2-Methylpiperidine	C ₁₈ H ₂₀ N ₂ O ₂	
ZM5	Hexamethyleneimine	C ₁₈ H ₂₀ N ₂ O ₂	
ZM6	2,6-Dimethylpiperidine	C ₁₉ H ₂₂ N ₂ O 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). ¹H NMR (DMSO-*d*₆): δ, 3.24 (t, 1H, *J* = 2.01 Hz, C≡CH), 4.32 (d, 2H, *J* = 2.01 Hz, N-CH₂-C≡), 7.76–7.8 (m, 4H, ArH). ¹³C NMR (DMSO-*d*₆): δ, 27 (C⁵), 74 (C⁷), 78 (C⁶), 123 (C^{1,1'}), 131 (C^{3,3'}), 135 (C^{2,2'}), 167 (C^{4,4'}). Anal. Calcd. (C₁₁H₇NO₂): 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). ¹H NMR (DMSO-*d*₆): δ, 1.9 (m, 4H, ¹⁰CH₂-¹⁰CH₂), 1.6 (m, 4H, ⁹CH₂-⁹CH₂), 3.05 (t, 2H, *J* = 2.4 Hz, ≡C-⁸CH₂-N), 3.66 (t, 2H, *J* = 2.4 Hz, ⁵CH₂), 7.7–8 (m, 4H, ArH). ¹³C NMR (DMSO-*d*₆): δ, 20 (C¹²), 24 (C¹¹), 26.2 (C¹⁰), 27.4 (C¹⁰), 54.4 (C⁹), 43.8 (C⁹), 52.9 (C⁸), 54.5 (C⁵), 78.2 (C⁶), 79.5 (C⁷), 123.8 (C^{1,1'}), 131.9 (C^{3,3'}), 135.2 (C^{2,2'}), 167.2 (C^{4,4'}). Anal. Calcd. (C₁₆H₁₆N₂O₂): 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). ¹H NMR (DMSO-*d*₆): δ, 1.53 (m, 2H, ¹¹CH₂-C), 1.59 (m, 4H, C-¹⁰CH₂-¹⁰CH₂), 1.64 (m, 4H, ⁹CH₂-⁹CH₂), 3.39 (t, 2H, *J* = 2.4 Hz, ⁸CH₂-N), 3.66 (t, 2H, *J* = 2.4 Hz, ⁵CH₂), 7.7–8 (m, 4H, ArH). ¹³C NMR (DMSO-*d*₆): δ, 24.03 (C¹¹), 25.8 (C^{10,10'}), 27.1 (C^{9,9'}), 52.9 (C⁸), 66.6 (C⁵), 74.2 (C⁶), 79.3 (C⁷), 123.8 (C^{1,1'}), 131.9 (C^{3,3'}), 135.2 (C^{2,2'}), 167 (C^{4,4'}). Anal. Calcd. (C₁₇H₁₈N₂O₂): 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). ¹H NMR (DMSO-*d*₆): δ, 1.12 (d, 3H, *J* = 4.2 Hz, ¹²CH₃), 1.5 (m, 6H, ¹⁰CH₂-¹⁰CH₂-¹¹CH₂), 1.5 (m, 2H, ⁹CH₂), 1.64 (m, 1H, ⁹CH), 3.05 (t, ⁵CH₂, *J* = 2.4 Hz), 3.66 (t, 2H, ⁵CH₂, *J* = 2.4 Hz), 7.7–8.0 (m, 4H, ArH). ¹³C NMR (DMSO-*d*₆): δ, 20.0 (C¹²), 24 (C¹¹), 26.2 (C¹⁰), 27.4 (C¹⁰), 43.8 (C⁹), 52.9 (C⁸), 54.5 (C⁵), 78.2 (C⁶), 79.5 (C⁷), 123.8 (C^{1,1'}), 131.9 (C^{3,3'}), 135.2 (C^{2,2'}), 167.2 (C^{4,4'}). Anal. Calcd. (C₁₈H₂₀N₂O₂): 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). ¹H NMR (DMSO-*d*₆): δ, 1.2–1.4 (m, 8H, ¹⁰CH₂-¹⁰CH₂-¹¹CH₂-¹¹CH₂), 1.6 (m, 4H, ⁹CH₂-⁹CH₂), 3.03 (t, 2H, *J* = 2.4 Hz, ⁸CH₂), 3.46 (t, 2H, *J* = 2.4 Hz, ⁶CH₂), 3.66 (t, 2H, *J* = 4.04 Hz, N-CH₂-C≡), 7.7–8 (m, 4H, ArH). ¹³C NMR (DMSO-*d*₆): δ, 26.8 (C^{11,11'}), 27.4 (C^{10,10'}), 28.15 (C^{9,9'}), 47.7 (C⁸), 54.7 (C⁵), 78.5 (C⁷), 80.8 (C⁶), 123.8 (C^{1,1'}), 131.9 (C^{3,3'}), 135.1 (C^{2,2'}), 167.2 (C^{4,4'}). Anal. Calcd. (C₁₈H₂₀N₂O₂): 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). ¹H NMR (DMSO-*d*₆): δ, 1.14 (d, 3H, *J* = 4.4 Hz, ¹²CH₃), 1.14 (d, 3H, *J* = 4.2 Hz, ^{12'}CH₃), 1.5 (m, 6H, ¹⁰CH₂-¹¹CH₂-^{10'}CH₂), 1.80 (m, 2H, ⁹CH-⁹CH), 3.4 (t, 2H, *J* = 2.4 Hz, ⁸CH₂), 3.68 (t, 2H, *J* = 2.4 Hz, ⁵CH₂), 7.6–7.8 (m, 4H, ArH). ¹³C NMR (DMSO-*d*₆): δ, 22.02 (C¹²), 24.03 (C¹¹), 25.8 (C^{10,10'}), 27.1 (C^{9,9'}), 52.9 (C⁸), 66.6 (C⁵), 74.2 (C⁶), 79.3 (C⁷), 128.8 (C^{2,2'}), 131.9 (C^{3,3'}), 135.2 (C^{1,1'}), 167.0 (C^{4,4'}). Anal. Calcd. (C₁₉H₂₂N₂O₂): 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).

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

188 3.2. Anti-inflammatory activity

189 The paw edema was induced by subcutaneous injection of
190 0.1 ml of 1% carrageenan solution into the plantar region of
191 the left hind paw of rats. The thickness of edema was measured
192 and recorded for each rat at 1, 3 and 5 h intervals, using an
193 electronic caliper (Mitutouo Corp., Japan). Different doses
194 of the tested compounds (0, 5, 10 and 20 mg/kg) in comparison
195 with Ibuprofen (5 and 10 mg/kg), Diclofenac (5 and 10 mg/kg),
196 and Celecoxib (3, 6 and 10 mg/kg) were given to the rats by
197 oral gavages 1 h prior to the administration of carrageenan.
198 The percent inhibition of paw edema thickness was calculated
199 using the following formula:

$$200 \text{ Percent inhibition} = 100 \times [1 - (x_2 - x_1)/(y_2 - y_1)]$$

203 where x_1 is the thickness of paw of rats before administration
204 of carrageenan and test or reference compounds, x_2 is the
205 thickness of paw of rats after administration of carrageenan
206 in the test group, y_1 is the thickness of paw of rats before
207 the administration of carrageenan in the control group and
208 y_2 is the thickness of paw of rats after administration of carra-
209 geenan in the control group.

210 3.3. COX-1 and -2 inhibition assay

211 This assay measures directly prostaglandin (PGF_{2α}) produced
212 by SnCl₂ reduction of Cox-derived prostaglandin H synthase
213 (Cayman, Chemical Co., MI, USA). Briefly, the PGF_{2α} are
214 produced using ovine Cox 1 and human recombinant Cox 2,
215 arachidonic acid and heme in a reaction buffer (0.1 M Tris-
216 HCl, pH 8.0 containing 5 mM EDTA and 2 mM phenol).
217 The reaction is stopped by adding 1 M HCl followed by adding
218 saturated stannous chloride solution which is used to re-
219 duce PGH₂ produced in the COX reaction to more stable
220 PGF₂. The produced PGF_{2α} was then assayed by an enzyme
221 immunoassay using a capture assay mouse anti-rabbit IgG
222 (captured antibody) for rabbit anti-prostaglandin antiserum.
223 Prostaglandin standards (15.6–2000 pg/ml) were used to con-
224 struct 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_{2α} 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 $p < 0.05$.

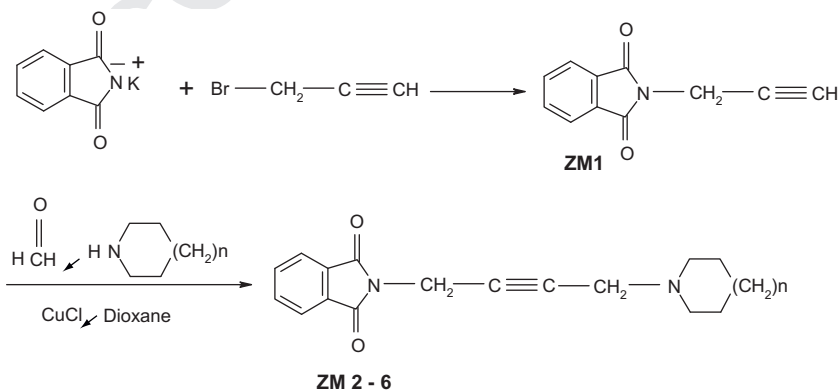
4. Results

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, ¹H NMR, ¹³C
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).

264 than ZM2 since the inhibition of inflammation induced by car-
265 rageenan is more pronounced and independent to dose. Its
266 activity was comparable to Ibuprofen and Diclofenac (5 and
267 10 mg/kg), and Celecoxib (12 mg/kg) ($p < 0.05$).

268 The anti-inflammatory activity of ZM4 was dose- and time-
269 dependent. A significant reduction of carrageenan-induced
270 inflammation was detected only at 20 mg/kg dose of ZM4 at
271 1, 3 and 5 h post-administration ($p < 0.01$). On the other
272 hand, ZM5 produced a varying degree of inhibition of inflam-
273 mation at 1, 3 and 5 h intervals post-carrageenan-induced
274 inflammation. The maximal inhibition of inflammation was
275 observed with 20 mg/kg after 3 and 5 h intervals, which was
276 equipotent to Diclofenac and Celecoxib (Table 3).

277 The COX-1 and COX-2 inhibition assay (IC₅₀ values) for
278 the tested compounds are presented in Table 4. The results
279 showed that all the compounds ZM2, ZM3, ZM4, and ZM5
280 showed inhibition of COX-1 and COX-2 with a maximum
281 inhibition at 5 μM (see Table 5).

282 However, the inhibition values were less than Diclofenac and
283 Celecoxib, as COX-1 and COX-2 inhibitors. All of the ZM com-
284 pounds exhibited a bell-shaped inhibition curve being the max-
285 imum at 5 μM and to a lesser extent at 2 and 10 μM. The best
286 response was by ZM4 for COX-2 inhibition ranging from
287 27.5%, 90.5%, and 44.0%, for 2, 5, and 10 μM, respectively.
288 Other ZM compounds such as ZM2, ZM3 and ZM5 exhibited
289 inhibitory responses for COX-2 more than COX-1 at 10 μM.

290 5. Discussion

291 The tested aminoacetylenic isoindoline-1,3-diones (ZM2–5),
292 except ZM6, were effective in reducing the inflammation in-

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

293 duced by carrageenan in the paw male rats. When the anti-
294 inflammatory activities of some newly synthesized compounds
295 (ZM2–ZM5) were compared with Ibuprofen, Diclofenac and
296 Celecoxib at a dose of 10 mg/kg at 3 and 5 h intervals post-oral
297 administration, it was evident that ZM3 was more effective
298 ($p < 0.05$) than Ibuprofen, Diclofenac, and equal to or slightly
299 more effective than Celecoxib. The order of activity for ZM
300 compounds at 10 mg/kg dose at 3 and 5 h intervals was as fol-
301 low: ZM3 ≥ ZM5 ≥ ZM4 ≥ ZM2. This variation in activity
302 may be attributed to the nature of the cyclic amino groups;
303 piperidine is preferred over hexamethyleneimine, pyrrolidine,
304 and the least with the 2-methyl piperidine. Such difference
305 may be rationalized on differences in ring size, lipophilicity
306 and conformational stability of the cyclic amine. Steric factor
307 neighboring the basic nitrogen may be the reason in decreasing
308 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 \pm SD.

Compound	Concentration		
	2 μ M	5 μ M	10 μ M
ZM2	4.0 \pm 1.5	79.0 \pm 8.0	34.0 \pm 5.0
ZM3	8.5 \pm 1.0	85.0 \pm 11.0	41.0 \pm 6.0
ZM4	27.5 \pm 1.5	90.5 \pm 6.1	44.0 \pm 4.0
ZM5	21.5 \pm 1.5	85.5 \pm 9.0	32.5 \pm 2.5
Celecoxib	70.0 \pm 0.0	97.5 \pm 1.5	93.5 \pm 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 μ M 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 $ZM2 \geq ZM5 \geq ZM3 \geq ZM4$ and $ZM4 \geq ZM5 \geq ZM3 \geq ZM2$, 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 (7). 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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