DISCOVERY

58(319), July 2022

In-vivo anti-inflammatory activity studies of some p-nitrophenyl hydrazones

Sodeeq Babalola[1*], Nosakhare Igie[2], Isaiah Odeyemi[3], Abdullahi Y. Idris[1], Sanni YM[1], Asmau N. Hamza[1]

To Cite:
Babalola S, Igie N, Odeyemi I, Idris AY, Sanni YM, Hamza AN. *In-vivo* anti-inflammatory activity studies of some p-nitrophenyl hydrazones. *Discovery*, 2022, 58(319), 698-706

Author Affiliation:
[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences Ahmadu Bello University Zaria, Nigeria
[2]Department of Chemistry and Biochemistry, The University of Texas at Dallas, USA. Email: nosakhare.igie@utdallas.edu; ORCID: 0000-0001-7590-0757
[3]Department of Chemistry and Biochemistry, The University of Texas at Dallas, USA. Email: odeyemiisaiah14@gmail.com; ORCID: 0000-0002-8369-9130

[*]**Corresponding author:**
Babalola Sodeeq Adewale, Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Email: wale.babalola91@gmail.com, Tel: +2347058199796; ORCID: 0000-0003-0580-0595

Peer-Review History
Received: 29 April 2022
Reviewed & Revised: 30/April/2022 to 02/June/2022
Accepted: 03 June 2022
Published: July 2022

Peer-Review Model
External peer-review was done through double-blind method.

© The Author(s) 2022. Open Access. This article is licensed under a Creative Commons Attribution License 4.0 (CC BY 4.0)., which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

ABSTRACT

The goal of this research was the discovery of newer anti-inflammatory drug. Four hydrazones with p-nitrophenyl moiety were synthesized by solvent-free and conventional synthetic methods with good to excellent yields. The synthesized hydrazones were evaluated for their anti-inflammatory activities using carrageenan-induced paw edema in mice. All the compounds indicated significant anti-inflammatory activity, demonstrating slow onset of action and longer duration of action compared to celecoxib and piroxicam; this characterized them as suitable for the treatment of chronic inflammatory diseases. Most of the compounds indicated superior anti-inflammatory activities compared to celecoxib and piroxicam after 4½ hours of inflammation induction. All the compounds demonstrated time-dependent anti-inflammatory activities. Compound 3b [1-(4-nitrophenyl)-2-(3,4,5-trimethoxybenzylidene) hydrazine] had the best activity, indicating time-dependent and dose-dependent activities during the studies. This suggests that the trimethoxy benzaldehyde moiety confers favorable pharmacokinetic properties to the compound. The hydrazones, especially 3b have been identified as lead compounds and are recommended for further *in-vivo* anti-inflammatory evaluations against other acute and chronic inflammatory animal models.

Keywords: Anti-inflammatory, carrageenan-induced paw edema, NSAIDs, p-nitrophenyl hydrazones.

1. INTRODUCTION

Inflammation is not a disease in and of itself, but rather a manifestation of disease. It has initially positive advantage, such as minimizing infection and stimulating regeneration. However, if extended over long period of time or spread excessively, it might aggravate disease by destroying tissue [1].

Chronic pain is a public health concern which affects roughly 30% of the world's population, is generally caused by inflammation. Current pain management therapy choices have increased complications associated with long-term usage, and there is a growing need to develop better and safer medicines [2].

Non-steroidal anti-inflammatory drugs (NSAIDs) have become widely used as analgesics, antipyretics, and anti-inflammatory therapies across the world. The market size for NSAIDs in the United States was estimated at $5.78 billion in

2021, and it was expected to reach $7.10 billion by 2025. In 2019, the worldwide NSAIDs market was worth $15.58 billion, with a forecast of $24.35 billion by 2027. One of the major reasons driving the NSAIDs market is the rising prevalence of pain and inflammation-causing disorders [3].

The major drawbacks of NSAIDs are their side effects, which include gastrointestinal toxicities, cardiovascular complications, renal failure, allergic reactions, and hypersensitivity. The bulk of emergency hospital admissions are due to NSAID-related GI bleeding. Furthermore, it is the most prevalent therapeutic adverse effect in the United States [3].

The local irritation caused by the carboxylic acid moiety found in most NSAIDs (topical effect) and decreased tissue prostaglandin production, which undermines the physiological role of cytoprotective prostaglandins in maintaining GI health and homeostasis, are the two main causes of GI damage caused by NSAIDs. The pharmacological effect of NSAIDs is linked to the inhibition of cyclooxygenases, which inhibit prostaglandin formation from arachidonic acid (COXs). Chronic usage of NSAIDs can cause significant GI damage [4]. Furthermore, newer NSAIDs, such as highly selective COX-2 inhibitors, have been linked to cardiovascular complications [2].

The hydrazones –NHN=CH– moiety is critical in the development of novel drugs; Levosimendan, dantrolene, nitrofurantoin, nitrofurazone, furazolidone, and nifuroxazide etc. Hydrazones exhibit anti-inflammatory properties because they have been reported as dual inhibitors of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (LOX-5) enzymes [5]. It prevent cellular arachidonic acid (AA) metabolism and prostaglandin synthesis, which would otherwise result in increased vascular permeability, edema, hyperalgesia, pyrexia, and inflammation.

Leukotrienes, which are generated via the 5-LOX enzyme pathway, may also play a role in inflammation and NSAID-related adverse effects. Co-inhibition of COX and 5-LOX may lessen cardiovascular and gastrointestinal adverse effects while maintaining COX-1/2 inhibitors' primary efficacy [6]. As a result, substances that are dual COX and 5-LOX inhibitors are being investigated as possible anti-inflammatory medicines with a better safety profile than NSAIDs [7]. Furthermore, investigations have shown that the hydrazone moiety found in certain compounds has a pharmacophoric feature for inhibiting COX and LOX enzymes with greater safety and efficacy than a few currently marketed medications [5].

The inhibition of H^+/K^+ ATPase enzyme by hydrazones have been previously reported [8]. As a result of the therapeutic features of this family of compounds, novel hydrazones as multi-target inhibitors with strong anti-inflammatory activity and a better efficacy and safety profile are designed and evaluated as a long-term therapy for inflammatory disorders.

2. MATERIALS AND METHODS

2.1. Chemistry

All the reagents used were purchased from Sigma Aldrich, and they were used with no further purification. Electrothermal Engineering LTD 9100 apparatus was employed in the melting points determination of the synthesized compounds. The FTIR spectra were recorded on Agilent technologies spectrometer model 543, and the 1H and 13C NMR spectra were obtained using a Brucker AMX 400 MHz spectrometer operating at 400 MHz and 101 MHz respectively with dimethyl sulfoxide (DMSO) used as the solvent. Chemical shifts (δ) are reported in parts per million and are referenced to the NMR solvent peak.

2.1.1. General procedure: Synthesis of p-nitrophenyl hydrazones 3a-d

Solvent-based synthesis

5.09mmol of each of the benzaldehydes 1a-d and p-nitrophenyl hydrazine 2 were dissolved in 30 ml of ethanol. This was followed by the addition of five drops of glacial acetic acid as a catalyst. The mixture was magnetically stirred for three hours. The reactions were carried out under room conditions. The progress of the reaction was monitored by TLC. Upon completion, the crude products were filtered, dried then transferred into a beaker.

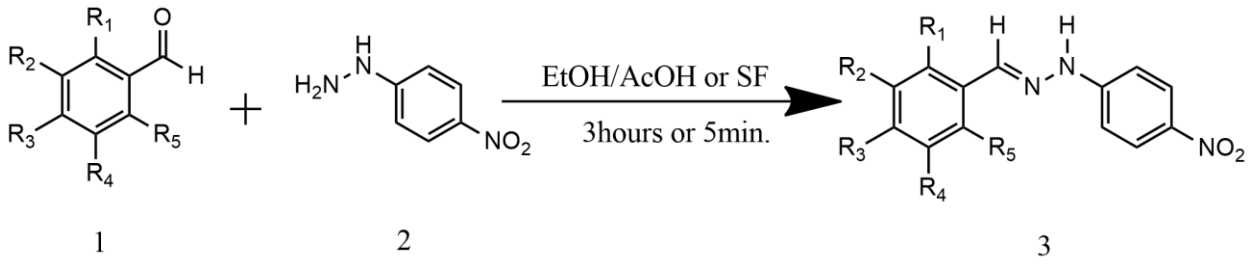
Solvent-free synthesis

A glass rod was used to grind equimolar amounts of p-nitrophenyl hydrazine 2 (1 mmol) and each of the aromatic aldehydes 1a-b, d (1 mmol) in a universal tube for 5 minutes. The reactions were performed under room condition. TLC was used to monitor the reaction's progress.

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

2.1.2. Purification (work-up)

20 ml of cold 2 M hydrochloric acid was added and stirred to scavenge the possible unreacted p-nitrophenyl hydrazine 2. The product precipitate was filtered off, dried, and subsequently washed with 30 ml of cold distilled water and 20 ml of cold 95% ethanol step-wisely to afford colored powdered products 3a-d in high to excellent yield.



1 2 3

Scheme 1: Synthesis of p-nitrophenyl hydrazones.

Compound	R1	R2	R3	R4	R5
3a	Cl	H	Cl	H	H
3b	H	OCH3	OCH3	OCH3	H
3c	H	H	OCH3	H	H
3d	H	Cl	H	Cl	H

2.2. Pharmacology Study

2.2.1. Experimental Animals

One hundred and five Swiss albino mice (15-34 g) were used in the experiments. The animals were procured within the Zaria community, Kaduna State. The mice were housed in single-sex cages under a 12-hour light:12 hour dark cycle (lights on at 6 am) in a controlled-temperature room (22 ± 2 °C with 50 ± 10% humidity) at the animal house. The mice were kept for 2 weeks to stabilize, habituate (acclimatize) and become more adult. After 7 weeks, all the animals were adults at the age of 7 weeks and weights of 15–34 g. Availability of standard diet and water was ad libitum. All animal experiments were performed as per the requirement of the bio-ethical committee protocols of the ABU Committee on Animal Use and Care in compliance with the guidelines for the care and use of laboratory animals provided by the National Institute of Health (NIH publication no. 85–23, revised 1985). Ethical approval was sought and obtained from ABU Committee on Animal Use and Care.

2.2.2. Oral Acute Toxicity Studies and lethality (LD_{50}) test

The acute toxicity and lethality (LD50) of the titled compounds were estimated in mice using the Fixed Dose Procedure (FDP) — OECD TG 420. This OECD guideline method does not use the death of animals as a clear sign of toxicity. Instead, any changes such as changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic, and central nervous systems, somatomotor activity, and behavior patterns. Attentions are particularly directed at observations of increased motor activity, anesthesia, tremors, arching and rolling, clonic convulsions, tonic extension, lacrimation, Straub reaction, salivation, muscle spasm, writhing, hyperesthesia, loss of righting reflex, depression, ataxia, stimulation, sedation, blanching, hypnosis, cyanosis and analgesia, diarrhea, lethargy, sleep, and coma were taken as a sign of toxicity (Deora *et al*., 2010)[9].

The limit test was employed in the experiment, a group (n = 3) was used for each test compound. The test animals were male Swiss albino mice with weights ranging between 20-25g. The test dose of each titled compound was prepared by dissolving 150mg of each titled compound in 3ml of deionized water to form an aqueous suspension of 50mg/ml concentration which is equivalent to 2000mg/kg per body weight for mice weighing 25g. Each one of these compounds was administered orally in the form of deionized water suspension (1% w/v) at an appropriate volume equivalent to a dose of 2000 mg/kg (n = 3). Animals were observed continuously for the first one hour for any toxic symptoms after administration and then for the next 24 hours, 48 hours, 7 days, and 14 days.

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

2.2.3. Anti-inflammatory activity evaluation

The anti-inflammatory activity of the titled compounds was evaluated using Carrageenan-induced hind paw edema in mice model. The method of Kasahara *et al*., 1985 was used with modifications in measuring periods. Seventy-five mice were randomly selected into fifteen different groups. Each group of mice (n=5) received a dose of the test compound. Each compound was evaluated for its anti-inflammatory activity at 10mg/kg, 30mg/kg and 50mg/kg doses respectively. A group of mice (n=5) received a dosing vehicle (normal saline) and was tagged as the control group. One other group of mice (n=5) received 10mg/kg of the reference drug Celecoxib while another group of mice (n=5) received 10mg/kg of another reference drug Piroxicam. The route of administration was intraperitoneal (i.p) at the left side of the lower abdominal cavity of each mouse. Immediately after the intraperitoneal (i.p) administration of the titled compounds, zero reading of the right hind paw was taken using pair of dial thickness gauge vernier caliper. Sixty minutes after the administration of test compounds, reference drugs, or dosing vehicle each mouse has injected with freshly prepared (40 mg/10 ml) suspension of carrageenan (Sigma, St. Louis, Missouri, USA) in normal saline into subplantar tissue of the right hind paw. Paw edema was measured every 90 minutes for 6 hours after induction of inflammation.

The measurement of the hind paw was carried out using a thickness gauge vernier caliper before each treatment (Vo) and in each interval (Vt) after the administration of the test compound, dosing vehicle, and the reference drug. The data were expressed as mean ± standard error of the mean (n=5). The data were analyzed statistically using a Two-way analysis of variance (ANOVA) with replication, residual error test, and Tukey's Multiple Comparison Test. The percentage of swelling inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = \{[(Vt - Vo)_{control} - (Vt - Vo)_{treated}]/(Vt - Vo)_{control}\} \times 100 \qquad \text{Eq. (4)}$$

Where Vt and Vo relate to the average volume in the hind paw of the mice after carrageenan injection and before carrageenan injection respectively. All the results were expressed as Mean ± Standard Error of Mean (S.E.M.) and percentage of inhibition.

3. RESULTS

3.1. Chemistry

1-(2,4-dichlorobenzylidene)-2-(4-nitrophenyl)hydrazine 3a. Yield 67.90%, chrome yellow powder, mp 226-228 °C. FTIR (KBr, cm^{-1}): 3265 (N-H), 3078 (C-H_{imine}), 1587 (C=N), 1498 (NO_2), 1461 (C=$C_{aromatic}$), 1300 (C-$N_{aniline}$), 1043 (C-Cl). H^1 NMR spectrum (400 MHz, DMSO-*d6*) δ, ppm: 7.18 d (2H_{arom}, *J* = 8.2 Hz), 7.47 d (1H_{arom}, *J* = 8.5 Hz), 7.65 d (1H_{arom}, *J* = 1.8 Hz), 8.05 d (1H_{arom}, *J* = 8.6 Hz), 8.13 d (2H_{arom}, *J* = 9.0 Hz), 8.29 s (1H_{imine}), 11.61 s (1H, NH). ^{13}C NMR spectrum (101 MHz, DMSO-*d6*), δ, ppm: 112.10, 126.54, 127.98, 128.29, 129.71, 131.39, 133.16, 134.36, 136.58, 139.43, 150.43.

1-(4-nitrophenyl)-2-(3,4,5-trimethoxybenzylidene)hydrazine 3b. Yield 70.27%, brick red powder, mp 198-201 °C. FTIR (KBr, cm^{-1}): 3283 (N-H), 2929 (C-H_{imine}), 2840 (C-$H_{methoxy}$), 1591 (C=N), 1494 (NO_2), 1412 (C=$C_{aromatic}$), 1125 (C-$N_{aniline}$). H^1 NMR spectrum (400 MHz, DMSO-*d6*) δ, ppm: 3.69 s (3H, OCH_3), 3.85 s (6H, OCH_3), 7.05 s (2H_{arom}), 7.19 d (2H_{arom}, *J* = 7.6 Hz), 7.97 s (1H_{imine}), 8.13 d (2H_{arom}, *J* = 8.7 Hz), 11.30 s (1H, NH). ^{13}C NMR spectrum (101 MHz, DMSO-*d6*), δ, ppm: 56.35, 60.54, 104.15, 111.69, 126.58, 130.65, 138.67, 139.02, 142.24, 150.98, 153.60.

1-(4-methoxybenzylidene)-2-(4-nitrophenyl)hydrazine 3c. Yield 53.49%, dark brown, mp 152-155 °C. FTIR (KBr, cm^{-1}): 3261 (N-H), 3000 (C-H_{imine}), 2832 (C-$H_{methoxy}$), 1595 (C=N), 1509 (NO_2), 1468 (C=$C_{aromatic}$), 1168 (C-$N_{aniline}$). ^{1}H NMR spectrum (400 MHz, DMSO-*d6*) δ, ppm: 3.79 s (3H, OCH_3), 6.99 d (2H, *J* = 8.2 Hz), 7.12 d (2H, *J* = 7.0 Hz, 2H), 7.66 d (2H, *J* = 8.2 Hz), 7.99 s (1H, CH_{imine}), 8.11 d (2H, *J* = 8.8 Hz), 11.18 s (1H, NH). ^{13}C NMR spectrum (101 MHz, DMSO-*d6*), δ, ppm: 55.70, 111.43, 114.74, 126.65, 127.68, 128.48, 138.39, 142.41, 151.15, 160.70.

1-(3,5-dichlorobenzylidene)-2-(4-nitrophenyl)hydrazine 3d. Yield 72.36%, bright yellow powder, mp 257-259 °C. FTIR (KBr, cm^{-1}): 3254 (N-H), 3075 (C-H_{imine}), 1580 (C=N), 1476 (NO_2), 1416 (C=$C_{aromatic}$), 1297 (C-$N_{aniline}$), 957 (C-Cl). H^1 NMR spectrum (400 MHz, DMSO-*d6*) δ, ppm: 7.25 d (2H_{arom}, *J* = 8.1 Hz), 7.59 s (1H_{arom}), 7.79 s (2H_{arom}), 7.98 s (1H_{imine}), 8.15 d (2H_{arom}, *J* = 9.1 Hz), 11.55 s (1H, NH). ^{13}C NMR spectrum (101 MHz, DMSO-*d6*), δ, ppm: 112.23, 124.96, 126.50, 128.38, 135.00, 138.80, 139.44, 145.24, 150.52.

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

3.2. Pharmacology

Table 1: Oral Acute Toxicity Studies and lethality (LD_{50}) results.

Compounds	Dose(mg/kg)	No. of Mice	Sign of toxicity	No. of dead mice
3a	2000	3	Lethargy	None
3b	2000	3	None	None
3c	2000	3	None	None
3d	2000	3	Lethargy	None

3.2.1. Anti-inflammatory activity results

Table 2: Mean ± S.E.M of hind paw thickness at 10mg/kg dose. n=5

Test sample	0hr	1hr 30min.	3hrs	4hrs 30min.	6hrs
3a	1.48 ± 0.06	1.98 ± 0.11	1.86 ± 0.11	1.65 ± 0.06	1.54 ± 0.03
3b	1.36 ± 0.02	1.79 ± 0.05	1.74 ± 0.06	1.59 ± 0.05	1.45 ± 0.03
3c	1.29 ± 0.01	1.69 ± 0.09	1.66 ± 0.01	1.56 ± 0.02	1.43 ± 0.02
3d	1.26 ± 0.04	1.74 ± 0.10	1.74 ± 0.07	1.56 ± 0.05	1.49 ± 0.06
Piroxicam	1.32 ± 0.03	1.66 ± 0.04	1.67 ± 0.04	1.53 ± 0.01	1.52 ± 0.02
Celecoxib	1.27 ± 0.01	1.62 ± 0.04	1.66 ± 0.05	1.56 ± 0.02	1.52 ± 0.02
Normal Saline	1.27 ± 0.03	1.80 ± 0.05	1.87 ± 0.07	1.81 ± 0.04	1.76 ± 0.03

Compounds: p-value = $5.46 \times 10^{-15} < \alpha = 0.05$, F = 15.01 > $F_{Critical}$ = 2.07
Time: p-value = $3.44 \times 10^{-43} < \alpha = 0.05$, F = 102.88 > $F_{Critical}$ = 2.43

Table 3: Mean ± S.E.M of hind paw thickness at 30mg/kg dose. n=5.

Test sample	0hr	1hr 30min.	3hrs	4hrs 30min.	6hrs
3a	1.36 ± 0.08	1.74 ± 0.05	1.89 ± 0.10	1.63 ± 0.02	1.52 ± 0.04
3b	1.35 ± 0.01	1.81 ± 0.06	1.69 ± 0.03	1.55 ± 0.03	1.45 ± 0.03
3c	1.27 ± 0.03	1.81 ± 0.02	1.67 ± 0.02	1.58 ± 0.04	1.39 ± 0.04
3d	1.35 ± 0.04	1.90 ± 0.05	1.63 ± 0.03	1.52 ± 0.05	1.41 ± 0.04

Compounds: p-value = 0.00064 < $\alpha = 0.05$, F = 5.31 > $F_{Critical}$ = 2.46
Time: p-value = $1.90 \times 10^{-34} < \alpha = 0.05$, F = 102.25 > $F_{Critical}$ = 2.46

Table 4: Mean ± S.E.M. of hind paw thickness at 50mg/kg dose. n=5.

Test sample	0hr	1hr 30min.	3hrs	4hrs 30min.	6hrs
3a	1.41 ± 0.06	1.83 ± 0.08	1.87 ± 0.11	1.62 ± 0.05	1.51 ± 0.05
3b	1.36 ± 0.03	1.74 ± 0.05	1.64 ± 0.02	1.54 ± 0.01	1.42 ± 0.02
3c	1.32 ± 0.02	1.75 ± 0.01	1.72 ± 0.03	1.55 ± 0.02	1.46 ± 0.01
3d	1.33 ± 0.02	1.83 ± 0.04	1.71 ± 0.03	1.55 ± 0.03	1.42 ± 0.04

Compounds: p-value = 0.0029 < $\alpha = 0.05$, F = 4.32 > $F_{Critical}$ = 2.46
Time: p-value = $1.08 \times 10^{-28} < \alpha = 0.05$, F = 72.47 > $F_{Critical}$ = 2.46

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

Table 5: Percentage inhibitions of hind paw edema at 10mg/kg dose.

Test sample	0hr	1hr 30min.	3hrs	4hrs 30min.	6hrs.
3a	0.00%	4.58%	34.80%	67.66%	87.30%
3b	0.00%	17.56%	35.81%	57.62%	80.33%
3c	0.00%	23.66%	37.84%	49.81%	70.90%
3d	0.00%	8.02%	18.92%	43.49%	52.05%
Piroxicam	0.00%	35.50%	42.06%	55.41%	60.25%
Celecoxib	0.00%	33.21%	34.80%	46.84%	49.18%
Normal Saline	0.00%	0.00%	0.00%	0.00%	0.00%

Table 6: Percentage inhibition of hind paw edema at 30mg/kg dose.

Test sample	0hr	1hr 30min.	3hrs	4hrs 30min.	6hrs
3a	0.00%	27.10%	11.49% 50.56%	71.31%	
3b	0.00%	12.98%	42.57% 62.83%	79.92%	
3c	0.00%	-3.82%	31.42% 42.01%	97.54%	
3d	0.00%	-5.73%	51.69% 68.40%	86.48%	

Table 7: Percentage inhibitions of hind paw edema at 50mg/kg dose.

Test sample	0hr	1hr 30min.	3hrs	4hrs 30min.	6hrs
3a	0.00%	20.61%	22.97%	60.60%	80.74%
3b	0.00%	27.48%	52.03%	67.29%	88.53%
3c	0.00%	27.48%	32.77%	57.62%	70.49%
3d	0.00%	4.58%	37.16%	59.48%	81.56%

4. DISCUSSION

4.1. Chemistry

We have previously reported the synthesis of the p-nitrophenyl hydrazones [11-12]. The synthesis followed the described procedures and as indicated by the scheme 1. All the synthesized p-nitrophenyl hydrazones were obtained in high yields by simple manipulations. The structures of the hydrazones were confirmed by FTIR, ^{1}H-NMR, and C-13 NMR and 2D-NMR. The formation of the hydrazone bridge (–CH=N–NH) was confirmed by the absorption signals at 1580 – 1595 cm^{-1} for C=N, 2929 – 3075 cm^{-1} for imine C–H, and 3254 – 3283 cm^{-1} for N–H in the FTIR spectra and singlet peaks at δ = 7.97 – 8.29 ppm and 11.18 – 11.61 ppm for N=C–H and N–H respectively. The chemical structures were confirmed with spectroscopic methods.

4.2. Pharmacology

4.2.1. Oral Acute Toxicity Studies and lethality (LD_{50})

Oral administration of 2000 mg/kg dose of the tested compounds in mice caused reduced motor activity and slight sleepiness which summed up as lethargy, was observed in mice that were administered with compounds 3a, and 3d within the first hour of oral administration as tabulated in table 1. These signs of acute toxicity, however, wear off after subsequent hours with no further symptoms over the fourteen days of observation. Compounds 3b and 3c caused no symptoms of acute toxicity in mice over the fourteen days of observation at this dose. Oral administration of all the tested compounds at 2000 mg/kg in mice caused no mortalities. Thus, the LD_{50} of the test compounds was found to be greater than 2000 mg/kg for each compound. This implies that they fall into Class V of the globally harmonized system (GHS) (2000mg/kg < LD50 ≤ 5000kg/kg) which translates as may be

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

harmful if swallowed. The overall examination implies a remote risk of acute intoxication and indicates a high degree of relative safety for the oral administration of these compounds.

4.2.2. Anti-inflammatory activity

All the synthesized compounds were evaluated for their anti-inflammatory activity at doses of 10 mg/kg, 30 mg/kg, and 50 mg/kg via induced inflammation of 10 mg/ml concentration of carrageenan. The route of administration was intraperitoneal (*ip*). All the tested compounds exhibited statistically significant ($p \leq 0.005$) anti-inflammatory activities at all doses in carrageenan-induced paw edema in mice model of inflammation as indicated in tables 2-4.

All the tested p-nitrophenyl hydrazones at a 10 mg/kg dose showed slow onset of action which was marked after the first 90 minutes of inflammation induction compared to the reference drugs celecoxib and piroxicam with rapid onset of action. This is indicated by their low percentage inhibition of the induced paw edema in table 5. The percentage inhibition of the induced paw edema is at the lowest with dichloro p-nitrophenyl hydrazones; 3a (4.58%) and 3d (8.02%), as compared to methoxy p-nitrophenyl hydrazones; 3b (17.56%) and 3c (23.66%) suggesting that methoxy group contributes more to inhibition of synthesis of first phase inflammatory mediators principally histamine and serotonin than chloro group. The percentage inhibition of the induced paw edema for piroxicam and celecoxib were found to be 35.50% and 33.21% respectively after the first 90 minutes after inflammation induction.

However, for the synthesized hydrazones as the dose increased, it lead to faster onset of action and better inhibition of phase I proinflammatory mediators as indicated by the percentage inhibition of all the tested compounds after the first 90 minutes of inflammation induction for doses at 30 mg/kg and 50 mg/kg viz; 3a (27.10% and 20.61%), 3b (12.98% and 27.48). It was observed that compounds 3c and 3d showed no activity -3.82% and -5.73% respectively at 30 mg/kg dose while compound 3d still exhibited even lower percentage inhibition (4.58%) at 50 mg/kg dose compared to it 8.02% inhibition at 10 mg/kg dose after the first 90 minutes of inflammation induction as indicated by the results in table 5. The inactivity of 3c and 3d at this dose and measuring time may be due to differences in the physiological response to treatment of the mice in the said groups or pharmacokinetics factors.

Compounds 3a, 3b, and 3c exhibited comparable percentage inhibition as to reference drugs piroxicam and celecoxib after three hours of inflammation induction at 10 mg/kg dose according to results in table 5. This indicated their comparable potencies as second phase prostaglandin synthase inhibitors to reference drugs. Compound 3a exhibited equal percentage inhibition (34.80%) as celecoxib (34.80%) but less than that of piroxicam (42.06%) at three hours after induction of inflammation. Moreover, compounds 3b and 3c exhibited greater percentage inhibition (35.81%) and (37.84%) respectively at a 10 mg/kg dose compared to celecoxib percentage inhibition (34.80%), but less than that of piroxicam (42.06%) at the third hour of inflammation induction. However, at higher doses 3b, unlike 3c, indicated superior percentage inhibition of 42.57% and 52.03% at 30 mg/kg and 50 mg/kg respectively to those of 10 mg/kg dose for piroxicam and celecoxib at the same hour according to resultings in table 6 and 7.

Furthermore, at a 10 mg/kg dose all the synthesized compounds exhibited comparable percentage inhibition to reference drugs at the fourth and half hours after inflammation induction as in indicated in the results in table 5. Furthermore, compounds 3a and 3b demonstrated significantly higher percentage inhibition of edema for all doses to that of piroxicam and celecoxib at the same measuring time. However, at 30 mg/kg dose, 3a which exhibited slightly lower percentage inhibition of 50.56% at the fourth and half-hour compared to 55.41% inhibition for piroxicam but greater than 46.84% inhibition for celecoxib at the fourth and half hour. Compound 3c exhibited lower percentage inhibition (49.81%) and (42.01%) at 10 mg/kg and 30 mg/kg respectively compared to piroxicam (55.41%) at 10 mg/kg dose, but greater percentage inhibition (57.62%) at 50 mg/kg compared to piroxicam and celecoxib at the fourth and half hour. In the same vein compound, 3c exhibited greater percentage inhibition (49.81%) at 10 mg/kg dose to celecoxib (46.84%) and lower percentage inhibition (42.01%) at 30 mg/kg to that of celecoxib (46.84%) at the fourth and half hour of inflammation induction.

All the synthesized p-nitrophenyl hydrazones exhibited a longer duration of action compared to piroxicam and celecoxib used as reference drugs. This is indicated by their greater percentage inhibition at all doses compared to the reference drugs at the sixth hour after induction of inflammation. The prolonged efficacy of the synthesized compounds is due to a lower extraction rate from systemic circulation as a consequence of the high plasma protein binding (PPB) leading to slow clearance and longer half-life ($T_{1/2}$) compared to reference drugs which was conferred by the presence of para nitro group (NO_2) [13].

Interestingly, a consistent increase in anti-inflammatory activity by all the tested compounds at each dose over the measured times suggests time-dependent inhibition. There is a progressive increase in in-vivo anti-inflammatory activity over the measuring times as the dose increases for compound 3b indicated dose-dependent activity. This suggested that increase in methoxy group on p-nitrophenyl hydrazone scaffold increases anti-inflammatory activity. The slow on-set of action and yet long duration of action of the synthesized p-nitrophenyl hydrazones characterized them as agents suitable for the treatment of chronic inflammatory diseases.

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

The synthesized compounds have been reported as multi-target inhibitors of COX-2, 5-LOX, and H^+/K^+ ATPase in in-silico studies [13]. Wherein compound 3b exhibited potent inhibitions of the enzymes with superb interactions forming five, four and five hydrogen bond interactions with the enzymes respectively.

5. CONCLUSION

All the tested compounds demonstrated significant anti-inflammatory activity, indicating longer duration of action compared to the reference drugs used. Compound 3b indicated the best anti-inflammatory activity which is comparable to those of piroxicam and celecoxib used as reference drugs. This suggests that increasing methoxy group on hydrazone scaffold increases anti-inflammatory activity. Therefore, compound 3b has been identified as a lead compound.

Acknowledgement

The authors expressed appreciation of the support from the department of pharmaceutical chemistry and medicinal chemistry, Ahmadu Bello University and staffs at the multi-user laboratory, Ahmadu Bello University.

Funding

This study has not received any external funding.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Marc Y. Donath, Elise Dalmas, Nadine S. Sauter, and Marianne Boni-Schnetzler. Inflammation in Obesity and Diabetes: Islet Dysfunction and Therapeutic Opportunity. Cell Metabolism, 2013. Elsevier Inc. http://dx.doi.org/10.1016/j.cmet.2013.05.001
2. Sara Zeeshan, Muhammad Naveed, Adnan Khan, Ayesha Atiq, Maryam Arif. N-Pyrazoloyl and N-thiopheneacetyl hydrazone of isatin exhibited potent anti-inflammatory and anti-nociceptive properties through suppression of NF-κB, MAPK and oxidative stress signaling in animal models of inflammation. Inflammation Research, 2019, 68:613–632 https://doi.org/10.1007/s00011-019-01245-9
3. Fortune Business Insight. Healthcare. (2020). Pharmaceutical. Global non-steroidal anti-inflammatory drugs (NSAIDs) market analysis, insights and forecast, 2016-2027. Report ID: FBI1028823
4. Mehtap Gokçe, Semra Utku, Esra Ku peli. Synthesis and analgesic and anti-inflammatory activities 6-substituted-3(2H)-pyridazinone-2-acetyl-2-(p-substituted/nonsubstituted benzal) hydrazone derivatives. European Journal of Medicinal Chemistry, 2009, 44, 3760–3764. DOI:10.1016/j.ejmech.2009.04.048
5. Asif M, Husain A. (2013). Analgesic, anti-Inflammatory, and antiplatelet profile of hydrazones containing synthetic molecules. J Appl Chem. 2013:1–7. https://doi.org/10.1155/2013/24720 3.
6. Nguyen Ha Thi, Thien-Y. Vu, Vishala Chandi, Haritha Polimati, Vinay Bharadwaj Tatipamula. (2020). Dual COX and 5-LOX inhibition by clerodane diterpenes from seeds of Polyalthia longifolia (Sonn.) Thwaites. 10:15965 https://doi.org/10.1038/s41598-020-72840-8
7. Kumar N. and Chauhan L. S. (2014). Analgesic and anti-inflammatory potential of hydrazones. Journal of Chemical and Pharmaceutical Research, 6(12):916-934. ISSN: 0975-7384 CODEN(USA) : JCPRC5.
8. Wang Shi-Meng, Gao-Feng Zha, K. P. Rakesh, N. Darshini, T. Shubhavathi, H. K. Vivek, N. Mallesha et al. (2017). Synthesis of benzo[d]thiazole-hydrazone analogues: molecular docking and SAR studies of potential H+/K+ ATPase inhibitors and anti-inflammatory agents. RCS MedChem. DOI: 10.1039/c7md00111h
9. Deora Paramveer S., Mishra Chanchal K., Mavani Paresh, Asha Rani, Shrivastava B *et al*. (2010). J. Chem. Pharm. Res., 2(6):450-453.
10. Kasahara Y, H. Hikino, S. Tsurufuji, M. Watanabe (1985). Planta Med. 51, 325.
11. Babalola SA, Idris AY, Sani YM, Hamza A, Igie N, Muhammad H, Erumiseli OG, Bakare LO. (2022). Solvent-free synthesis of polymethoxy and dichloro p-nitrophenyl hydrazones. Drug Discovery, 16(37), 63-67
12. Babalola SA, Igie N, Idris AY, Hamza A, Sanni YM, Muhammad HY, Erumiseli OG, Bakare LO. (2022).

Antimicrobial activities of hydrazones with 2,4-dichloro moiety. Drug Discovery, 16(37), 53-62

13. Babalola S, Igie N, Odeyemi I. Structure-based discovery of multitarget directed anti-inflammatory p-nitrophenyl hydrazones; molecular docking, drug-likeness, in-silico pharmacokinetics, and toxicity studies. (2022). ChemRxiv. Cambridge: Cambridge Open Engage; DOI: 10.26434/chemrxiv-2022-mrptw

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS