Synthesis and Biological Activity of Novel Acyl Hydrazone Derivatives of 3-(4,5-diphenyl-1,3-oxazol-2-yl) propanoic acid as Anticancer, Analgesic and Anti-inflammatory Agents

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ABSTRACT

A series of acyl hydrazone derivatives of 3-(4,5-diphenyl-1,3-oxazol-2-yl) propanoic acid have been synthesized and screened for in vivo anti-inflammatory, analgesic and in vitro anti cancer activities. The key intermediate N-acylhydrazine is prepared in good yield from NSAID oxaprozin, was coupled with a variety of aromatic aldehydes under conventional as well as microwave irradiation conditions. The microwave approach utilized solvent free condition, required short time and resulted in very good yields. The newly synthesized compounds have been characterized by IR, ¹H NMR, ¹³C NMR and Mass analysis. The results of the biological activities showed that the compounds 5b, 5d and 5e exhibited significant in vivo analgesic and anti inflammatory activities than reference compound oxaprozin. Compounds 5a, 5b, 5e, 5g, 5h, 5i and 5j revealed promising in vitro cytotoxicity than reference compound Cisplatin.

KEYWORDS: Acyl hydrazones, Analgesic activity, Anti-inflammatory activity, Cytotoxicity, Oxaprozin.

1. INTRODUCTION

Pharmacological properties of NSAIDs (non steroidal anti-inflammatory drugs) are widely used for the treatment of different types of arthritis and other musculoskeletal disorders [1-6]. The biological response of NSAIDs results from the suppression of prostaglandin (PG) biosynthesis in which the cyclooxygenase (COX) enzyme is the key intermediate in the biosynthesis of prostaglandin from arachidonic acid [7, 8]. It was discovered during early 1990s, that COX exists in two isoforms, namely constitutive COX-1, which provides cytoprotection in the Gastro-intestinal (GI) and the other inducible COX-2 which mediates inflammation [9-11].

It was observed that traditional NSAIDs in current use non-selectively inhibit COX-1 and COX-2. Consequently, long term therapy with non-selective NSAIDs results in appreciable GI irritation, bleeding and ulceration [12]. GI damage from NSAIDs is generally due to two factors i.e. local irritation by the direct contact of carboxylic acid (–COOH) moiety of NSAID with GI mucosal cells and decreased tissue prostaglandin production [12]. Commonly used anti-inflammatory drugs such as NSAIDs and analgesic agents are associated with some side effects such as myocardial infarction, dyspepsia, congestive heart failure, gastric ulceration, diarrhea and hypertension [13]. The NSAIDs may also cause renal failure when used in combination with some diuretic and ACE inhibitors, interstitial nephritis, nephrotic syndrome, acute tubular necrosis and acute renal failure and also causing analgesic nephropathy when used in combination with phenacetin and paracetamol, premature birth, hepatotoxicity, raised liver enzymes, bronchospasm, hyperkalaemia, headache, rash and allergy [14]. These adverse clinical manifestations have attracted medicinal chemists to synthesis newer NSAIDs by chemical modification with the aim of improving safety profile.

Hydrazones have attracted considerable attention in medicinal chemistry due to their broad range of pharmacological response such as antimicrobial, anticonvulsant, analgesic, anti inflammatory, antiplatelet, antitubercular and antitumor activity (Fig. 1) [15-18]. It has been reported that hydrazones derived from diclofenac (Fig. 2) have shown...
antimycobacterial activities when tested in vitro and in vivo [19]. Recently, a series of acyl hydrazones based on mefenamic acid (Fig. 2) were synthesised which are associated with strong cytotoxic properties in vitro [20, 21]. Moreover the presence of azomethine proton (HN-N=CH-) in hydrazones has gained special interest for the development of newer drugs. These observations lead to the development of new hydrazones that possess varied biological responses. In continuation of the previous efforts to identify more potent anti-inflammatory and cytotoxic agents, we have explored the structural features of other anti-inflammatory agents that can be incorporated into our target molecules. Accordingly, potent anti-inflammatory agent, oxaprozin was selected which belong to group of NSAIDs that are commonly used to treat pain and other inflammatory diseases. We expect that these different novel substituted acyl hydrazone derivatives of oxaprozin would display cytotoxicity, analgesic and anti-inflammatory activities. Herein, we report the synthesis, structure analysis, in vitro studies and in vivo pharmacological evaluation of a series of oxaprozin hydrazone derivatives.

2. EXPERIMENTAL

2.1. Material and Methods

All the chemicals were purchased from SRL-India, Merck, Finar and have been carried forward without further purification. Melting points were determined by open glass capillary method on a Cintex melting point apparatus. IR spectra were recorded on a JASCO FT/IR-5300 in KBr pellets. 1H NMR spectra were recorded on a Varian 300 MHz spectrometer using CDCl3 and DMSO as a solvent. Chemical shift (δ) values are presented as singlet (s), doublet (d), triplet (t), quartet (q) or multiplet (m). Mass spectra were recorded on a LC-MSD-Trap-SL instrument in the electrospray ionization (ESI) mode. All the reactions were monitored by TLC on pre-coated silica gel plates (60F 254; Merck).

2.2. Synthetic Procedure

2.2.1. Synthesis of methyl 3-(4,5-diphenyloxazol-2-yl) propionate (2a) : Compound 2 (3.07 g, 0.01 mol) was taken in a 100 mL single necked round bottomed flask followed by the addition of methanol (30 mL) to obtain clear solution. The resulting solution was refluxed to afford crude mass and has been purified by column chromatography with ethylacetate : hexane (3 : 7) as eluent. The crude product was dried over anhydrous sodium sulphate and the solvent was concentrated under reduced pressure to afford colorless crystals of compound (2). M.p.150-152 ºC; IR (KBr) νmax (cm⁻¹) 3052, 3030, 2953, 2843, 1736, 1583, 1495, 1435, 1358, 1331, 1227, 1167, 1063; 1H NMR (500 MHz, CDCl3) δ 2.93 (t, 2H, CH2), 3.20 (t, 2H, CH2), 3.74 (s, 3H, OCH3), 7.26-7.38 (m, 6H, Ph-H), 7.56-7.64 (dd, 4H, Ph-H); Molecular formula C19H17O3N; MS [M+1]+: m/z 308.

2.2.2. Synthesis of 3-(4,5-diphenyloxazol-2-yl) propane hydrazide (3): Compound 2 (3.07 g, 0.01 mol) was taken in a 100 mL single necked round bottomed flask followed by the addition of methanol (30 mL) to obtain clear solution. To the resulting solution hydrazine hydrate (0.185 g, 0.001 mol) was added and stirred at ambient temperature, 4.77 (s, 2H, -NH2), 4.72 (s, 1H, -NH, D2O exchange), 4.77 (s, 2H, -NH, D2O exchange) 7.26-7.38 (m, 6H, Ph-H), 7.54-7.65 (dd, 4H, Ph-H); Molecular formula C18H17O2N2; MS [M+1]+: m/z 308.

2.3. General Methods of Synthesis of Compounds (5a-5j)

2.3.1. Method (III), (Conventional heating method) Compound 3 (0.307 g, 0.001 mol) was dissolved in 10 mL of ethanol in a 25 mL round bottomed flask. To the resulting solution 3-bromo benzaldehyde (4a) (0.185 g, 0.001 mol) was added and stirred at ambi-

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**Fig. (1).** Design of hybrid acyl hydrazone molecules of potential pharmacological interest.

**Fig. (2).** Some of NSAIDs having cytotoxicity and anti-mycobacterial activity.
ent temperature for 5 h. Progress of the reaction was monitored by TLC using ethylacetate : hexane (4 : 6) as eluent. After the completion of the reaction, methanol was removed by distillation. The obtained crude product was diluted with water, the resulting precipitate was filtered and then dried, followed by recrystallisation in hot ethanol to furnish colorless N’-(3-bromobenzylidene)-3-(4,5-diphenyloxazol-2-yl)propane hydrazide (5a).

\[ \text{M.p.} 128.4, 128.6, 128.7, 128.8, 129.0, 129.3, 130.2, 132.5, 133.4, 135.1, 138.8, 142.3, 145.3, 157.9, 162.4, 173.3; \]
\[ \text{Molecular Formula } C_{29}H_{23}N_2O_3; MS [M+H]^+; m/z 441.2. \]

\[ \text{N’-(2, 5-dimethoxybenzylidene)-3-(4,5-diphenyloxazol-2-yl)propane hydrazide (5d)} \]

\[ \text{M.p.} 1599, 1435, 1342, 1254, 1139, 1019; \]
\[ \text{\[M+H\]}^+; m/z 456.2. \]

\[ \text{3-(4,5-diphenyloxazol-2-yl)-N’-(3,4,5-trimethoxybenzylidene) propane hydrazide (5e)} \]

\[ \text{M.p.} 147-148 \degree C; \]
\[ \text{IR (KBr)} \nu_{max} (cm^{-1}) 3441, 3172, 3068, 2947, 2838, 1665; \]
\[ \text{\[M+H\]}^+; m/z 486.3. \]

\[ \text{3-(4,5-diphenyloxazol-2-yl)-N’-(4-methoxymethylbenzylidene) propa nehydrazide (5f)} \]

\[ \text{M.p.} 152-153 \degree C; \]
\[ \text{\[M+H\]}^+; m/z 410.2. \]
N’-(2,6-dichlorobenzylidene)-3-(4,5-diphenyloxazol-2-yl) propanehydrazide (5b)

M.p.150-151 °C; IR (KBr) ν max (cm⁻¹) 3319, 3180, 3059, 2972, 2920, 1678, 1604, 1579, 1554, 1417, 1134, 1057; ¹H NMR (300 MHz, CDCl₃) δ 3.25-3.40 (m, 4H, CH₂-CH₂), 7.30-7.40 (m, 9H, Ph-H), 7.60-7.72 (m, 4H, Ph-H), 8.15 (s, 1H, N=CH-), 9.23 (s, 1H, O=C=NH); ¹³C NMR (75 MHz, CDCl₃) δ 23.0, 29.9, 30.3, 126.3, 127.8, 128.2, 128.4, 128.5, 129.0, 129.5, 130.0, 132.4, 135.0, 138.6, 145.2, 162.2, 174.3; Molecular Formula CₙHₙN₂O₂Cl₂; MS [M+1]+; m/z 464.1.

3-(4,5-diphenyloxazol-2-yl)-N’-(4-nitrobenzylidene) propanehydrazide (5i)

M.p.160-161 °C; IR (KBr) ν max (cm⁻¹) 1574, 1549, 1423, 1145, 1105; ¹H NMR (500 MHz, CDCl₃) δ 3.28-3.41 (m, 4H, CH₂-CH₂), 7.28-7.37 (m, 6H, Ph-H), 7.58 (dd, 4H, J=6.9, 5.9 Hz, Ph-H), 7.76 (d, 2H, J=7.9 Hz, Ph-H), 7.81 (s, 1H, N=CH-), 8.18 (d, 2H, J=8.9 Hz, Ph-H), 9.81 (s, 1H, O=C=NH); ¹³C NMR (75 MHz, CDCl₃) δ 23.1, 29.9, 126.9, 126.3, 127.7, 127.8, 128.1, 128.5, 128.6, 128.9, 132.3, 133.5, 131.6, 141.6, 145.4, 148.3, 162.1, 175.0; Molecular Formula CₙHₙN₂O₄; MS [M+1]+; m/z 441.2.

N’-(5-bromo-2-methoxybenzylidene)-3-(4,5-diphenyloxazol-2-yl) propanehydrazide (5j)

M.p.160-161 °C; IR (KBr) ν max (cm⁻¹) 3244, 3051, 2964, 2928, 2852, 1674, 1585, 1523, 1421, 1145, 1105; ¹H NMR (500 MHz, CDCl₃) δ 3.24-3.40 (m, 4H, CH₂-CH₂), 3.82 (s, 3H, OCH₃), 6.77 (d, 1H, J=8.87 Hz, Ph-H), 7.27-7.36 (m, 7H, Ph-H), 7.60 (dd, 4H, J=9.2, 6.5 Hz, Ph-H), 7.98 (d, 1H, J=2.6 Hz, Ph-H), 8.06 (s, 1H, N=CH-), 9.07 (s, 1H, O=C=NH); ¹³C NMR (75 MHz, CDCl₃) δ 23.0, 29.8, 55.7, 112.7, 113.3, 124.0, 126.4, 127.9, 127.9, 128.1, 128.2, 128.4, 128.5, 128.6, 128.9, 132.4, 133.6, 138.5, 156.8, 173.8; Molecular Formula CₙHₙN₂O₃Br; MS [M+1]+; m/z 504.

2.5. Biological Assay

2.5a. Anti-inflammatory activities (in vivo) of test compounds

The anti-inflammatory activities of synthesised compounds were screened by Carrageenan induced paw oedema method [22, 23]. Female Swiss albino mice weighing 20 g to 30 g were used for the experiment. They were housed in the clean polypropylene cages and kept under room temperature (25 °C), relative humidity (60-70%) in 12 h of light dark cycle. The animals were given standard laboratory diet and water ad libium. Food was withdrawn 12 h before and during experimental hours.

Swiss albino mice divided into eight groups with each group containing six animals. A mark was made on the right hind paw just below the tibia-tarsal junction. So that every time the paw was dipped in the mercury column up to fixed mark to ensure constant paw volume, the initial paw volume of each mice was noted by plethysmometrically. The group I was kept as control and received only 0.5% carboxy methyl cellulose (CMC) solution. Group II was kept as standard and received oxaprozin (10 mg kg⁻¹ p.o). Group III to VIII were kept as test and received test compounds of a dose 10 mg kg⁻¹ (suspended in 0.5% CMC given p.o). Carrageenan solution (0.1% in sterile 0.9% NaCl solution) in a volume of 0.1 mL was injected subcutaneously into the sub-plantar region of the right hind paw of each mice. 2 h after the administration of the test compounds and standard drug. The right hind paw volume was measured by means of a plethysmometer. The percentage inhibition by the drugs was calculated according to the following formula.

Percentage of paw oedema inhibition = 100 - Vₜ/Vₐ X 100

Where Vₐ = volume of paw edema in control group
Vₜ = volume of paw edema in the treated group with test compounds

The results were expressed as % inhibition of oedema over the untreated control group.

2.5b. Analgesic activity (in vivo) of the test compounds

Analgesic activity was carried out by Eddy’s Hot Plate Method using Female swiss albino mice of weighing 20 g to 30 g [24]. In this method heat is used to induce pain. Animals are individually placed on a hot plate maintained at constant temperature (55 ± 1 °C) and the reaction of animals, such as licking of the paw or jump response is taken as the end point. Swiss albino mice are divided into twelve groups with each group containing six animals [25]. Group I was kept as control, group II was kept as standard and group III to XII were kept as test and analgesic activity was evaluated after oral administration of the test compounds. The test compounds and standard drug are administered orally at the dose of 10 mg kg⁻¹ body weight, as suspension in 0.5% CMC (Carboxy Methyl Cellulose) solution.

The analgesic activity was observed as the reaction time of animals at 30 min, 60 min, 90 min and 120 min after compound administration. The percentage analgesic activity shown by the tested compounds is presented.

2.5c. Anticancer activity (in vitro) of test compounds

All fine chemicals/reagents used in this study were of cell culture grade and obtained from Sigma-Aldrich, Merck. Human colon cancer cell line (HCT-15) was obtained from National Centre for Cell Science, Pune, India. The cells were grown in DMEM (Dulbecco’s Modified Eagle Medium) culture medium supplemented with 2 mM L-glutamine, 10% FBS (Foetal Bovine Serum), Pencillin (50 IU mL⁻¹) and Streptomycin (50 µg mL⁻¹) at a temperature of 37 °C in a humidified incubator with a 5% CO₂ atmosphere and passaged twice weekly to maintain a sub confluent state.

The viability of the cells was assessed by MTT [(3,4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [26, 27]. This is based on
the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product cisplatin a known anticancer drug was used as a reference compounds in the assay \[29\]. Cells (1 x 10^6) were placed in a 96-well plate. After 24 h, they were treated with different concentration (2.0-25 µg mL^-1) of different test compounds diluted appropriately with culture media for 48 h. Cells grown in media containing equivalent amount of DMSO served as positive control and cells in medium without any supplementation were used as negative control. After the treatment, media containing compound were carefully removed by aspiration. 100 µL of DMSO was added to each well and kept in an incubator for 4 h for dissolution of the formed formazan crystals. Amount of formazan was determined by measuring the absorbance at 540 nm using an ELISA plate reader. The data were presented as percent of dead cells, whereas absorbance from non-treated control cells was defined as 100% live cells.

3. RESULTS AND DISCUSSION

3.1 Synthesis

Acyl hydrazone derivatives of 3-(4,5-diphenyloxazol-2-yl) propanoic acid were synthesised via efficient synthetic route transformations and microwave irradiation method from 3 and 4a-4j to form product 5a-5j. The key intermediate 3 was prepared in 79% yield from methyl 3-(4,5-diphenyloxazol-2-yl) propionate (2) and hydrazine hydrate employing methanol as solvent. Reaction of 3 with one equivalent of different aromatic aldehydes (4a-4j) in the presence of ethanol at ambient temperature for 5h afforded the title compounds 5a-5j in good yields (method (III)\_A, scheme 1). In order to reduce the reaction time as well as use of solvents significantly, the reaction of 3 with different aldehydes were conducted using microwave radiation. Organic transformations, under solvent free microwave irradiation conditions, have gained extensive applications due to many convenient advantages coupled with superior reaction rates, high yields and improved selectivity and environment friendly protocols. Solvent free synthesis afforded the desired products 5a-5j in few minutes with excellent yields (Method (III)\_B, Scheme 1). Results of the study along with the time/yields obtained by two methods have been presented in Table 1.

![Scheme 1](image-url)

**Scheme (1). Synthetic route of the Compounds (5a-5j).**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Aldehydes</th>
<th>Products</th>
<th>Conventional heating Time/Yield (%)</th>
<th>Microwave irradiation Time/Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4a</td>
<td>5a</td>
<td>6.0 h / 69</td>
<td>2.5 min / 75</td>
</tr>
<tr>
<td>2</td>
<td>4b</td>
<td>5b</td>
<td>5.0 h / 72</td>
<td>2.5 min / 78</td>
</tr>
<tr>
<td>3</td>
<td>4c</td>
<td>5c</td>
<td>6.0 h / 72</td>
<td>3.0 min / 84</td>
</tr>
<tr>
<td>4</td>
<td>4d</td>
<td>5d</td>
<td>7.0 h / 78</td>
<td>4.0 min / 80</td>
</tr>
<tr>
<td>5</td>
<td>4e</td>
<td>5e</td>
<td>6.0 h / 75</td>
<td>2.5 min / 82</td>
</tr>
<tr>
<td>6</td>
<td>4f</td>
<td>5f</td>
<td>7.0 h / 67</td>
<td>3.0 min / 74</td>
</tr>
<tr>
<td>7</td>
<td>4g</td>
<td>5g</td>
<td>5.0 h / 73</td>
<td>4.0 min / 81</td>
</tr>
<tr>
<td>8</td>
<td>4h</td>
<td>5h</td>
<td>6.0 h / 70</td>
<td>3.0 min / 78</td>
</tr>
<tr>
<td>9</td>
<td>4i</td>
<td>5i</td>
<td>6.0 h / 82</td>
<td>3.5 min / 87</td>
</tr>
<tr>
<td>10</td>
<td>4j</td>
<td>5j</td>
<td>5.0 h / 70</td>
<td>2.5 min / 80</td>
</tr>
</tbody>
</table>

Table 1. Synthesis of Novel Acyl Hydrazone Derivatives of 3-(4,5-diphenyl-1,3-oxazol-2-yl) propanoic acid (5a-5n) under conventional and microwave irradiation condition from acid hydrazide (3) and aromatic aldehydes (4a-4n)
3.2. Biological assay

3.2a. Analgesic activity (in vivo)

The synthesised compounds 5a to 5j were screened for analgesic activity by Eddy’s Hot Plate Method taking oxaprozin as standard. The compounds 5a to 5j and standard were tested at an equimolar oral dose relative to 10 mg kg\(^{-1}\) body weights. The compounds showed good analgesic activity in comparison to their respective standard oxaprozin drug. The compounds showed analgesic activity ranging from 64.96% to 90.88% inhibition at 120 min, where as standard drug showed 79.28% inhibition (Table 2, Fig. 3). The compound 5b (3-(4,5-diphenyloxazol-2-yl)-N’-(2-nitrobenzylidene) propanehydrazide), showed highest activity 90.88% inhibition. Replacement of nitro group by 2-methoxy substituent 5c (3-(4,5-diphenyloxazol-2-yl)-N’-(2-methoxybenzylidene) propanehydrazide) resulted in slight decrease of analgesic activity (86.18%). The compound 5e (3-(4,5-diphenyloxazol-2-yl)-N’-(3,4,5 trimethoxy benzilidene) Propanehydrazide) showed 87.37% inhibition. The compounds 5d (N’-(2, 5-dimethoxybenzylidene)-3-(4,5-diphenyloxazol-2-yl) Propanehydrazide) and 5f (3-(4,5-diphenyloxazol-2-yl)-N’-(4-methylbenzilidene) propane hydrazide) showed slightly decreased 85.21% and 85.75% respective. The compound 5h (N’-(2,6-dichlorobenzylidene)-3-(4,5-diphenyloxazol-2-yl) propane hydrazide) showed least activity (64.96%) compared to other compounds and standard oxaprozin drug. The results indicate that the ortho positions in the aromatic ring should be occupied preferably with electron withdrawing groups like nitro. Presence of OCH\(_3\) in other than ortho position also favored the analgesic activity. Substitution with chloro groups in the aromatic ring drastically decreased the biologically activity. In general the presence of 2-nitro, 2-methoxy, 2-methoxy-5-bromo, 2,5-dimethoxy, 3,4,5-tri methoxy and 4- methyl substituent of aldehyde derivatives resulted in high analgesic activity.

Table 2. Analgesic activity data of 5a-5j.

<table>
<thead>
<tr>
<th>Compds.</th>
<th>Dose (mg kg(^{-1}))</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>10</td>
<td>11.92 ± 0.11</td>
<td>32.19 ± 1.04</td>
<td>71.52 ± 0.50</td>
<td>79.28 ± 0.57</td>
</tr>
<tr>
<td>5a</td>
<td>10</td>
<td>58.61 ± 0.52</td>
<td>73.20 ± 0.96</td>
<td>84.79 ± 0.90</td>
<td>85.21 ± 0.50</td>
</tr>
<tr>
<td>5b</td>
<td>10</td>
<td>64.96 ± 0.80</td>
<td>80.88 ± 0.64</td>
<td>86.06 ± 0.52</td>
<td>90.88 ± 0.76</td>
</tr>
<tr>
<td>5c</td>
<td>10</td>
<td>69.53 ± 0.76</td>
<td>81.80 ± 0.50</td>
<td>84.36 ± 0.57</td>
<td>86.18 ± 0.57</td>
</tr>
<tr>
<td>5d</td>
<td>10</td>
<td>64.96 ± 0.76</td>
<td>74.68 ± 0.57</td>
<td>81.04 ± 0.76</td>
<td>85.21 ± 0.50</td>
</tr>
<tr>
<td>5e</td>
<td>10</td>
<td>58.61 ± 0.90</td>
<td>85.29 ± 0.50</td>
<td>86.97 ± 0.51</td>
<td>87.37 ± 0.60</td>
</tr>
<tr>
<td>5f</td>
<td>10</td>
<td>78.30 ± 0.55</td>
<td>80.88 ± 1.53</td>
<td>84.85 ± 0.28</td>
<td>85.75 ± 1.00</td>
</tr>
<tr>
<td>5g</td>
<td>10</td>
<td>11.92 ± 1.24</td>
<td>24.21 ± 3.11</td>
<td>82.47 ± 2.64</td>
<td>83.72 ± 1.21</td>
</tr>
<tr>
<td>5h</td>
<td>10</td>
<td>12.84 ± 1.02</td>
<td>33.82 ± 1.02</td>
<td>41.92 ± 1.04</td>
<td>64.96 ± 1.38</td>
</tr>
<tr>
<td>5i</td>
<td>10</td>
<td>49.42 ± 0.57</td>
<td>74.72 ± 0.76</td>
<td>80.88 ± 0.57</td>
<td>84.80 ± 0.51</td>
</tr>
<tr>
<td>5j</td>
<td>10</td>
<td>29.10 ± 1.15</td>
<td>64.68 ± 1.02</td>
<td>75.21 ± 1.25</td>
<td>81.80 ± 0.57</td>
</tr>
</tbody>
</table>

All results expressed as MEAN ± SEM, n=6 in each group, Results are analyzed by student’s t-test; Oxaprozin is used as the standard.

3.2b. Anti-inflammatory activity (in vivo)

The compounds which showed good analgesic activity (5a-5e and 5i) were further tested for their anti-inflammatory activity by Carrageenan induced paw oedema method of winter et al., at same oral dose as used for the analgesic activity. The compounds showed anti-inflammatory activity ranging from 34.25% to 100% inhibition of paw oedema, where as standard drug oxaprozin showed 88.15% inhibition after 2 h (Table 3, Fig. 4a, 4b). The compounds 5b (3-(4,5-diphenyloxazol-2-yl)-N’-(2-nitrobenzylidene) propane hydrazide), 5d (N’-(2, 5-dimethoxybenzylidene)-3-(4,5-diphenyloxazol-2-yl) Propanehydrazide) and 5e (3-(4,5-diphenyloxazol-2-yl)-N’-(3,4,5-trimethoxybenzylidene) Propanehydrazide) showed highest anti-inflammatory activity i.e., 100% inhibition of paw oedema in comparison to oxaprozin taken as standard (88.15%). The compound 5e (3-(4,5-diphenyloxazol-2-yl)-N’-(2-methoxybenzylidene) propanehydrazide) showed very least activity 34.25% and 5a (N’-(3-bromobenzylidene)-3-(4,5-diphenyloxazol-2-yl) propane hydrazide), 5i (3-(4,5-diphenyloxazol-2-yl)-N’-(4-nitrobenzylidene) propanehydrazide) showed moderate anti-inflammatory activity (49.02% and 50.65%) respectively. It is clear that oxaprozin acyl hydrazine derivative which had the substituent like p-nitro, 2,5-dimethoxy, 3,4,5-trimethoxy substituent groups of aldehydes resulted in good anti-inflammatory activity.

Table 3. Anti-inflammatory activity data of 5a-5e and 5i.

<table>
<thead>
<tr>
<th>Compds.</th>
<th>Dose (mg kg(^{-1}))</th>
<th>Increase in paw volume (MEAN ± SEM)</th>
<th>% Inhibition of paw oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>10</td>
<td>0.102 ± 0.002</td>
<td>49.02</td>
</tr>
<tr>
<td>5b</td>
<td>10</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>5c</td>
<td>10</td>
<td>0.1 ± 0.0438</td>
<td>34.21</td>
</tr>
<tr>
<td>5d</td>
<td>10</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>5e</td>
<td>10</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>5f</td>
<td>10</td>
<td>0.075 ± 0.0034</td>
<td>50.65</td>
</tr>
<tr>
<td>5i</td>
<td>10</td>
<td>0.152 ± 0.0031</td>
<td>88.15</td>
</tr>
<tr>
<td>Control</td>
<td>0.1</td>
<td>0.018 ± 0.0027</td>
<td>-</td>
</tr>
</tbody>
</table>

All results expressed as MEAN ± SEM, n=6 in each group, Results are analyzed by student’s t-test; Oxaprozin is used as the standard, CMC= carboxy methyl cellulose as a suspending agent.
The authors confirm that this article content has no conflict of interest.

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Conflict of interest

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