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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, ^{1}H NMR, ^{13}C 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

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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.

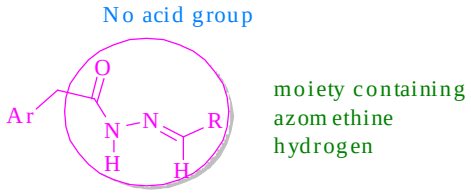


Fig. (1). Design of hybrid acyl hydrazone molecules of potential pharmacological interest.

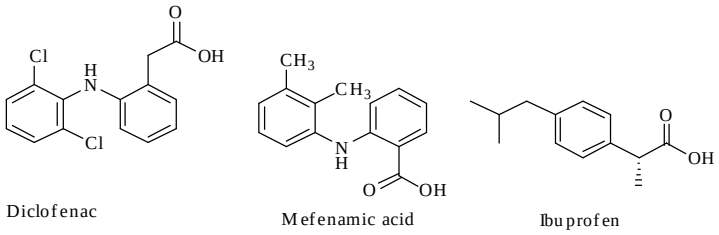


Fig. (2). Some of NSAIDs having cytotoxicity and anti-mycobacterial activity.

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. ^{1}H NMR spectra were recorded on a Varian 300 MHz spectrometer using $CDCl_3$ and DMSO as a solvent. Chemical shift (d) 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). Column chromatography was performed on 100-200 mesh silica gel (SRL, India) using 10-20 fold excess (by weight) of the crude product. The organic extracts were dried over anhydrous sodium sulphate.

2.2. Synthetic Procedure

2.2a. Synthesis of methyl 3-(4,5-diphenyloxazol-2-yl) propionate (2) : 3-(4,5-diphenyloxazol-2-yl) propanoic acid (oxaprozin) (1) (2.92 g, 0.01 mol) was dissolved in methanol (25 mL) in a 100 mL round bottomed flask and few drops of conc.H_2SO_4 were added. The resulting solution was refluxed for 5 h. Progress of the reaction was monitored by TLC using ethylacetate : hexane (3 : 7) as eluent. After completion of the reaction as indicated by TLC, methanol was removed under reduced pressure and the crude product was diluted with ice water and extracted with ethyl acetate (3 X 10 mL). The organic layer was dried over anhydrous sodium sulphate and the solvent was concentrated. The crude product purified by column chromatography with ethylacetate : hexane (1 : 5) as eluent. The solid obtained was recrystallized from ethylacetate to afford colorless crystals of compound **(2)**. M.p.150-152 ºC; IR (KBr) ν_{max} (cm^{-1}) 3052, 3030, 2953, 2843, 1736, 1583, 1495, 1435, 1358, 1331, 1227, 1167, 1063; ^{1}H NMR (500 MHz, $CDCl_3$) *d* 2.93 (t, 2H, CH_2), 3.20 (t, 2H, CH_2), 3.74 (s, 3H, OCH_3), 7.26-7.38 (m, 6H, Ph-H), 7.56-7.64 (dd, 4H, Ph-H); Molecular formula $C_{19}H_{17}O_3N$; MS $[M+1]^+$: *m/z* 308.

2.2b. 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 (3 mL, 0.06 mol) was added drop wise and refluxed for 7 h. Progress of the reaction was monitored by TLC using ethylacetate : hexane (3 : 7) as eluent. After the completion of the reaction, methanol was distilled off and the reaction mass was diluted with cooled water and extracted with chloroform (3 X 15 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford crude mass and has been purified by column chromatography to afford the compound as light yellow crystalline solid. M.p. 166-168 °C; IR (KBr) ν_{max} (cm^{-1}) 3484, 3282, 3057, 1654, 1610, 1435, 1210, 1057; ^{1}H NMR (500 MHz, $CDCl_3$) *d* 2.73 (t, 2H, -CH_2), 3.20 (t, 2H, -CH_2), 4.72 (s, 1H, -NH, D_2O exchange), 4.77 (s, 2H, -NH_2, D_2O exchange) 7.26-7.38 (m, 6H, Ph-H), 7.54-7.65 (dd, 4H, Ph-H); Molecular formula $C_{18}H_{17}O_2N_3$; MS $[M+1]^+$: *m/z* 308.

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

2.3a. Method (III)$_A$ (Conventional heating method)

Compound **3** (0.307 g, 0.001mol) 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, 0.001mol) was added and stirred at ambi-

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**)*. M.p. 149-150 °C; IR (KBr) ν_{max} (cm^{-1}) 3462, 3183, 3057, 2964, 2909, 1660, 1572, 1402, 1358, 1265, 1216, 1068; ^{1}H NMR (300 MHz, $CDCl_3$) *d* 3.26-3.40 (m, 4H, CH_2-CH_2), 7.20 (t, 1H, *J* = 7.9, 7.7 Hz, Ph-H), 7.28-7.40 (m, 6H, Ph-H), 7.49 (t, 2H, *J* =8.4, 7.7 Hz, Ph-H), 7.60 (dd, 4H, *J* =6.4, 5.6 Hz, Ph-H), 7.70 (s, 1H, Ph-H), 7.78 (s, 1H, N=CH-), 10.07 (S, 1H, O=C-NH); ^{13}C NMR (75 MHz, $CDCl_3$) *d* 23.1, 29.9, 122.9, 125.9, 126.4, 127.9, 128.0, 128.3, 128.5, 128.6, 128.7, 129.0, 129.7, 130.2, 132.5, 132.9, 135.1, 135.7, 142.3, 145.3, 162.3, 174.3; Molecular Formula $C_{25}H_{20}N_3O_2Br$; MS $[M+1]^+$: *m/z* 474.2.

2.3b. Method (III)$_B$ (Microwave irradiation method)

The conversion of compound **3** to **5a-5j** was also carried out under microwave conditions using commercially available domestic microwave oven (ONIDA Power Grill 20) 600w. The reactants **3** (0.001 mol) and **4a-4j** (0.001 mol) were mixed thoroughly in a china dish in the absence of solvents and irradiated with microwave radiation at 600w-800w power for 2.5-4 min. The reaction mixture was allowed to react in neat fashion to furnish **5a-5j** in good yields without any polymerization of aldehydes.

3-(4,5-diphenyloxazol-2-yl)-N'-(2-nitrobenzylidene) propanehydrazide (5b)

M.p.147-148 °C; IR (KBr) ν_{max} (cm^{-1}) 3451, 3199, 3019, 2914, 1660, 1523, 1523, 1435, 1353, 1249, 1172, 1068; ^{1}H NMR (300 MHz, $CDCl_3$) *d* 3.26-3.40 (m, 4H, CH_2-CH_2), 7.30-7.39 (m, 6H, Ph-H), 7.51 (d, 1H, *J* = 8.3 Hz, Ph-H), 7.53-7.65 (m, 5H, Ph-H), 8.03 (d, 1H, *J* = 8.3 Hz, Ph-H), 8.10 (d, 1H, *J* = 6.7 Hz, Ph-H), 8.34 (s, 1H, N=CH-), 9.27 (S, 1H, O=C-NH); ^{13}C NMR (75 MHz, $CDCl_3$) *d* 23.0, 30.0, 124.8, 126.4, 127.8, 127.9, 128.0, 128.4, 128.6, 128.7, 128.8, 129.0, 129.3, 130.2, 132.5, 133.4, 135.1, 138.8, 145.4, 148.0, 162.2, 173.6; Molecular Formula $C_{25}H_{20}N_4O_4$; MS $[M+1]^+$: *m/z* 441.2.

3-(4,5-diphenyloxazol-2-yl)-N'-(2-methoxybenzylidene) propanehydrazide (5c)

M.p.160-161 °C; IR (KBr) ν_{max} (cm^{-1}) 3440, 3172, 3068, 2947, 2838, 1665, 1599, 1435.5, 1380.7, 1342.4, 1254.7, 1139.6, 1019.1; ^{1}H NMR (500 MHz, $CDCl_3$) *d* 3.27-3.38 (m, 4H, CH_2-CH_2), 3.85 (s, 3H, OCH_3), 6.90 (d, 1H, *J* = 7.9 Hz, Ph-H), 6.95 (t, 1H, *J* = 7.9, 6.9 Hz, Ph-H), 7.28-7.39 (m, 7H, Ph-H), 7.57 (d, 2H, *J* = 5.9 Hz, Ph-H), 7.63 (d, 2H, *J* = 6.9 Hz, Ph-H), 7.91 (d, 1H, *J* = 7.9 Hz, Ph-H), 8.15 (s, 1H, N=CH-), 8.71 (S, 1H, O=C-NH); ^{13}C NMR (75 MHz, $CDCl_3$) *d* 23.1, 30.1, 55.5, 111.0, 120.9, 122.0, 126.3, 126.4, 127.9, 127.9, 128.3, 128.5, 128.5, 128.6, 129.1, 131.4, 132.6, 139.6, 145.3, 157.9, 162.4, 173.3; Molecular Formula $C_{26}H_{23}N_3O_3$; MS $[M+1]^+$: m/z 426.2.

N'-(2, 5-dimethoxybenzylidene)-3-(4,5-diphenyloxazol-2-yl) propanehydrazide (5d)

M.p.169-170 °C; IR (KBr) ν_{max} (cm^{-1}) 3440, 3172, 3068, 2947, 2838, 1665, 1599, 1435, 1342, 1254, 1139, 1019; ^{1}H NMR (300 MHz, $CDCl_3$) *d* 3.26-3.40 (m, 4H, CH_2-CH_2), 3.76 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 6.84 (d, 1H, *J* = 7.9 Hz, Ph-H), 6.91 (dd, 1H, *J* = 3.0 Hz, Ph-H), 7.28-7.40 (m, 6H, Ph-H), 7.43 (d, 1H, *J* = 3.0 Hz, Ph-H), 7.60 (dd, 4H, *J* = 8.1, 5.2 Hz, Ph-H), 8.12 (s, 1H, N=CH-), 8.73 (S, 1H, O=C-NH); ^{13}C NMR (75 MHz, $CDCl_3$) *d* 23.1, 30.1, 55.5, 56.1, 111.0, 120.9, 122.5, 126.3, 126.4, 127.9, 128.3, 128.4, 128.6, 129.1, 131.4, 132.6, 139.5, 145.3, 157.9, 162.2, 173.4; Molecular Formula $C_{27}H_{25}N_3O_4$; MS $[M+1]^+$: *m/z* 456.2.

3-(4,5-diphenyloxazol-2-yl)-N'-(3,4,5-trimethoxybenzylid ene) propanehydrazide (5e)

M.p.178-179 °C; IR (KBr) ν_{max} (cm^{-1}) 3441, 3172, 3068, 2947, 2838, 1665, 1599, 1435, 1380, 1254, 1139, 1019; ^{1}H NMR (300 MHz, $CDCl_3$) *d* 3.26-3.42 (m, 4H, CH_2-CH_2), 3.86 (s, 9H, OCH_3), 6.87 (s, 2H, Ph-H), 7.30-7.40 (m, 6H, Ph-H), 7.58 (dd, 4H, *J* = 9.8, 7.5 Hz, Ph-H), 7.69 (s, 1H, N=CH-), 9.42 (S, 1H, O=C-NH); ^{13}C NMR (75 MHz, $CDCl_3$) *d* 23.1, 29.9, 56.0, 56.1, 60.8, 104.2, 104.6, 126.3, 127.7, 127.8, 128.0, 128.3, 128.4, 128.5, 128.6, 128.9, 129.0, 132.3, 135.0, 139.8, 144.1, 145.3, 153.3, 162.3, 174.2; Molecular Formula $C_{28}H_{27}N_3O_5$; MS $[M+1]^+$: *m/z* 486.3.

3-(4,5-diphenyloxazol-2-yl)-N'-(4-methylbenzylidene) propanehydrazide (5f)

M.p.152-153 °C; IR (KBr) ν_{max} (cm^{-1}) 3177, 3051, 2958, 2909, 1671, 1610, 1408, 1271, 1216, 1062; ^{1}H NMR (500 MHz, $CDCl_3$) *d* 2.36 (s, 3H, CH_3), 3.26-3.40 (m, 4H, CH_2-CH_2), 7.15 (d, 2H, *J* 6.9, Ph-H), 7.27-7.40 (m, 7H, Ph-H), 7.50-7.65 (m, 5H, Ph-H), 7.73 (s, 1H, N=CH-), 9.46 (S, 1H, O=C-NH); ^{13}C NMR (75 MHz, $CDCl_3$) *d* 21.5, 23.2, 30.0, 126.4, 127.2, 127.9, 128.3, 128.5, 128.6, 128.7, 129.4, 130.6, 132.5, 135.1, 140.4, 144.1, 145.3, 162.4, 174.0; Molecular Formula $C_{26}H_{23}N_3O_2$; MS $[M+1]^+$: *m/z* 410.2.

3-(4,5-diphenyloxazol-2-yl)-N'-(4-hydroxy-3-methoxy benz ylidene) propanehydrazide (5g)

M.p.182-183 °C; IR (KBr) ν_{max} (cm^{-1}) 3512, 3202, 3009, 2924, 2845, 1655, 1649, 1512, 1429, 1280, 1174, 1055; ^{1}H NMR (500 MHz, $CDCl_3$) *d* 3.26-3.40 (m, 4H, CH_2-CH_2), 3.86 (s, 3H, OCH_3), 5.45 (s, 1H, OH), 7.15 (d, 2H, *J* = 6.9 Hz, Ph-H), 7.27-7.40 (m, 6H, Ph-H), 7.50-7.65 (m, 5H, Ph-H), 7.73 (s, 1H, N=CH-), 9.46 (S, 1H, O=C-NH); ^{13}C NMR (75 MHz, $CDCl_3$) *d* 23.1, 30.1, 55.5, 111.0, 120.9, 122.0, 126.3, 126.4, 127.9, 128.3, 128.5, 128.6, 128.6, 129.1, 131.8, 132.6, 139.6, 145.3, 157.9, 162.4, 173.3; Molecular Formula $C_{26}H_{23}N_3O_4$; MS $[M+1]^+$: *m/z* 442.2.

N'-(2,6-dichlorobenzylidene)-3-(4,5-diphenyloxazol-2-yl) propanehydrazide (5h)

M.p.150-151 °C; IR (KBr) ν_{max} (cm^{-1}) 3319, 3180, 3059, 2972, 2920, 1678, 1604, 1579, 1554, 1417, 1134, 1057; ^{1}H NMR (300 MHz, $CDCl_3$) *d* 3.25-3.40 (m, 4H, CH_2-CH_2), 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); ^{13}C NMR (75 MHz, $CDCl_3$) *d* 23.0, 29.9, 30.3, 126.3, 127.8, 128.2, 128.4, 128.5, 128.6, 129.0, 129.5, 130.0, 132.4, 135.0, 138.6, 145.2, 162.2, 174.3; Molecular Formula $C_{25}H_{19}N_3O_2Cl_2$; MS $[M+1]^+$: *m/z* 464.1.

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

M.p.170-171 °C; IR (KBr) ν_{max} (cm^{-1}) 3470, 3188, 3061, 2964, 2928, 2852, 1674, 1585, 1523, 1421, 1145, 1105; ^{1}H NMR (500 MHz, $CDCl_3$) *d* 3.28-3.41 (m, 4H, CH_2-CH_2), 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); ^{13}C NMR (75 MHz, $CDCl_3$) *d* 23.1, 29.9, 126.9, 126.3, 127.7, 127.8, 128.1, 128.5, 128.6, 128.9, 132.3, 135.1, 139.6, 141.6, 145.4, 148.3, 162.1, 175.0; Molecular Formula $C_{25}H_{20}N_4O_4$; MS $[M+1]^+$: *m/z* 441.2.

N'-(5-bromo-2-methoxybenzylidene)-3-(4,5-diphenyloxa zol -2-yl) propanehydrazide (5j)

M.p.160-161 °C; IR (KBr) ν_{max} (cm^{-1}) 3244, 3051, 2964, 2852, 1693, 1568, 1537, 1433, 1259, 1176, 1064; ^{1}H NMR (500 MHz, $CDCl_3$) *d* 3.24-3.40 (m, 4H, CH_2-CH_2), 3.82 (s, 3H, OCH_3), 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); ^{13}C NMR (75 MHz, $CDCl_3$) *d* 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 $C_{26}H_{22}N_3O_3Br$; 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 ^{0}C), relative humidity (60-70%) in 12 h of light dark cycle. The animals were given standard laboratory diet and water ad labium. 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 upto 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 - V_T / V_C) X 100

Where V_C = volume of paw edema in control group
V_T = 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 Eagler 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% CO_2 atmosphere and passaged twice weekly to maintain a sub confluent state.

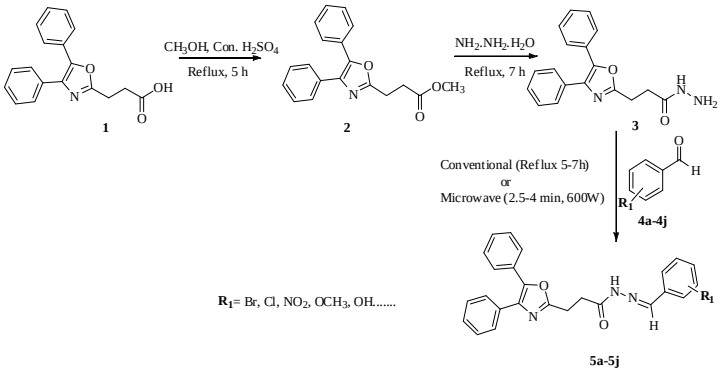
The viability of the cells was assessed by MTT ((3,4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [26, 27]. This is based on

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 [28]. Cells (1 x 10^4) 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). Synthetic route of the Compounds (5a-5j).

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)

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

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 benzlidene) 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-methylbenzylidene) 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, 4-nitro, 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.

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

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.

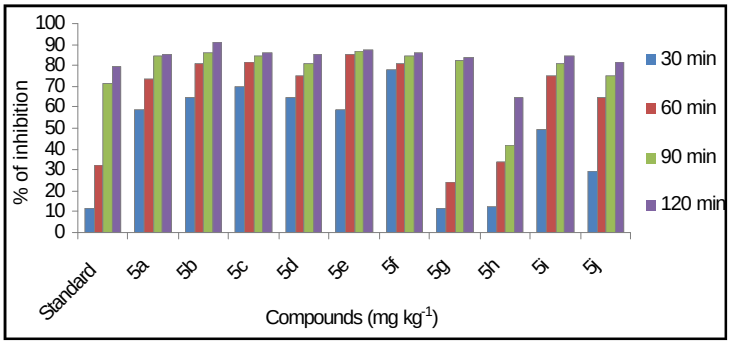


Fig. (3). Analgesic activity of Compounds 5a-5j.

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 **5c** (3-(4,5-diphenyloxazol-2-yl)-N'-(2-methoxybenzylidene) propa nehydraz ide) 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-nitroben zylidene) propanehydrazide) showed moderate anti-inflammatory activity (49.02% and 50.65%) respectively. It is clear that oxaprozin acyl hydrazone 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.

Compds	Dose (mg kg^{-1})	Increase in paw volume (MEAN ± SEM)	% Inhibition of paw oedema
5a	10	0.102 ± 0.002	49.02
5b	10	0	100
5c	10	0.1 ± 0.0438	34.21
5d	10	0	100
5e	10	0	100
5i	10	0.075 ± 0.0034	50.65
Control	0.1	0.152 ± 0.0031	-
Standard	10	0.018 ± 0.0027	88.15

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.

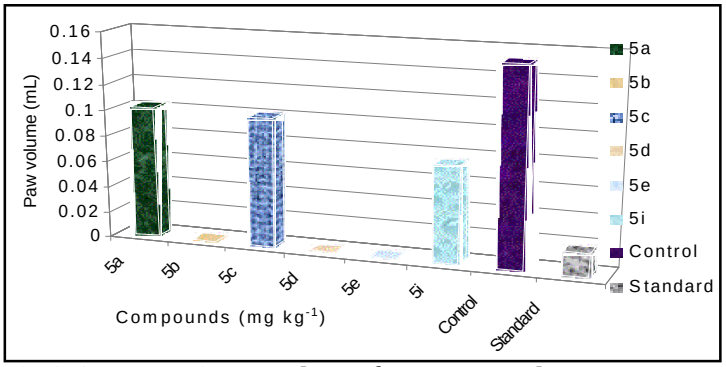


Fig. (4a). Increase in paw volume of test compounds.

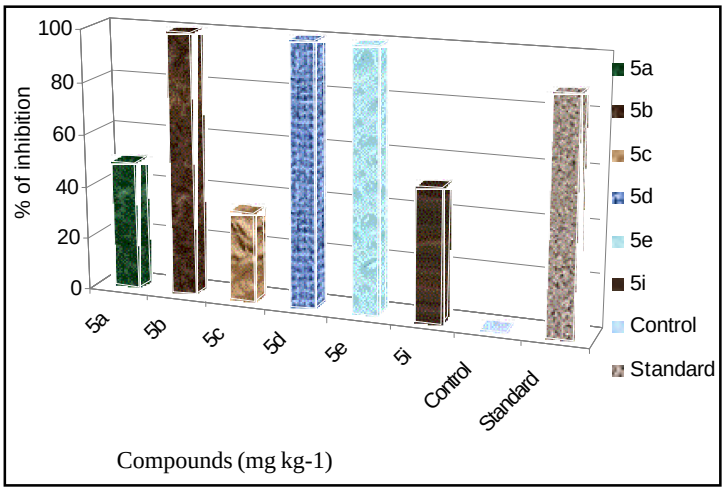


Fig. (4b). % inhibition of anti-inflammatory activity of test

(half maximal inhibitory concentration) values of compounds are shown in Table **4** (Fig. **5**) cisplatin was used as a reference compound. The fact that IC_{50} is inversely proportional to the cytotoxicity indicates that all these compounds are associated with promising cytotoxic effects and the compounds **5a, 5b, 5e, 5g, 5h, 5i** and **5j** are most notable among them as they showed IC_{50} values between 4.2 to 6.8 µg mL^{-1} at low concentration.

Table 4. Cytotoxicity of hydrazone derivatives against human colon cancer cell line (HCT-15).

Compds	% of cell deaths at various concentration				IC_{50}
	2.0 (µg mL^{-1})	5.0 (µg mL^{-1})	10.0 (µg mL^{-1})	25.0 (µg mL^{-1})	(µg mL^{-1})
5a	47.95 ± 0.015	51.15±0.012	52.42±0.001	56.94 ± 0.035	4.2
5b	48.25 ± 0.012	57.81 ± 0.007	58.14 ± 0.026	59.71 ± 0.008	4.4
5c	28.92 ± 0.039	38.72 ± 0.013	50.73 ± 0.031	61.76 ± 0.003	10.5
5d	29.65 ± 0.023	37.74 ± 0.015	46.32 ± 0.016	51.47 ± 0.012	18.3
5e	33.33±0.018	42.89 ± 0.026	51.27 ± 0.014	61.27 ± 0.018	6.8
5f	30.88 ± 0.025	35.04 ± 0.019	44.11 ± 0.006	61.76 ± 0.018	15.2
5g	20.34 ± 0.073	36.37 ± 0.071	40.31 ± 0.038	41.91 ± 0.113	4.5
5h	52.13 ± 0.005	44.71 ± 0.021	48.93 ± 0.064	52.38 ± 0.070	4.3
5i	43.13 ± 0.022	50.24 ± 0.014	53.18 ± 0.011	69.85 ± 0.007	5.0
5j	30.08 ± 0.084	42.40 ± 0.084	45.39 ± 0.010	56.09 ± 0.027	4.3
Cisplatin	22.38 ± 0.048	44.24 ± 0.040	49.36 ± 0.022	64.17 ± 0.021	25

All results expressed as MEAN ± SD, n=3, Cisplatin is used as the standard.

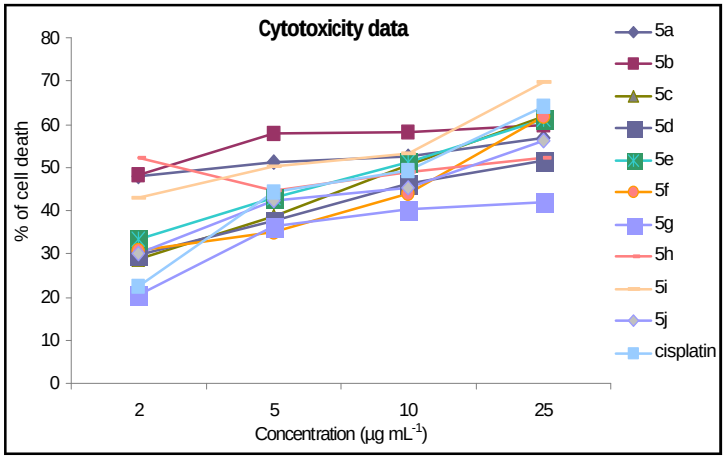


Fig. (5). IC_{50} (µg mL^{-1}) Values of new chemical entities 5a to 5j

4. CONCLUSION

In conclusion, we have described the synthesis, *in vitro* and *in vivo* pharmacological properties of a number of acyl hydrazone derivatives of oxaprozin a well known NSAIDs. All compounds were found to be active when tested against human colon cancer (HCT-15) cell line *in-vitro* and nearly all the compounds exhibited 6 fold more potent cytotoxic activity compared to reference compound cisplatin. *In vivo* studies, many of them exhibited potent analgesic and anti inflammatory activity compared to reference compound oxaprozin. Among these **5b, 5d** and **5e** presented maximum anti inflammatory, analgesic and anticancer activity. Finally, we believe that this call of hydrazone derivatives presents an interesting profile for further experimental investigations especially in the area of anti cancer as well as anti-inflammatory research.

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Conflict of interest

The authors confirm that this article content has no conflict of interest.

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