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SYNTHESIS, EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF SUBSTITUTED 1,2-BENZOXAZOLONE AND 3-CHLORO-1,2-BENZOXAZOLE DERIVATIVES

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ABSTRACT: Herein method for the synthesis of substituted 1,2-benzoxazolone **(8a-f)** and 3-chloro-1,2-benzoxazole **(9a-f)** derivatives has been described. A new scheme has been adapted for the construction of 1,2-benzoxazole ring from salicylic acid and its derivatives which leads to the formation of series of 5,6 and 7 substituted title compounds. Synthesized compounds were characterized by IR, ^{1}H-NMR and Mass spectral analysis. Spectral data confirmed the compounds formation. Final compounds **(8a-f)** and **(9a-f)** were screened for their *in-vivo* analgesic activity by acetic acid induced writhing method in rats and anti-inflammatory activity by carrageenan-induced paw edema model. Among the compounds screened compound **8a** and compound **9c** showed good analgesic activity of about 45% (writhing mean 8.9) and 54% (writhing mean 7.5) inhibition respectively at 5 mg/Kg po dosage. Compounds **8b** and **9b** (both having inhibition edema of 66.1%) showed significant anti-inflammatory activity. Other derivatives exhibit moderate to good analgesic and anti-inflammatory activities.

INTRODUCTION: Benzoxazole derivatives are highly potent and extremely useful in medicinal, industrial and pharmaceutical fields. These compounds have received considerable attention during last few decades as they are endowed with variety of biological activities and have wide range of therapeutic properties with low toxicity.

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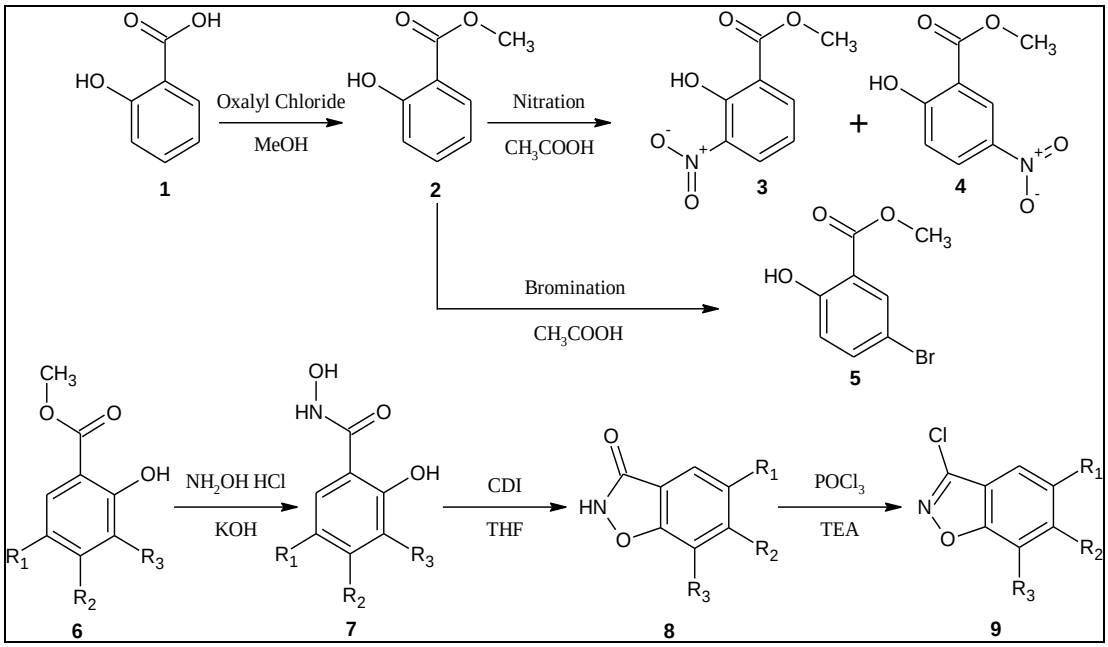
The derivatives of benzoxazole were found to be show antimicrobial [1, 2], antitumour [3], antifungal [4], antihistaminic [5], anticancer [6], antiviral [7], imaging probes [8] and anti-inflammatory [9] activities. Hence synthesis of biologically active benzoxazole derivatives is a demanding work in the present scenario.

Most common and simple way to build benzoxazole skeleton is by the rapid condensation reaction of 2-aminophenol with various aldehydes to afford 2-substituted benzoxazoles[10-14]. However, molecules containing benzoxazole moiety could also be synthesized in many ways as reported earlier [15-22]. Even though several reports available

for the synthesis of benzoxazole derivatives, the present route is found to be new one, which ease us to synthesize 5, 6 and 7 substituted 1,2-benzoxazole derivatives.

The present work describes the synthesis, characterization and evaluation of biological activities of 1,2-benzoxazole derivatives. A series of substituted 1,2-benzoxazolone **(8a-f)** and 3-chloro-1,2-benzoxazole **(9a-f)** derivatives was synthesized from salicylic acid (**Scheme 1**) and its analogues and screened for their *in-vivo* analgesic and anti-inflammatory activities.

MATERIALS AND METHODS: Melting points were determined using open capillary and were uncorrected. IR spectra were recorded by using JASCO FTIR-4100 spectrophotometer by KBr pellet method. ^{1}H NMR spectra were recorded on JEOL-400 MHz NMR instrument using $CDCl_3$/DMSO-d_6 as solvent. Chemical shift values were expressed in δ (ppm) relative to TMS as an internal standard. Mass spectra were recorded on Shimadzu LC-2010EV with ESI probe. The reaction progress was monitored by thin layer chromatography using silica gel G for TLC (Merck) plates and spots were visualized under ultraviolet light (254nm). Silica gel (60-120 mesh) was used for column chromatography. Animal experiments were done with the permission of the concerned authority.



a : R_1=H, R_2=H, R_3=H
b : R_1= NO_2, R_2= H, R_3=H
c : R_1=H, R_2=H, R_3=NO_2
d : R_1=Br, R_2=H, R_3=H
e : R_1=H, R_2= CH_3, R_3=H
f : R_1=H, R_2=F, R_3=F

SCHEME 1: SYNTHESIS OF SUBSTITUTED 1,2-BENZOXAZOLONE (8a-f) AND 3-CHLORO-1,2-BENZOXAZOLE (9a-f) DERIVATIVES

Detailed procedure for the synthesis of substituted 1,2-benzoxazolone and 3-chloro-1,2-benzoxazole derivatives:

Methyl 2-hydroxy benzoate (2):

2-Hydroxy benzoic acid, **1** (5g, 36 mmol) was dissolved in a mixture of DCM (30mL) and DMF (1mL) and stirred the mixture on ice bath until it reaches to 0°C. Oxalyl chloride (6.2mL) was added to the stirring mixture and stirring was continued for about 3 h. Then methanol (5.8mL) was added drop wise and kept stirring overnight at room temperature. The progress of the reaction was monitored by TLC using mobile phase pet ether: ethyl acetate (7:3). DCM was removed under vacuum and the solution was poured into the ice cold water to get gel type thick viscous liquid. The liquid was extracted to diethyl ether and dried over anhydrous sodium sulphate. Removal of solvent under vacuum gives the title compound as colorless liquid. The compounds **6e** and **6f** were similarly synthesized.

Methyl 2-hydroxy benzoate (2):
Colorless Viscous Liquid (Yield 94.5%); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 3.92 (s, 3H), 6.88 (t, J = 16.40 Hz, 1H), 6.98 (d, J = 8.00 Hz, 1H), 7.46 (t, J = 17.20 Hz, 1H), 7.84 (d, J = 10.00 Hz, 1H), 10.76 (s, 1H), IR (KBr, cm^{-1}) 3348.2 (OH), 2961.2 (CH_3), 1786.7 (C=O), 1631.5 (C=C aromatic),1340.4(C-O).

Methyl 2-hydroxy-4-methylbenzoate (6e):
The title compound was prepared according to the general procedure as described in **2**. Pale yellow Viscous Liquid (Yield 98.2%); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 2.34 (s, 3H), 3.89 (s, 3H), 5.34 (s, 1H), 6.90-7.76 (m, 3H), IR (KBr, cm^{-1}) 3334.6 (OH), 2981.2 (CH_3), 1756.3 (C=O), 1631.5 (C=C aromatic).

Methyl 3,4-difluoro-2-hydroxybenzoate (6f):
The title compound was prepared according to the general procedure as described in **2**. White Solid (Yield 84.2%); mp: 42-45 ^{o}C, ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 3.97 (s, 3H), 6.67-6.73 (m, 1H), 7.59-7.63 (m, 1H), 11.01 (s, 1H);. IR (KBr, cm^{-1}) 3357.5 (OH), 2961.2 (CH_3), 1786.7 (C=O), 1631.5 (C=C aromatic), 1340.4 (C-O).

Methyl-2-hydroxy-3-nitro benzoate (3) and methyl-2-hydroxy-5 nitro benzoate (4):
Methyl-2-hydroxyl benzoate, **2** (5.2 g, 36 mmol) in 25mL of acetic acid was taken in 250mL round bottom flask and stirred at 0°C. By maintaining the same temperature, nitrating mixture (6.6mL) was added drop wise and stirred the reaction mixture for about 2h at room temperature. After the completion of reaction, the mixture was poured into a crushed ice with stirring and filtered through Buckner funnel. The crude yellow mass obtained was washed with ice cold water and dried.

Crude compound was a mixture of methyl 2-hydroxy-3-nitro benzoate, **3** and methyl 2-hydroxy-5-nitro benzoate, **4** and was separated by column chromatography using silica gel (60-120 mesh). A mixture of crude products were loaded into the column using pet ether solvent and eluted with ethyl acetate/petroleum ether mixtures. Solvent fractions consisting of **3** and **4** were separately dried under reduced pressure to afford corresponding pure products.

Methyl 2-hydroxy-3-nitrobenzoate (3):
Yellow solid; mp: 124-126 °C,^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 3.89 (s, 3H), 5.35 (s, 1H), 7.78 (d, J = 404.00 Hz, 1H), 7.83 (t, J = 368.00 Hz, 1H), 7.80 (d, J = 416.00 Hz, 1H); IR (KBr, cm^{-1}) 3105.0 (OH), 1681.4 (C=O), 2919.5 (CH_3), 1620.6 (C=C aromatic).

Methyl 2-hydroxy-5-nitrobenzoate (4):
Pale Yellow Solid; (Yield 60%) mp:114-117^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 3.89 (s, 3H), 5.35 (s, 1H), 7.36 (d, J = 32.00 Hz, 1H), 8.33 (d, J = 20.00 Hz, 1H), 8.43 (s, 1H); IR (KBr, cm^{-1}) 3107.7 (OH), 1680.6 (C=O), 2919.7 (CH_3), 1622.8 (C=C aromatic).

Synthesis of methyl-2-hydroxy-5-bromobenzoate (5): Methyl-2-hydroxy benzoate, **2** (2 g, 131 mmol) was dissolved with stirring in 20mL of glacial acetic acid. 20% bromine in acetic acid was added to this mixture in small portions with stirring till the solution becomes pale yellow. The reaction mixture was allowed to stand for 30 min and then poured into ice cold water. The methyl-2-hydroxy-5-bromo benzoate (**5**) separates out and was filtered through Buchner funnel, washed with sodium bisulphite solution, water and then dried.

Methyl-2-hydroxy-5-bromo benzoate (5):
Pale Yellow Solid (Yield 90%); mp:122-125^{o}C; ^{1}H-NMR(400 MHz, $CDCl_3$): δ 3.98 (s, 3H), 6.88 (d, 1H), 7.53 (d, J = 12.00 Hz, 1H), 7.95 (d, J = 12.00 Hz, 1H), 10.68 (s, 1H); IR (KBr, cm^{-1}) 3154.3 (OH), 2961.2 (CH_3), 1786.7 (C=O), 1631.5 (C=C) (aromatic), 1340.4 (C-O).

N,2-dihydroxy-3-nitrobenzamide (7c):
Hydroxylamine hydrochloride (1.42 g, 20mmol) and KOH (2.29 g, 40.9mmol) were added to a stirring solution of 2-hydroxy-3-nitrobenzoate, 3 (1.0g, 513mmol) in 80 mL methanol. benzoate, **3** (1.0g, 513mmol) in 80mL methanol were added with stirring hydroxylamine hydrochloride (1.42 g, 20mmol) and KOH (2.29 g, 40.9mmol). The mixture was stirred for 4 h at room temperature. After the completion of reaction, solvent was removed under vacuum and added to ice cold water with stirring. The pH of the solution was adjusted to 2.0 using 1.5 N HCl and a pale yellow solid

obtained was filtered and dried. Compounds **7a, 7b** and **7(d-f)** were similarly synthesized.

N,2-dihydroxybenzamide (7a):
White Solid (Yield 82.9%); mp:202-205^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 5.35 (s, 1H), 6.95-7.86 (m, 4H), 11.68 (s, 1H), 13.12 (s, 1H); IR (KBr, cm^{-1}) 3327.6 (NH), 3156.3 (OH), 1700.8 (C=O, amide).

N,2-dihydroxy-5-nitrobenzamide (7b):
Pale Yellow Solid (Yield 73.8%); mp: 235-238^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.35 (s, 1H), 7.41 (d, J = 12.00 Hz, 1H), 8.37 (d, J = 20.00 Hz, 1H), 8.41 (s, 1H), 11.92 (s, 1H), 13.42 (s, 1H); IR (KBr, cm^{-1}) 3326.9 (NH), 3116.3 (OH), 1690.8 (C=O, amide).

N,2-dihydroxy-3-nitrobenzamide (7c):
Pale Yellow Solid (Yield 82.8%); mp: 215-218^{o}C; 1H-NMR(400 MHz, $CDCl_3$): δ 5.35 (s, 1H), 7.45-8.34 (m, 3H), 11.71 (s, 1H), 13.11 (s, 1H); IR (KBr, cm^{-1}) 3443.3 (NH), 1681.6 (C=O, amide), 3323.7 (OH, aromatic), 1619.9 (C=C, aromatic), 3082.6 (C-H, aromatic).

5-bromo-N,2-dihydroxybenzamide (7d):
Pale Yellow Solid (Yield 78.9%); mp: 239-241^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 5.35 (s, 1H), 7.12 (d, J = 80.00 Hz, 1H), 7.70 (d, J = 16.00 Hz, 1H), 8.05 (s, 1H), 11.68 (s, 1H), 13.12 (s, 1H); IR (KBr, cm^{-1}) 3296.9 (NH), 3016.3 (OH), 1690.8 (C=O, amide).

N,2-dihydroxy-4-methylbenzamide (7e):
Pale Yellow Solid (Yield 80.3%); mp: 222-225^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 2.34 (s, 3H), 5.35 (s, 1H), 6.99 (d, J = 12.00 Hz, 1H), 7.77 (d, J = 20.00 Hz, 1H), 7.54 (s, 1H), 11.52 (s, 1H), 13.01 (s, 1H); IR (KBr,cm^{-1}) 3356.8 (NH), 3156.7 (OH), 1730.3 (C=O, amide).

3,4-difluoro-*N*,2-dihydroxybenzamide (7f):
White Solid (88.32%); mp:165-168^{o}C, ^{1}H-NMR (400 MHz, DMSO-*d6*): δ (ppm) 6.92-6.99 (m, 1H), 7.51-7.55 (m, 1H), 9.54 (s, 1H), 11.68 (s, 1H), 13.12 (s, 1H); IR (KBr, cm^{-1}) 3316.9 (NH), 3136.6 (OH), 1730.8 (C=O, amide), 1503.2 (C-C, aromatic).

1,2-benzoxazol-3(2*H*)-one (8a):
N,2-dihydroxybenzamide, **7a** (0.68 g, 4.2 mmol) in THF (5.46 mL) was taken in a round bottom flask and stirred on magnetic stirrer until it reaches to 65°C. CDI (1.2 g, 7.6 mmol) dissolved in 4.0 mL of THF was added slowly at the same temperature and continued to stirring for further 3 hr. After completion of the reaction, solvent was removed under pressure and added to ice cold water with stirring and acidify with 1.5 N HCL till solid formed. Buff color solid obtained was collected by filtration and dried. The compounds **8(b-f)** were similarly synthesized.

1,2-benzoxazol-3(2*H*)-one (8a):
White Solid (Yield 76.8%); mp:187-191^{o}C, ^{1}H-NMR (400 MHz, $CDCl_3$): δ(ppm) 7.13-7.86 (m, 4H), 8.00 (s, 1H); IR (KBr, cm^{-1}) 1702.5 (C=O), 3117.2 (NH), 1652.8 (C=C, aromatic).

5-Nitro-1,2-benzoxazole-3(2H)-one (8b):
Pale Yellow Solid (Yield 73.5%); mp182-185^{o}C; ^{1}H-NMR(400 MHz, DMSO-d6): δ (ppm) 7.08 (d, J = 9.20 Hz, 1H), 8.28 (d, J = 9.20 Hz, 1H), 8.56 (s, 1H), 13.04 (s, 1H); IR (KBr, cm^{-1}) 1680.6 (C=O), 3107.2 (NH), 1622.8 (C=C, aromatic).

7-Nitro-1,2-benzoxazole-3(2H)-one (8c):
Pale Yellow Solid (Yield 76.8%); mp:174-179^{o}C; ^{1}H-NMR(400 MHz, DMSO-d6): δ (ppm) 7.57 (t, J = 16.00 Hz, 1H), 8.23 (d, J = 8.00 Hz, 1H), 8.47 (d, J = 8.00 Hz, 1H), 13.11 (s, 1H); IR (KBr, cm^{-1}) 1746.2 (C=O), 3108.7 (NH), 1623 (C=C, aromatic).

5-bromo-1,2-benzoxazol-3(2H)-one (8d):
Pale Buff Solid (Yield 76.8%); mp:181-183^{o}C; ^{1}H-NMR(400 MHz, $CDCl_3$): δ (ppm) 7.24 (s, 1H), 7.64 (dd, J = 32.00, Hz, 2H); IR (KBr, cm^{-1}) 1696.2 (C=O), 3128.7 (NH), 1643.2 (C=C, aromatic).

6-Methyl-1,2-benzoxazole-3(2H)-one (8e):
White Solid (Yield 70.6%); mp:123-126^{o}C; ^{1}H-NMR(400 MHz, $CDCl_3$): δ (ppm) 2.51 (s, 3H), 6.96 (d, J = 0.80 Hz, 1H), 7.12-7.14 (m, 1H), 7.66 (d, J = 8.00 Hz, 1H), 7.97 (d, J = 8.00 Hz, 1H), IR (KBr, cm^{-1}) 2967.0 (C-H), 1764.0 (C=O), 1257.3 (C-N), 801.2 (C-H, aromatic).

6,7-difluoro-1,2-benzoxazol-3(2H)-one (8f):
White Solid (Yield 82.43%); mp: 147-150^{o}C; ^{1}H-NMR (400 MHz, DMSO-*d6)*: δ (ppm) 7.39-7.46 (m, 1H), 7.59-7.62 (m, 1H), 12.90 (s, 1H); Mass m/z: 170.2; IR (KBr.cm^{-1}) 1645.9 (C=O), 3015.1 (NH), 1477.2 (C=C, aromatic).

3-chloro-1,2-benzoxazole (9a):
$POCl_3$ (0.68 g, 4.4 mmol) and triethylamine (0.14 g, 1.4 mmol) were added to an ice cold mixture of 1,2-benzoxazol-3(2H)-one, 8a (0.2 g, 1.4 mmol)and the mixture was heated at 140°C in a sealed tube for 2h. Reaction mass was cooled to room temperature and then poured slowly into crushed ice with vigorous stirring. The brown color solid obtained was filtered, washed and dried. The compounds **9(b-f)** were similarly synthesized.

3-chloro-1,2-benzoxazole (9a):
Brown Solid (54.1%); mp: 56-59^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 7.39 (d, 1H), 7.74 (t, 1H), 8.10 (t, Hz, 1H), 8.42 (d, 1H); ESI MS (*m/z*) 153.4 (M^+); IR (KBr, cm^{-1}) 727.7 (C-Cl), 1623.02 (C=C, aromatic), 3115.7 (CH, aromatic).

3-Chloro-5-nitro-1,2-benzoxazole (9b):
Brown Solid (54.1%); mp 64-68^{o}C,^{1}H-NMR(400 MHz, $CDCl_3$): δ 7.76 (d, J = 11.20 Hz, 1H), 8.56 (d, J = 2.00 Hz, 1H), 8.68 (s, 1H)**,** ESI MS (*m/z*) 198.3 (M^+); IR (KBr, cm^{-1}) 734.74 (C-Cl), 1617.02 (C=C, aromatic), 3106.76 (CH, aromatic).

3-Chloro-7-nitro-1,2-benzoxazole (9c):
Brown Solid (64.40%); mp 74-77^{o}C,^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.62 (t, J = 16.00 Hz, 1H), 8.07 (dd, J = 8.80, Hz, 1H), 8.52 (dd, J = 8.80, Hz, 1H), ESI MS (*m/z*) 198.3 (M^+); IR (KBr, cm^{-1}) 742.32 (C-Cl), 1620.66 (C=C, aromatic), 3076.92 (CH, aromatic).

5-bromo-3-chloro-1,2-benzoxazole (9d):
Brown Solid (61.59%); mp 43-46^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.24 (s, 1H), 7.64 (dd, J = 32.00, Hz, 2H), ESI MS (*m/z*) 232.9 (M^+); IR (KBr, cm^{-1}) 722.42 (C-Cl), 1640.56 (C=C, aromatic), 3106.22 (CH, aromatic).

3-chloro-6-methyl-1,2-benzoxazole (9e): Brown Solid (68.67%) mp 49-52^{o}C; ^{1}H-NMR(400 MHz, $CDCl_3$): δ 2.34 (s, 3H), 7.23-7.45 (m, 3H), ESI MS (*m/z*) 166.9 (M^+); IR (KBr, cm^{-1}) 729.5 (C-Cl), 1640.7 (C=C, aromatic), 3092.1 (CH, aromatic).

3-chloro-6,7-difluoro-1,2-benzoxazole (9f):
Brown Solid (58.62%) mp 35-38^{o}C; ^{1}H-NMR(400 MHz, $CDCl_3$): δ 7.25-7.31 (m, 1H), 7.41-7.45 (m, 1H); ESI MS (*m/z*) 189.5 (M^+); IR (KBr, cm^{-1}) 757.88 (C-Cl), 1645.95(C=C, aromatic), 3095.19 (CH, aromatic).

Analgesic activity evaluation:
The analgesic activity was determined using acetic acid induced writhing in rats. Sprague-Dawley rats (100 to150 g) either sex, were randomly divided into fourteen groups of each containing five and fasted overnight before the experiment with free access to water. The test samples were administered orally at the dose of 5mg/kg, prior to the injection of 0.1mL acetic acid (1% v/v) solution intraperitoneally. All the test samples were suspended in saline solution. The first group received saline as a control, second group received diclofenac sodium as a standard drug, and rest of the groups received test samples. After the acetic acid injection, number of writhes and extension of hind limbs as well as the number of animals showing such response during a period of 10min was recorded and analyzed. The analgesic activity was expressed in % inhibition.

% Analgesic activity = [(Normal activity - Inhibited activity) / (Normal activity)] x 100.

Anti-inflammatory activity evaluation:
The anti-inflammatory activity was determined using a carrageenan-induced paw edema model in Sprague-Dawley rats (100-150 g) of either sex. The animals were housed under standard conditions of temperature (25±2ºC) and 12 hr/12 hr light/dark cycles for one week before start of the experiments and allowed food and water *ad libitum*.

The animals were randomly divided into fourteen groups of each containing five and fasted overnight before the experiment with free access to water. The test samples were administered at the dose of 5 mg/kg, one hour prior to the subcutaneous injection of carrageenan (0.1mL of 1% w/v in Normal Saline Solution) into the plantar surface of the left hind paw. The group I received saline as control, group

II received standard drug diclofenac sodium, and all other groups received the test samples. After the carrageenan injection, the paw volumes were measured at 1,3 and 6 h using a pleythysmometer (Dolphin, India). Edema was expressed as the mean increase in paw volume relative to control animals. The percentage inhibition of edema was calculated by the following equation:

% Inhibition of edema = (Vc-Vt/Vc) x100,

Where Vc is the edema volume in the control group and Vt is the edema volume in the tested group.

RESULTS AND DISCUSSION: Preparation of substituted 1,2-benzoxazol-3(2*H*)-ones and 3-chloro-1,2-benzoxazoles from salicylic acid (**1**) and its analogues has been described in **Scheme 1**. The compound 2-hydroxy benzoic acid (**1**) was first converted into its corresponding ester, methyl 2-hydroxy benzoate (**2**) and then subjected to nitration and bromination. Nitration of **2** gives intermediates methyl 2-hydroxy-3-nitro benzoate (**3**) and methyl 2-hydroxy-5-nitro benzoate (**4**) whereas bromination of **2** resulted in exclusively single major compound methyl 2-hydroxy-5-bromo benzoate (**5**) in 90% yield. The resulted esters (**6a-f**) were converted into corresponding N,2-dihydroxybenzamide derivatives (**7a-f**) using hydroxyl amine hydrochloride and KOH in methanol[23]. Cyclization of the benzamides using CDI[24] in THF gives 1,2-benzoxazol-3(2*H*)-one derivatives (**8a-f)** which subsequently chlorinated using $POCl_3$ and triethyl amine in sealed tube condition at 140 °C to get 3-chloro 1,2-benzoxazoles (**9a-f**).

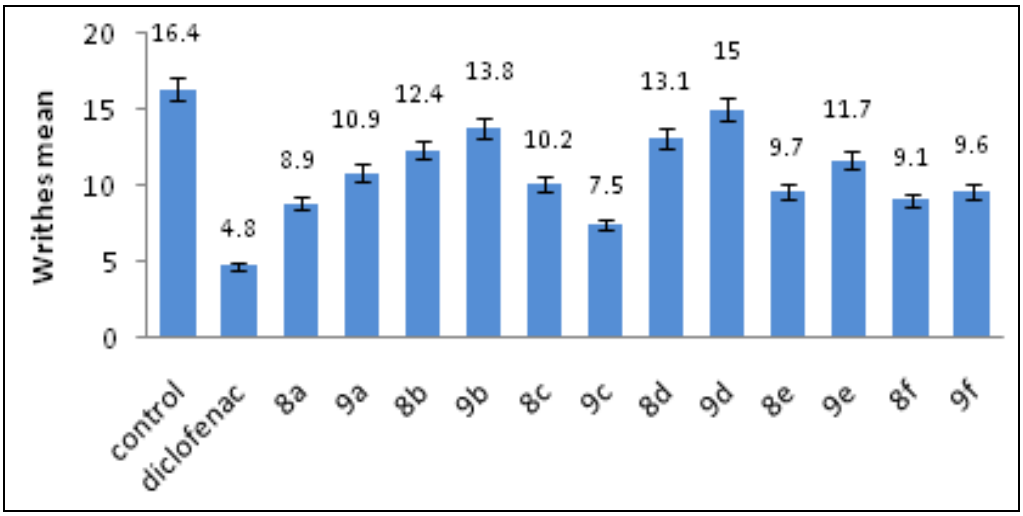


FIG. 1: ACETIC ACID INDUCED WRITHING TEST OF COMPOUNDS 8(a-f) AND 9(a-f).

For the synthesis of 6-methyl-1,2-benzoxazole-3(2*H*)-one (**8e**) and 6,7-difluoro-1,2-benzoxazole-3(2*H*)-one (**8f**), the starting materials 4-metylsalicylic acid and 3,4-difluorosalicylic acid were employed respectively. A detailed procedure for all the synthesized compounds is given in experimental section. The intermediates and final compounds were characterized using IR, ^{1}H-NMR and Mass spectral analysis.

Title compounds were subjected to *in-vivo* analgesic and anti-inflammatory activity studies. Acetic acid induced writhing in rats [25] method was followed for the determination of analgesic activity and carrageenan-induced paw edema model [26] was followed for anti-inflammatory activity. The results are expressed as means ± S.E.M differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.05$. In case of analgesic activity, the entire test samples were shown significant percentage inhibition in acetic acid induced writhing test in mice. The compounds 1,2-benzoxazol-3(2H)-one **8a** and 3-chloro-7-nitro-1,2-benzoxazole **9c** showed good analgesic activity of about 45% (writhing mean 8.9) and 54% (writhing mean 7.5) inhibition respectively. The analgesic activity data is depicted in **Fig.1**. In general the 1,2-benzoxazolones show higher activity than the corresponding 3-chloro-1,2-benzoxazole analogues.

TABLE 1: ANTI-INFLAMMATORY ACTIVITY OF THE SYNTHESIZED COMPOUNDS [a] 8(a-f) AND 9(a-f).

Compounds	Change in paw volume (mL) Mean±SEM (% inhibition)		
	1 hr	3 hr	6 hr
control	0.49±0.02	0.54±0.02	0.62±0.03
Diclofenac	0.21±0.01 (57.1)	0.22±0.02 (59.6)	0.18±0.02 (71.0)
8a	0.28±0.02 (42.9)	0.28±0.01 (48.1)	0.29±0.01 (53.2)
9a	0.31±0.03 (36.7)	0.35±0.01 (35.2)	0.32±0.02 (48.4)
8b	0.25±0.01 (49.0)	0.35±0.01 (35.2)	0.21±0.01 (66.1)
9b	0.29±0.02 (40.8)	0.32±0.02 (40.7)	0.21±0.02 (66.1)
8c	0.38±0.01 (22.4)	0.40±0.01 (25.9)	0.39±0.02 (37.1)
9c	0.26±0.03 (46.9)	0.30±0.02 (44.4)	0.23±0.01 (62.9)
8d	0.29±0.01 (40.8)	0.30±0.02 (44.4)	0.32±0.02 (48.4)
9d	0.33±0.02 (32.7)	0.32±0.02 (40.7)	0.30±0.02 (51.6)
8e	0.31±0.02 (36.7)	0.31±0.01 (42.6)	0.37±0.01 (40.32)
9e	0.25±0.02 (49.0)	0.27±0.01 (50.00)	0.24±0.01 (61.3)
8f	0.29±0.02 (40.8)	0.27±0.01 (50.00)	0.24±0.01 (61.3)
9f	0.32±0.01 (34.7)	0.33±0.01 (38.9)	0.30±0.02 (51.6)

[a] Drug dosage 5 mg/kg; The inhibition of edema shown in %

In case of anti-inflammatory activity, edema developed following injection of carrageenan serves as an index of acute inflammatory changes determined from differences in the paw volume measured immediately after carrageenan injection and then every hour for 6 hours. Edema induced by carrageenan is believed to be biphasic: the first phase (1h) involves the release of serotonin and histamine and the second phase (over 1h) is mediated by prostaglandins, cyclooxygenase products. Continuity between the two phases is provided by kinins. Results showed that all the test samples were shown good percentage inhibition in Carrageenan-induced paw edema model in rats. However compounds like 5-nitro-1,2-benzoxazol-3(2H)-one, **8b** and 3-chloro-5-nitro-1,2-benzoxazole, **9b** were found to be show significant anti-inflammatory activity compared to other derivatives. Unlike analgesic activity there is no general trend is observed here. The anti-inflammatory activity data is reported in **Table 1**.

CONCLUSION: In summary, we have synthesized **5, 6** and **7** substituted 1,2-benzoxazolone and 3-chloro-1,2-benzoxazole derivatives from salicylic acid and its analogues. Synthesized compounds were found to be show prominent analgesic and anti-inflammatory activities.

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