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Anti-inflammatory evaluation of isoxazole derivatives

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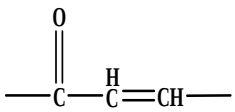
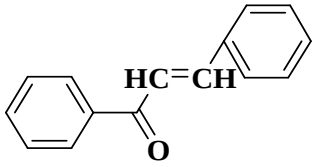
ABSTRACT

A new series of isoxazole derivatives were synthesized by synthesizing various chalcone derivatives and cyclizing them to give resultant isoxazole derivatives (TPI1- TPI20).. All the synthesized derivatives were characterized with FTIR and NMR spectral analysis and evaluated for their anti-inflammatory activity in vivo (carrageenan induced paw ooedema method in rats). The isoxazole derivatives ***TPI-7*** *and* ***TPI-13*** *were found to be the most active compounds due to the presence of the methoxy group at para position.. In future study, further investigation on the mechanism of action of some of our compound may reveal new compounds with potent anti-inflammatory action. The structures of the newly synthesized compounds were elucidated by using IR,* ^{1}H *NMR.*

INTRODUCTION

Inflammation is a complex biological process in which the body's immune cells provide protection from injury, infection and foreign substances. It is a protective attempt, a defense mechanism by the body to remove the injurious substances and to initiate the healing process [1]. The chalcones are open chain flavonoids where two rings are joined by 3-carbon α-β unsaturated carbonyl system. The chalcone compounds are more biologically active due to the presence of α, β-unsaturated carbonyl system. Chalcones and their derivatives have been reported to possess many useful biological activities such as antibacterial, antifungal, antiviral, antioxidant, anti-tubercular, insecticidal, antiprotozoal, nitric oxide inhibition, ulcerogenic, anti-inflammatory, anticancer and antihyperglycemic etc [2].





The chalcones are also known as benzylidene acetophenones or benzal acetophenones. These re colored compounds because of the presence of the chromospheres -CO-CH=CH [3]. Chalcones are used to synthesize different heterocyclic ring systems like isoxazoles, cyanopyridines, pyrimidines and pyrazolines [4]. Isoxazoles known to have broad spectrum of pharmacological and biological activities which include anti-HIV [5], GABA antagonist [6], anticancer [7], antinociceptive [8], antithrombotic [9], antifungal [10], antibacterial [11], dopamine D4 receptors antagonist [12], immunomodulatory [13]. Inspired by the above facts, we planned to synthesize some more derivatives of isoxazole and evaluated their anti- inflammatory potential.

MATERIALS AND METHODS

Chemistry

All the chemicals and reagents were of analytical grade and were used without further purification. Melting points were determined by capillary tube method and are uncorrected. All the reaction and purity of synthesized

compounds was deduced by thin layer chromatography(TLC) using silica-G plate. The plates were developed by exposing to the iodine vapours. Infrared spectra were recorded by perkin elmer spectrophotometer using KBr pellets. Proton nuclear magnetic resonance spectra were recorded on BRUKER AVANCE ÌÌ 400 NMR spectrophotometer.

Synthesis of 2, 4, 5-trisubstituted-1*H*-imidazole derivatives (4)

The synthesis of 2,4,5-trisubstituted-1*H*-imidazoles **(4)** was carried out by refluxing benzil (**1)** (10mmol) with different aromatic aldehydes (**2)** (12mmol**)** in the presence of ammonium acetate (**3)** (40mol) and sulphanilic acid (10mol%, 1.7gm) catalyst in ethanol (20mL) in round bottom flask at 80°C for 2hours. The completion of reaction was checked by thin layer chromatography (TLC) using solvent system having toluene, ethyl acetate and formaldehyde in ratio 4:4:2. Then reaction mixture was cooled to room temperature and poured on ice-cold water (50mL) to get the solid precipitated out. It was collected by filtration, washed with cold water and recrystallized with ethanol [14].

Synthesis of acetylated 2,4,5-trisubstituted-1*H* imidazole derivatives (5)

The synthesis of acetylated 2,4,5-trisubstituted-1*H*-imidazoles **(5)** was carried out by refluxing mixture of 0.0M of **(4)** and 0.01M of chloroacetone into a 250mL round bottom flask. Then 150mL of dry acetone and 30g of anhydrous potassium carbonate were added and the reaction mixture was refluxed for 6h at 75°C. Filtrate obtained was concentrated under vacuum. The product **(5)** was dried and recrystallized from acetone. The purity of the compound was checked by TLC and melting point [15].

Synthesis of chalcone derivatives (6)

In a flat bottom flask, placed a solution of sodium hydroxide (0.013mol) in 5ml of water and 3ml of ethanol and then placed the flask on the stirrer. Immersed the flask in a bath of crushed ice and acetylated 2, 4, 5-trisubstituted-1*H*-imidazolyl (0.01mol) and substituted benzaldehyde (0.01mol) were added. The mixture was stirred for 12-15 hr. until the entire mixture becomes very cloudy or thick. The mixture was kept in refrigerator overnight. Then the mixture was poured slowly into the water with the constant stirring, and acidified with dil HCl. Then precipitate was obtained, filtered, washed and recrystallized from ethanol. The formation of the chalcone derivative was then confirmed by performing TLC using Hexane: Ethyl acetate (8:2 v/v) as mobile phase [16].

Sythesis of isoxazole derivatives by cyclization (7)

A mixture of chalcone **6** (0.02mol) and hydroxylamine hydrochloride (0.02mol) were dissolved in 25ml of ethanol, then 0.01mol of sodium acetate was added and the mixture was heated under reflux for 6hr. The mixture was concentrated by distilling out the solvent under reduced pressure and poured into ice water. Then, precipitate obtained was filtered, washed and recrystallized from ethanol. The completion of reaction was confirmed by TLC using chloroform: methanol (9:1 v/v) as mobile phase [17].

Table 1 Physicochemical characterization of synthesized compounds

Sr. No.	Product Code	Mol. Formula	Mol. Wt.	M.P(˚C)	Rf Value	% Yield
1	**TPI-1**	$C_{33}H_{27}N_3O_2$	497.59	220-223	0.62	55.41
2	**TPI-2**	$C_{33}H_{26}N_4O_5$	558.58	217-219	0.53	59.62
3	**TPI-3**	$C_{33}H_{27}N_3O_3$	513.59	225-228	0.48	65.72
4	**TPI-4**	$C_{34}H_{30}N_4O$	510.63	205-207	0.59	62.34
5	**TPI-5**	$C_{33}H_{27}N_5O_3$	541.6	227-229	0.64	66.41
6	**TPI-6**	$C_{32}H_{24}BrN_3O$	546.46	224-226	0.45	58.05
7	**TPI-7**	$C_{34}H_{29}N_3O_5$	559.61	213-216	0.51	73.22
8	**TPI-8**	$C_{32}H_{25}N_3O_2$	483.56	219-221	0.43	63.64
9	**TPI-9**	$C_{32}H_{24}ClN_3O$	502.01	210-214	0.47	61.30
10	**TPI-10**	$C_{32}H_{24}ClN_3O_3$	534	221-223	0.67	65.09
11	**TPI-11**	$C_{34}H_{30}N_4O_2$	526.63	225-227	0.57	57.87
12	**TPI-12**	$C_{32}H_{24}ClN_3O_2$	518	216-219	0.52	54.98
13	**TPI-13**	$C_{36}H_{33}N_3O_6$	603.66	213-215	0.65	64.32
14	**TPI-14**	$C_{34}H_{28}ClN_3O_4$	578.06	207-210	0.44	68.26
15	**TPI-15**	$C_{35}H_{31}N_3O_4$	557.64	226-228	0.58	71.39
16	**TPI-16**	$C_{31}H_{21}N_5O_5$	543.53	221-224	0.52	67.85
17	**TPI-17**	$C_{32}H_{24}N_4O_5$	544.56	215-218	0.63	66.83
18	**TPI-18**	$C_{31}H_{22}FN_3O_2$	487.52	222-225	0.49	60.94
19	**TPI-19**	$C_{32}H_{24}FN_3O_2$	501.55	209-212	0.50	59.77
20	**TPI-20**	$C_{33}H_{27}BrN_4O$	575.5	214-216	0.68	60.68

Spectral data of synthesized isoxazole compounds

2-(4-methoxyphenyl)-4, 5-diphenyl-1-((5-p-tolylisoxazol-3-yl) methyl)-1*H*-imidazole (TPI-1): IR (KBr Pellets) cm^{-1}: 3006(-C-H), 3115(=C-H aromatic), 1605(C=N *str*.), 1253(N-O *str*.), 1146(C-O *str*.). **^{1}H-NMR (DMSO)δ**

ppm: 7.24-7.59 (14H, m, rest of aromatic proton on benzene ring), 6.85-7.12 (4H, m, H-3", H-5", H-3"', H-5"'), 6.74 (1H, s, isoxazole), 4.82 (2H, s, imidazole-N-CH_2-isoxazole ring), 3.76 (3H, s, -OCH_3) and 2.34 (3H, s, -CH_3)

2-(3, 5-dimethoxyphenyl)-1-((5-(4–nitrophenyl) isoxazol-3-yl)methyl)-4, 5-diphenyl-1*H*-imidazole (TPI-2): IR (KBr Pellets) cm^{-1}: 3120(=C-H aromatic), 3010(-C-H), 1611(C=N *str*.), 1358, 1541(NO_2), 1341(C-O *str*.), 1267(N-O *str*.). **^{1}H-NMR (DMSO)δ ppm:** 7.22-7.52(14H, m, rest of aromatic proton on benzene ring), 6.89-7.09(2H, H-3"', H-5"'), 5.04(2H, s, imidazole-N-CH_2-isoxazole ring), 6.76(1H, s, isoxazole), 3.88(3H, s, -OCH_3)

4-(4, 5-diphenyl-1-((5-p-tolylisoxazol-3-yl)methyl)-1*H*-imidazole-2-yl)-2-methoxyphenol (TPI-3): IR (KBr Pellets) cm^{-1}: 3438-3311(OH str., br), 3106(=C-H aromatic), 3015(-C-H), 1602(C=N *str*.), 1245(N-O *str*.), 1237(C-O *str*.).**^{1}H-NMR (DMSO)δ ppm:** 7.24-7.59(14H, m, rest of aromatic proton on benzene ring), 6.95-7.17(3H, H-5", H-3"', H-5"'), 6.74-6.77(1H, s, isoxazole), 4.82-5.19(2H, s, imidazole-N-CH_2-isoxazole ring), 3.71(3H, s, -$0CH_3$),5.78(-OH) and 2.39(3H, s, -CH_3)

4-(4, 5-diphenyl-1-((5-p-tolylisoxazol-3-yl) methyl)-1*H*-imidazole-2-yl)-N, N-dimethylbenzenamine (TPI-4): IR (KBr Pellets) cm^{-1}: 3124(=C-H aromatic), 3013(-C-H), 1601(C=N *str*.), 1260(N-O *str*.), 1251(C-O *str*.). **^{1}H-NMR (DMSO)δ ppm:** 7.24-7.59(14H, m, rest of aromatic proton on benzene ring), 6.95-7.17(4H, H-3", H-5",H-3"',H-5"'), 6.74-6.77(1H, s, isoxazole), 4.82-5.19(2H, s, imidazole-N-CH_2-isoxazole ring), 2.98(6H, s, -$N(CH_3)_2$ and 2.45(3H, s, -CH_3).

N, N-dimethyl-4-(1-((5-(4-nitrophenyl)isoxazol-3-yl)methyl)-4, 5-diphenyl-1*H*-imidazole-2-yl)benzenamine (TPI-5): IR (KBr Pellets) cm^{-1}: 1350, 1543(OH *str*., br.), 3118(=C-H aromatic), 3014(-C-H), 1615(C=N *str*.), 1255(N-O *str*.), 1252(C-O *str*.).

1-((5-(3-bromophenyl) isoxazol-3-yl)methyl)-4, 5-diphenyl-2-*p*-tolyl-1*H*-imidazole (TPI-6): IR (KBr Pellets) cm^{-1}: 3123(=C-H aromatic), 3015(-C-H), 1597(C=N *str*.), 1278(N-O *str*.), 1238(C-O *str*.), 677(C-Br).

3-(4, 5-diphenyl-1-((5-(3, 4, 5-trimethoxyphenyl) isoxazole-3-yl) methyl)-1*H*-imidazole-2-yl)phenol (TPI-7): IR (KBr Pellets) cm^{-1}: 3447-3292(OH *str*., Br), 3120(=C-H aromatic), 3013(-C-H), 1615(C=N *str*.), 1271(N-O *str*.), 1256(C-O *str*.).

2-(4, 5-diphenyl-1-((5-*p*-tolylisoxazole-3-yl)methyl)-1*H*-imidazole-2-yl) phenol (TPI-8): IR (KBr Pellets) cm^{-1}: 3449-3298(OH *str*., br.), 3128(=C-H aromatic), 3008(-C-H), 1612(C=N *str*.), 1261(C-O *str*.), 1239(N-O *str*.).

2-(4-chlorophenyl)-4, 5-diphenyl-1-((5-*p*-tolylisoxazole-3-yl)methyl)-1*H*-imidazole (TPI-9): IR (KBr Pellets) cm^{-1}: 3130(=C-H aromatic), 3018(-C-H), 1618(C=N *str*.), 1254(C-O *str*.), 1248(N-O *str*.), 779(C-Cl).

4-(3-((2-(3-chlorophenyl)-4, 5-diphenyl-1*H*-imidazole-1-yl)methyl) isoxazole-5-yl)-2-methoxyphenol (TPI-10): IR (KBr Pellets) cm^{-1}: 3429-3320(OH *str*., br.), 3134(=C-H aromatic), 3009(-C-H), 1601(C=N *str*.), 1236(C-O *str*.), 1232(N-O *str*.), 782(C-Cl).

4-(3-((2-(4-methoxy)-4, 5-diphenyl-1*H*-imidazol -1-yl)methoxy) isoxazole-5-yl)-*N*, *N*-dimethylbenzenamine (TPI-11): IR (KBr Pellets) cm^{-1}: 3124(=C-H aromatic), 3012(-C-H), 1256(C-O *str*.), 1240(N-O *str*.), 1611(C=N *str*.).

1-((5-(4-chlorophenyl) isoxazol-3-yl) methyl)-2-(4-methoxyphenyl)-4, 5-diphenyl-1*H*-imidazole(TPI-12): IR (KBr Pellets) cm^{-1}: 3132(=C-H aromatic), 3001(-C-H), 1657(C=N *str*.), 1267(N-O *str*.), 1236(C-O *str*.), 782 (C-Cl).

2-(3, 5-dimethoxyphenyl)-4, 5-diphenyl-1-((5-(3,4,5-trimethoxyphenyl)isoxazol-3-yl)methyl)-1*H*-imidazole (TPI-13): IR (KBr Pellets) cm^{-1}: 3116(=C-H aromatic), 3018(-C-H), 1619(C=N *str*.), 1257(C-O *str*.), 1254(N-O *str*.).

1-((5-(4-chlorophenyl) isoxazol-3-yl)methyl)-4, 5-diphenyl-2-(3, 4, 5-trimehoxyphenyl)-1*H*-imidazole (TPI-14): IR (KBr Pellets) cm^{-1}: 3127(=C-H aromatic), 3019(-C-H), 1601(C=N *str*.), 1255(C-O *str*.), 1226(N-O *str*.), 782(C-Cl).

4, 5-diphenyl-1-((5-*p*-tolylisoxazol-3-yl)methyl)-2-(3, 4, 5-trimethoxyphenyl)-1*H*-imidazole (TPI-15): IR (KBr Pellets) cm^{-1}: 3134(=C-H aromatic), 3021(-C-H), 1589(C=N *str*.), 1268(N-O *str*.), 1237(C-O *str*.).

2-(2,4-dinitrophenyl)-4,5-diphenyl-1-((5-phenylisoxazol-3-yl)methyl)-1*H*-imidazole (TPI-16): IR (KBr Pellets) cm^{-1}: 3123(=C-H aromatic), 3022(-C-H), 1592(C=N *str*.), 1362, 1553(NO_2)1239(C-O *str*.), 1236(N-O *str*.).

2-methoxy-4-(3-((2-(4-nitrophenyl)-4, 5-diphenyl-1*H*-imidazol-1-yl) methyl) isoxazol-5-yl) phenol (TPI-17): IR (KBr Pellets) cm^{-1}: 3427-3291(OH *str*., br.), 3108(=C-H aromatic), 3010(-C-H), 1612(C=N *str*.), 1345, 1558(NO_2), 1259(C-O *str*.), 1221(N-O *str*.).

2-(3-((2-(4-fluorophenyl)-4, 5-diphenyl-1*H*-imidazol-1-yl)methyl)isoxazol-5-yl)phenol (TPI-18): IR (KBr Pellets) cm^{-1}:. 3445-3311(OH *str*., br.), 3136(=C-H aromatic), 2989(-C-H), 1616(C=N *str*.), 1253(C-O *str*.), 1249(N-O *str*), 976 (C-F).

2-(4-fluorophenyl)-1-((5-(4-methoxyphenyl)isoxazol-3-yl)methyl)-4,5-diphenyl-1*H*-imidazole (TPI-19): IR (KBr Pellets) cm^{-1}: 3115(=C-H aromatic), 2990(-C-H), 1614(C=N *str*.), 1256(C-O *str*.), 1224(N-O *str*.), 960 (C-F).

4-(3-((2-(3-bromophenyl)-4, 5-diphenyl-1*H*-imidazol-1-yl)methyl) isoxazol-5-yl)-N, N-dimethylbenzenamine (TPI-20): IR (KBr Pellets) cm^{-1}: 3127(=C-H aromatic), 3021(-C-H), 1611(C=N *str*.), 1259(C-O *str*.), 1251(N-O *str*.), 682(C-Br).

ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory activity of the synthesized compounds was carried out *in vivo* by the carrageenan- induced rat paw edema method. In this the Nimesulide was used as the standard drug for evaluation of anti-inflammatory activity. Carrageenan induced paw edema in an important method in assessing the contribution of mediators involved in vascular changes associated with acute inflammation. It based upon the ability of agents to inhibit the edema produced in the hind paw of the rat after injection of a phlogiston agent. Many phlogiston agents employed for this study such as brewer's yeast, egg albumin, kaolin, dextran, formaldehyde and sulfated polysaccharides like carrageenan. Many methods have been described how to measure the paw volume by simple and less accurate and by more sophisticated electronically devised method. It has been documented that carrageenan induced rat paw edema is a simple and suitable method to predict the value of anti-inflammatory agents, which act by inhibiting the mediators of acute inflammation [19].

ANIMAL PROTOCOL

Adult Wistar albino rats weighing around 165-220g weight procured from the Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary Sciences (LUVAS), Hisar, Haryana (INDIA). They were housed in a natural light-dark cycle (12 h each) and controlled conditions of temperature. Water boiled wheat porridge (dalia) was administered to the animals as food. Experiment was carried out between 09:00am to 5:00pm. Separate groups of rats were used for each set of experiment which was performed in the Animal House G.J.U.S & T Hissar. The experimental protocol was approved by the Institutional Animals Ethical Committee (IAEC). The care of animals was taken as per guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (Registration No. 0436).

CARRAGEENAN INDUCED PAW EDEMA MODEL EMPLOYED FOR ANTI-INFLAMMATORY ACTIVITY

Drugs: Carrageenan (1%w/v solution and injected 0.1ml underneath the plantar region). Nimesulide (dose 50 mg/kg i.p. prepared a stock solution containing 10 mg/ml of drug and injected 0.5 ml/100 g of the body weight of the animal) and test drug (dose 100mg/kg orally) [20]**.**

- ❖ Weighed and numbered the albino rat of either sex.
- ❖ Mark was made on the hind paw just beyond the tibio-tarsal junction & the rat were divided into different groups, each group containing six rat individuals and the initial paw volume was noted.
- ❖ Control group received the solvent, standard group received the drug Nimesulide suspended in the solvent and the other groups received the test compounds suspended in the solvent.
- ❖ Thirty minutes later, rats were challenged by subcutaneous injection of 0.05 ml of 1 % solution of carragenan into plantar side of hind paw. The paw volume was measured by Plethysmometer (initial paw volume) after injection at 30, 60, 120 and 180 minutes interval.
- ❖ Ten compounds were evaluated for anti-inflammatory activity simultaneously using 10 different animals groups. These animals were reused after one week for the rest of ten other compounds.

% inhibition was calculated by the formula

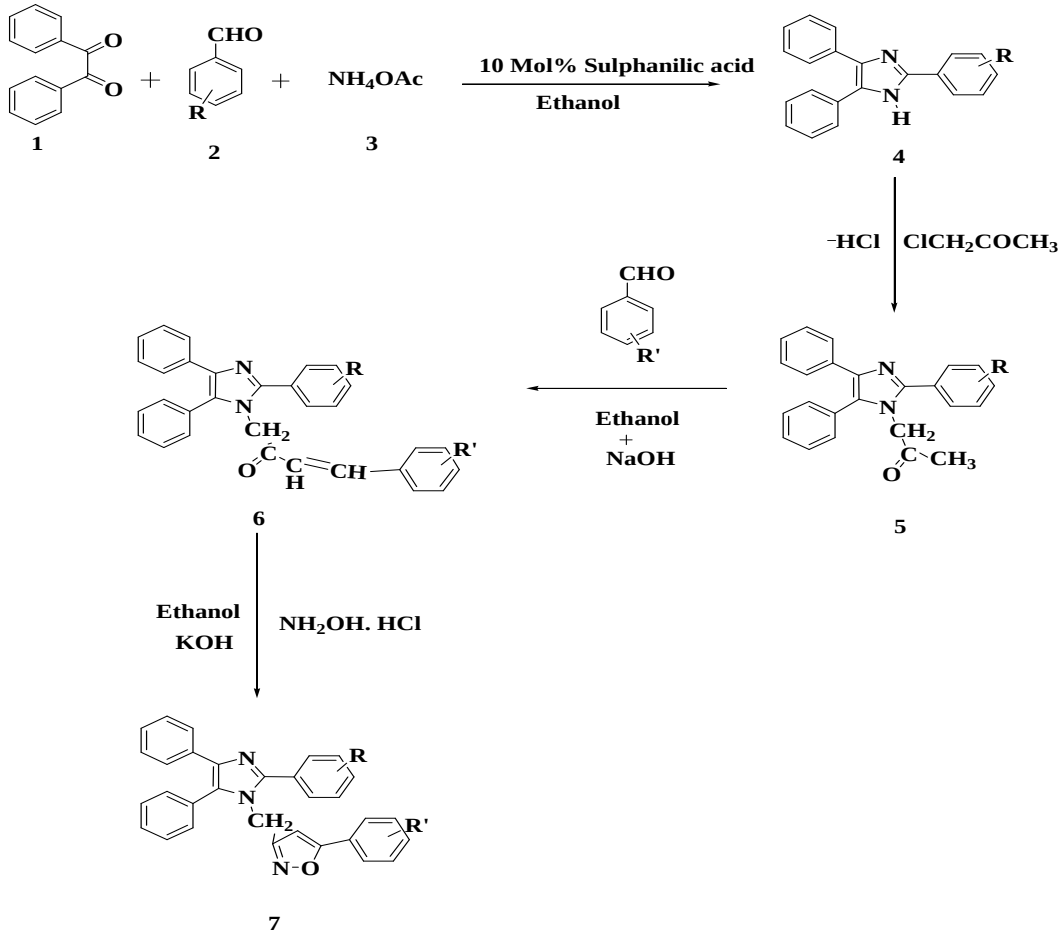
$$\% \text{ inhibition} = \left[1 - {}^{Vt}/_{Vc}\right] * 100$$

Where, ***Vt*** = volume of edema in paw of rats in the drug treated
Vc = volume of edema in paw of rats in the control group

RESULTS AND DISCUSSION

Chemistry

Scheme 1 was followed for synthesis of isoxazole derivatives. Reflucation of benzil with aromatic aldehyde gave compounds 1 which further acetylation with chloroacetone to yielded compound 2. The heating of compound 2 with NaOH in the presence of ethanol yielded chalcone compound which further reacted with hydoxyalamine for cyclization yielded isoxazole derivatives. All the derivatives were characterized by FTIR and ^{1}H-NMR spectra. All the derivatives were synthesized in good yields and physicochemical parameters are summarized in table 1.



Scheme 1. General synthetic pathway for the synthesis of the derivatives

Compounds	R	R'
TPI-1	4-OCH_3	4-CH_3
TPI-2	3,5-OCH_3	4-NO_2
TPI-3	4-OH, 3-OCH_3	H
TPI-4	4-$N(CH_3)_2$	4-CH_3
TPI-5	4-$N(CH_3)_2$	4-NO_2
TPI-6	4-CH_3	3-Br
TPI-7	3-OH	3,4,5-OCH_3
TPI-8	2-OH	4-CH_3
TPI-9	4-Cl	4-CH_3
TPI-10	3-Cl	4-OH, 3-OCH_3
TPI-11	4-OCH_3	4-$N(CH_3)_2$
TPI-12	4-OCH_3	4-Cl
TPI-13	3,5-OCH_3	3,4,5-OCH_3
TPI-14	3,4,5-OCH_3	4-Cl
TPI-15	3,4,5-OCH_3	4-CH_3
TPI-16	2-NO_2	H
TPI-17	4-NO_2	4-OH, 3-OCH_3
TPI-18	4-F	2-OH
TPI-19	2-F	4-OCH_3
TPI-20	3-Br	4-$N(CH_3)_2$

Anti-inflammatory activity

The anti-inflammatory activity of synthesized derivatives was performed by carrageenan induced paw edema model. The activity of synthesized compounds was done on paw of Wister albino rats and compared with Nimuslide as a standard drug. The paw volumes were recorded within firstly at 30 minutes and after that one hours time duration and measured by plethysmometer (initial paw volume). Percentage inhibition was calculated by this formula

$$\% \text{ inhibition} = \left[1 - {}^{Vt}/_{Vc}\right] * 100$$

Where, ***Vt*** = volume of edema in paw of rats in the drug treated
Vc = volume of edema in paw of rats in the control group

Table: 2 Anti-inflammatory activities (Paw volume)

Group	Paw volume (mean ± S.E.M.)				
	0min	30 minutes	60 minutes	120 minutes	180 minutes
Control	0.981±0.024	1.451±0.045	1.682±0.036	1.852±0.053	1.886±0.071
Standard	0.978±0.063	1.215± 0.083	1.285±0.011	1.348±0.026	1.321±0.049
TPI-1	0.988±0.090	1.250±0.054**	1.366±0.023**	1.429±0.096**	1.448±0.032**
TPI-2	0.992±0.014	1.273± 0.042*	1.396±0.058**	1.472±0.019**	1.455±0.053**
TPI-3	0.986±0.055	1.236±0.063**	1.354± 0.051**	1.424±0.014**	1.433±0.068**
TPI-4	0.984±0.024	1.317±0.050	1.466± 0.068*	1.552±0.057**	1.558±0.043**
TPI-5	0.981±0.039	1.375±0.042	1.577± 0.060	1.695± 0.037	1.732± 0.014
TPI-6	0.985±0.033	1.324±0.017	1.480± 0.032*	1.571± 0.020**	1.565± 0.034**
TPI-7	0.990±0.091	1.219±0.016**	1.327± 0.048**	1.399± 0.059**	1.393± 0.023**
TPI-8	0.988±0.074	1.291±0.012	1.431± 0.043**	1.509±0.012**	1.501± 0.019**
TPI-9	0.982±0.029	1.391±0.052	1.597± 0.016	1.719±0.02	1.733±0.039
TPI10	0.980±0.052	1.279± 0.020*	1.427± 0.052**	1.475±0.011**	1.471± 0.053**
TPI-11	0.983±0.015	1.296±0.032	1.440±0.061**	1.518 ±0.047**	1.528±0.023**
TPI-12	0.991±0.025	1.307±0.031	1.461± 0.048**	1.544±0.057**	1.538±0.033**
TPI-13	0.984±0.031	1.217±0.028**	1.321±0.033**	1.381±0.025**	1.383±0.056**
TPI-14	0.981±0.042	1.262±0.031*	1.393±0.024**	1.437±0.013**	1.452±0.012**
TPI-15	0.987±0.045	1.221±0.011**	1.329 ±0.030**	1.411±0.028**	1.418±0.028**
TPI-16	0.988+0.072	1.338±0.042	1.496±0.025*	1.579±0.031*	1.570± 0.036**
TPI-17	0.985±0.053	1.299±0.010	1.446± 0.045**	1.528±0.038**	1.543 ±0.037**
TPI-18	0.984±0.045	1.345±0.029	1.50±0.041*	1.587±0.011**	1.584±0.015**
TPI-19	0.987±0.016	1.303±0.026	1.449±0.036**	1.534±0.023**	1.546±0.011**
TPI-20	0.990±0.051	1.349±0.031	1.546±0.024	1.639±0.055*	1.657±0.037*

*The observation are mean ±SEM**P<0.01,*P<0.05 as compared to control*
(ANOVA followed by Durnett's test)

Table 3 Percentage inhibition in paw volume

Sr. No.	Compound	Percentage inhibition of paw volume			
		30minutes	60 minutes	120minutes	180minutes
1.	Standard	16.26	23.60	27.18	29.92
2.	TPI-1	13.84	18.73	22.83	23.18
3.	TPI-2	12.23	16.98	20.48	22.81
4.	TPI-3	14.81	19.48	23.08	23.97
5.	TPI-4	9.17	12.84	16.17	17.34
6.	TPI-5	5.21	6.22	8.31	8.15
7.	TPI-6	8.74	11.98	15.14	16.97
8.	TPI-7	15.98	21.43	24.42	26.10
9.	TPI-8	11.02	14.87	18.47	20.40
10.	TPI-9	4.08	5.02	7.14	8.10
11.	TPI-10	11.84	15.12	20.34	21.96
12.	TPI-11	10.68	14.36	17.99	18.98
13.	TPI-12	9.88	13.12	16.58	18.45
14.	TPI-13	16.08	21.43	25.41	26.67
15.	TPI-14	12.97	17.14	22.36	23.01
16.	TPI-15	15.79	20.98	23.81	24.77
17.	TPI-16	7.74	11.05	14.74	16.74
18.	TPI-17	10.42	14.01	17.49	18.17
19.	TPI-18	7.24	10.82	14.27	16.01
20.	TPI-19	10.17	13.84	17.13	17.99
21.	TPI-20	7.01	8.07	11.48	12.14

The synthesized isoxazole derivatives (TPI-1 to TPI-20) exhibited good anti-inflammatory activity as compared with the standard drug Nimesulide sodium. The compound **TPI-3**, **TPI-7**, **TPI-13** and **TPI-15** showed the excellent

activity after 3 hours with percentage inhibition **23.97%, 26.10%, 26.67%** and **24.77%** respectively. These four compounds are the most potent of all the compounds tested for anti-inflammatory activity. These compounds **TPI-1**, **TPI-2**, **TPI-3** and **TPI-10** showed good anti-inflammatory activity. Other substitution like methyl, hydroxyl and dimethylamino has displayed moderate activity

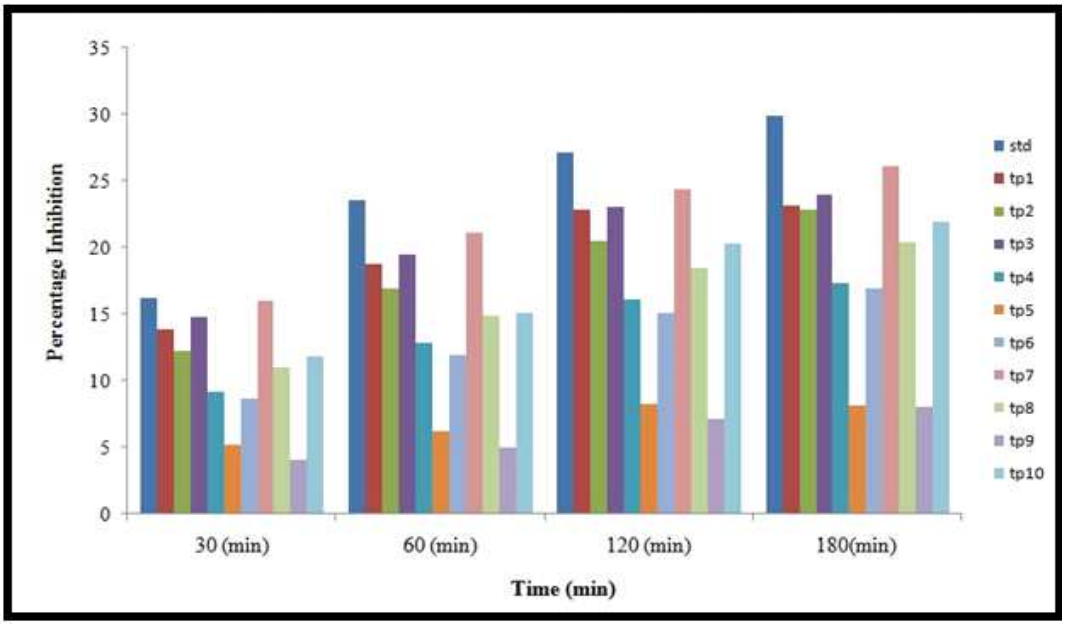


Figure 1. Percentage inhibition of the anti-inflammatory activity of tested compounds TPI-1 to TPI-10 and standard drug

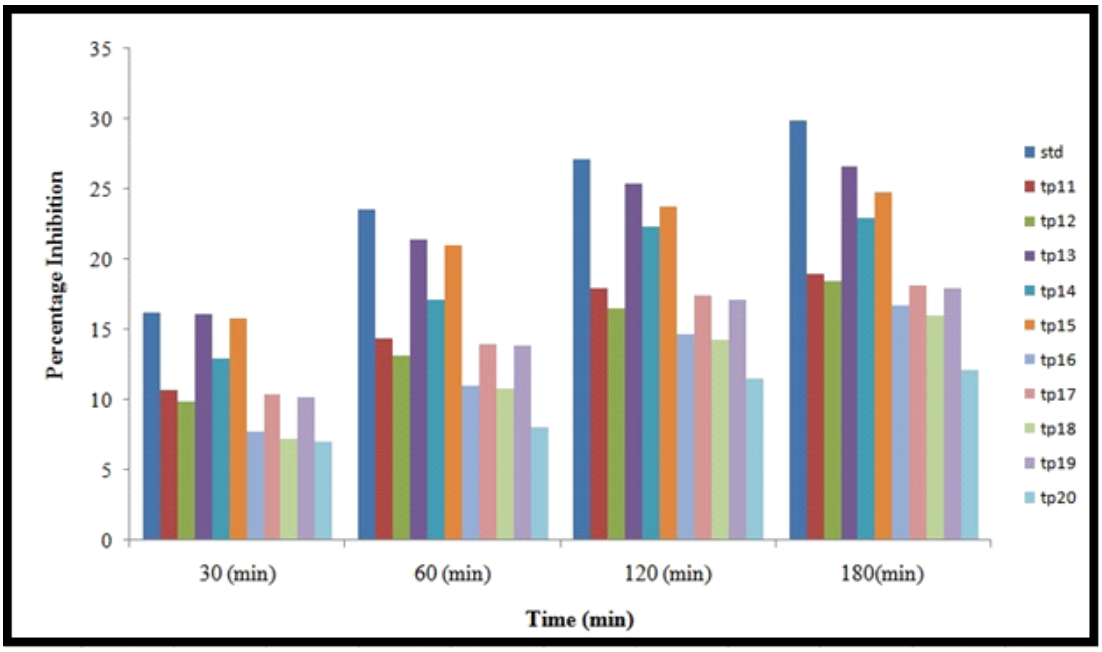


Figure 2. Percentage inhibition of the anti-inflammatory activity of tested compounds TPI-11 to TPI-20 and standard drug

CONCLUSION

Present study described the synthesis of some new isoxazole derivatives. The synthesized compounds were characterized by FTIR and ^{1}H-NMR spectra. All the compounds were evaluated for *in-vivo* anti-inflammatory activity by carrageenan induced paw oedema method. The results indicated that compound TPI-7 and TPI-13 was the most active compound due to the presence of the methoxy group at *para* position.. In future study, further investigation on the mechanism of action of some of our compounds may reveal new compounds with potent anti-inflammatory action.

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