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RESEARCH ARTICLE

Analgesic, Anti-Inflammatory and Anti-arthritic Activity of Newly-Synthesized Bicyclothieno 1, 2, 3 – Triazines

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ABSTRACT

The novel bicyclo thieno 1,2,3-triazines (BTT) namely BTT-1, BTT-2, BTT-3 and BTT-4 were evaluated for analgesic, anti-inflammatory and anti-arthritic activity. Analgesic and anti-inflammatory activity was evaluated using hot plate test, formalin-induced paw licking test and formalin-induced paw edema test respectively. Complete fruend's adjuvant (CFA)-induced arthritis model was used for anti-arthritic activity. All test drugs showed significant analgesic activity by increasing the reaction latency time in hot plate test and decreasing the number of lickings in formalin test. BTT-3 was found to be effective in both early and late phase, while all other test drugs were found to be effective only in late phase of nociception. In anti-inflammatory studies, the BTT-3 (25 and 50 mg/kg, i.p.) had significantly reduced the formalin-induced paw edema. In CFA-induced arthritis models, the BTT-3 has showed activity from the 4th day of the treatment, while all the other test drugs have showed significant inhibition of CFA-induced paw edema from the 7th day of the treatment by decreasing the elevated levels of WBC, % Hb, ESR, along with decreasing the serum levels of C-reactive protein (CRP) and rheumatoid factor (RF). In conclusion, all test drugs were found to possess very good analgesic, anti-inflammatory and anti-arthritic activity and BTT-3 was found to be more potent compared to other compounds.

Keywords: *Bicyclo thieno 1,2,3-triazines, Analgesic, Anti-inflammatory activity, Anti-arthritic activity, CFA-induced arthritis, Fomalin-induced paw licking test*

Triazines are the 6-membered ring compounds containing 3 nitrogen atoms. Theoretically, three different triazines are possible: 1,3,5 triazines, 1,2,4-triazines and 1,2,3-triazines. The 1,2,3-triazines are the novel class of heterocycles. Recently-discovered triazine derivatives are more efficacious drugs with less side effects, reported to possess the various biological activities like purine antagonism [1], xanthine oxidase inhibition [2], anti-allergic [3], anti-cancer and trypanocidal activity [4], anti-neoplastic activity [5], 5HT3 receptor antagonists with gastric motility enhancement activity [6], anti-anaphylatics [7], anti-platelet activity [8, 9], anti-viral/anti-tumour activity [10], inotropics and anti-platelet aggregation activity [11], fungicidal activity [12], thrombotic and elastase inhibition activity [13], analgesic and anti-inflammatory activity [14], nitric oxide and eicosanoid biosynthesis inhibition activity [15]. To verify our hypothesis, the present work is intended to carry out analgesic, anti-inflammatory and anti-arthritic activity of the structurally-established novel bicyclo thieno 1,2,3-triazines.

MATERIAL AND METHODS

Test Compounds

General method for the synthesis of thienotriazines

A mixture of the required 2-amino-3-N-(substituted carboxamido)-4,5-substituted thiophenes (0.01 M) in 30 ml of glacial acetic acid were warmed until the starting material dissolved. The mixture was cooled to room temperature, 20 ml of concentrated HCl was added and the reaction was cooled to a temperature below 5°C. To the cold mixture, an ice cold solution of $NaNO_2$ (0.03 M) in water (25 ml) was added drop-wise with constant stirring. Temperature was maintained below 5°C throughout the addition. The product separated as bright

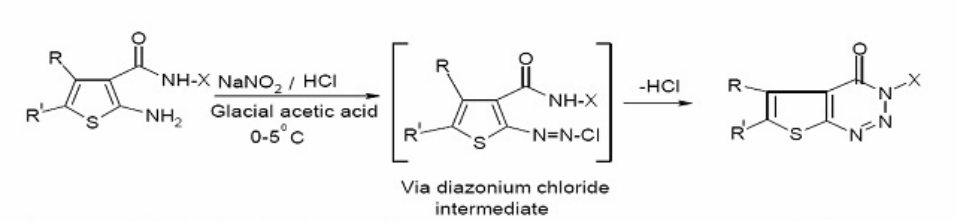
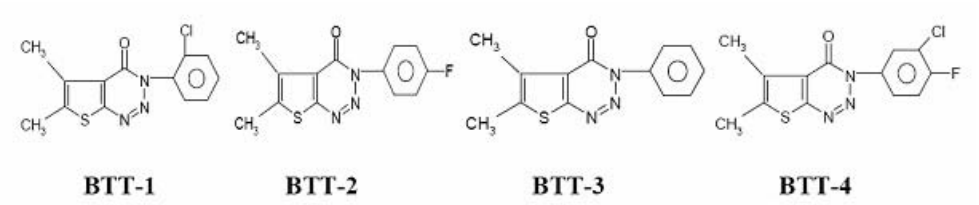


Fig 1. General experimental scheme for the synthesis of thienotriazines



IUPAC Names

BTT-1:- N_3 – (O-chlorophenyl) 4, 5-dimethyl thieno-1, 2, 3-trazin-4 -one.

BTT-2:- N_3 – (P-fluorophenyl) 4, 5-dimethyl thieno-1, 2, 3-trazin-4 -one.

BTT-3:- N_3 – (Phenyl) 4, 5-dimethyl thieno-1, 2, 3-trazin-4 -one.

BTT-4:- N_3 – (m-chloro-p-fluoro) 4, 5-dimethyl thieno-1, 2, 3-trazin-4 -one.

Fig 2. Structures of Synthesized thienotriazine derivatives.

yellow solid, which was filtered, dried and washed with methanol to obtain pure triazines. General experimental scheme for the synthesis of thienotriazines is given in Fig1.

In case of bicyclothieno triazines, the R and R1 groups are replaced by methyl groups (-CH_3) and the X group varies from one compound to other compound. The structures of synthesized thieno triazine derivatives namely BTT-1, BTT-2, BTT-3 and BTT-4 are given in Fig 2.

Drugs and chemicals

CRP turbilatex kit (Spinreact, Spain), RF turbilatex kit (Spinreact, Spain), all drugs, chemicals and solvents were purchased from local firms (India) and they were of highest purity and analytical grade. The test compounds BTT-1, BTT-2, BTT-3, and BTT-4 were synthesized at the Department of Pharmaceutical Chemistry, P.E.S College of Pharmacy, Bangalore, as a part of academic collaboration; Prof. Dr. J. Saravanan had generously supplied these drugs for Pharmacological evaluation.

Experimental animals

Swiss albino mice of 18-25 g and Wistar rats of 180-200 g weight were procured from Bioneeds limited, Nelamangala, Tumkur for experimental purpose. They were housed in separate room in animal facility of PES College of Pharmacy. Mice were maintained in polypropylene cages, while Guinea pigs were maintained in stainless steel cages at a temperature of 25 ± 1°C and relative humidity of 45 to 55% in clean environment under 12 h light-dark cycle. The animals had free access to food pellets (Pranav Agro Industry, Bangalore, India) and purified water *ad libitum*. All the experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) of PES College of Pharmacy (No. PESCP/IAEC/04/2005-06) and were conducted according to the guidelines of CPCSEA, India.

Experimental protocol

Acute toxicity study

The acute intra-peritoneal toxicity for the test compounds was determined in female, nulliparous and non-pregnant Swiss Albino mice weighing 18-22 g. After administration of different doses of test compounds, the mortality with each dose was noted at 48 h (acute) and 14 days (chronic) as per OECD guideline no. 425. LD_{50} was calculated using AOT425 stat program [16].

Determination of Analgesic activity

Hot plate method (INCO, Ambala, India)

Swiss Albino mice of either sex weighing 18 to 25 g were used for the study. The temperature of the hot plate was controlled between 55 to 56°C. The animals were placed into the perspex cylinder on the heated surface and the time (sec) taken to show the discomfort

Table 1. Effect of bicyclo thieno 1,2,3-triazines on hot plate test

Sl. no.	Groups	Dose (mg/kg, i.p.)	Reaction latency in minutes on hot plate			
			30	60	120	150
1	Vehicle control	10 ml/kg	2.33 ± 0.21	2.5 ± 0.22	3.5 ± 0.22	3.5 ± 0.22
2	Pentazocin	4	8.83 ± 0.40***	8.66 ± 0.33***	9.66 ± 0.33***	5.66 ± 0.42***
3	BTT-1	25	2.48 ± 0.32	2.7 ± 0.25	2.83 ± 0.16	3.0 ± 0.25
4	BTT-1	50	5.66 ± 0.33**	5.3 ± 0.21**	5.33 ± 0.21***	5.13 ± 0.16**
5	BTT-2	25	3.5 ± 0.22	3.66 ± 0.21	3.9 ± 0.25	3.83 ± 0.16
6	BTT-2	50	7.83 ± 0.30***	5.16 ± 0.16***	4.33 ± 0.33	3.2 ± 0.21
7	BTT-3	25	4.66 ± 0.21*	5.33 ± 0.21**	5.45 ± 0.21**	4.66 ± 0.21**
8	BTT-3	50	8.96 ± 0.21***	8.73 ± 0.21***	9.5 ± 0.20***	6.35 ± 0.19***
9	BTT-4	25	3.6 ± 0.42	3.5 ± 0.42	3.2 ± 0.36	2.33 ± 0.21
10	BTT-4	50	7.6 ± 0.33**	5.83 ± 0.30***	5.66 ± 0.33***	5.36 ± 0.23***

Values are expressed as mean ± SEM; n=6 *$p < 0.05$, ***$p < 0.001$ compared with vehicle-treated group using one-way ANOVA followed by Tukey- Kramer test.

reaction (licking paws or jumping) was recorded as response latency, prior to and 30, 60, 120, and 150 min following intra-peritoneal administration of the vehicle, standard and test drug. A latency period of 15 sec was defined as complete analgesia and if it exceeded the latency period, the measurement was terminated in order to avoid the injury [17].

Formalin-induced paw licking test in mice

This test was performed according to Dubuisson and Dennis (1977) to evaluate the analgesic activity of test drugs. In brief, Swiss Albino mice of either sex weighing 18-25 g were used for the study; they were divided into ten groups (n = 6). The animals were injected intra-peritoneally with vehicle or Diclofenac sodium (10 mg/kg, i, p) or test drugs. About 30 minutes after the drug administration, 20 µl of 1% formalin was injected subcutaneously under the dorsal surface of hind paw. Followed by administration of formalin, all the animals were individually observed in the glass chambers; the number of licks in the injected paw was counted which was considered as pain stimuli. In general, 0 to 10 min was considered as first phase and 20-30 min was considered as second phase of nociceptive response after formalin injection, the first phase represents neurogenic response and the second phase represents inflammatory response [18].

Determination of Anti-inflammatory Activity

Formalin-induced paw edema:

Wistar rats of 180-200 g weight were used for the study. Vehicle, diclofenac sodium and test drugs were injected intraperitoneally to the animal of respective groups. Thirty min after the treatment, all the animals were challenged by injection of 50 µl of 2.5% formalin into the plantar region of the left hind paw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume is measured plethysmographically immediately after injection, 1, 2, 3, 4 and 24 h after the challenge. From the data obtained, mean paw edema and mean percentage reduction in oedema was calculated [19, 20].

Percentage reduction in edema was calculated using the following formula:

% Inhibition of paw edema = [(Control – Test) / Control] × 100

Anti-arthritic activity

Complete Freund's adjuvant-induced arthritis in rats

Wistar rats of either sex were randomly divided into ten groups of six animals each (n = 6), arthritis was induced by injecting 50 µl (0.5% w/v) of CFA into the left hind paw; 0.5% w/v of CFA was prepared by triturating 5 mg of dead spores of *Mycobacterium tuberculai* in 10 ml of liquid paraffin. Drug treatment was started from the day of CFA injection (0 day), i.e. 30 min before CFA injection and continued till 21st day. Paw thickness was measured on 1st, 2nd, 4th, 7th, 14th, 21st days by using verneir callipers [21-23]. The mean changes in injected paw edema with respect to initial paw volume, were calculated on respective days and the percentage inhibition of paw edema with respect to untreated group was calculated on respective days using this formula:

% Inhibition of paw edema = [(Control – Test) / Control] × 100

On 21st day after the measuring the paw thickness, body weights were recorded. All the animals were anaesthetized and blood samples were collected by retro-orbital puncture for the estimation of various hematological parameters namely RBC count, total WBC count, %Hb, ESR and other serum parameters such as CRP and RF. finally all the animals were sacrificed, thymus and spleen were collected and weighed to see the effect of test drugs on body weight to organ weight ratio.

Statistical analysis

The results were subjected to statistical analysis by using one- way ANOVA followed by Tukey- Kramer test to calculate the significance difference, if any among the groups. The $p < 0.05$ was considered significant.

Table 2. Effect of bicyclo thieno 1,2,3-triazines on formalin induced paw licking test

Sl. no.	Groups	Dose (mg/kg, i.p.)	Paw licking early phase 0-10 min		Paw licking late phase 20-30 min	
			No. of licking	% inhibition	No. of licking	% inhibition
1	Vehicle control	10 ml/kg	142.0 ± 9.70	--	230.16 ± 3.32	--
2	Diclofenac Sodium	5	89.10 ± 6.34*	36.73	63.66 ± 2.18***	72.33
3	BTT-1	25	128.50 ± 8.26	9.50	180.23 ± 5.33	21.67
4	BTT-1	50	83.00 ± 7.75*	41.54	110.51 ± 13.32**	51.98
5	BTT-2	25	120.50 ± 9.72	15.50	182.22 ± 4.00	20.82
6	BTT-2	50	98.13 ± 8.02*	30.89	143.83 ± 3.83*	37.50
7	BTT-3	25	103.42 ± 1.14*	27.17	160.33 ± 4.80	30.34
8	BTT-3	50	69.66 ± 2.69**	50.34	54.89 ± 16***	76.15
9	BTT-4	25	119.32 ± 1.66	15.97	187.64 ± 6.28	18.47
10	BTT-4	50	93.41 ± 4.19*	34.21	117.98 ± 4.81**	48.94

Values are expressed as mean ± SEM; n = 6 ***$p < 0.001$ compared with vehicle treated group using one-way ANOVA followed by Tukey-Kramer test.

RESULTS

Acute Toxicity

Toxicity studies were carried out according to OECD guideline no. 425. At 550 mg/kg i.p., no mortality was observed and at 2000 mg/kg i.p., 100% mortality was observed. LD_{50} was calculated using AOT 425 Stat Programme. The LD_{50} was found to be 1098 mg/kg for all the four test compounds.

Analgesic activity:

Hot plate method

Pretreatment with BTT series of compounds and pentazocin (4 mg/kg, i.p.) increased the response latency at various time points in the hot plate test. Except BTT-3, none of the test drugs significantly increased response latency, while at higher dose levels, all the test drugs have showed significantly increase in response latency time and the BTT-3 was found to be more potent compared to all other test drugs. Results are shown in Table 1.

Formalin induced paw-licking test in mice

In this test, all the test drugs have shown significant inhibition of formalin-induced licking in both early- and late-phase at 50 mg/kg, i.p.dose, while the BTT-3 has shown siginificant inhibition in both 25 and 50 mg/kg doses. The inhibition offered by the BTT-3 (50 mg/kg, i.p.) was more than that of standard drug, diclofenac sodium (5 mg/kg, i.p.). These results are shown in Table 2.

Anti-inflammatory Activity

Formalin-induced paw edema test in rats

All the test drugs have offered siginificant inhibition of formalin-induced paw edema at only high dose (50 mg/kg, i.p.), while the BTT-3 has shown significant inhibition at both the dose levels (25 and 50 mg/kg, i.p.). The results of given in the Table 3.

Anti-arthritic activity

Freund's adjuvant induced arthritis

In this model, all the BTT (25 and 50 mg/kg, i.p.) series of compounds on chronic treatment for 21 days showed significant inhibition ($p < 0.001$) of adjuvant-induced increase in paw thickness. The inhibition offered by the tests drugs was found to be significant from 2nd day onwards ($p < 0.05$) at 50 mg/kg,i.p, whereas at 25 mg/kg, i.p. the inhibition was significant from 14th day onwards; exceptionally BTT-3 has showed significant activity in both the doses (25 and 50

Table 3. Effect of bicyclo thieno 1,2,3-triazines on Formalin induced paw oedema in rats

Sl. no.	Group	Dose (mg/kg,i.p.)	Difference in Paw oedema volume (ml)							
			After 1st hour		After 2nd hour		After 3rdhour		After 4th hour	
			PV	% RPV	PV	% RPV	PV	% RPV	PV	% RPV
1	Vehicle Control	--	0.17 ± 0.01	--	0.495 ± 0.02	--	0.65 ± 0.019	--	0.85 ± 0.036	--
2	Diclofenac Sodium	10	0.14 ± 0.01[c]	17.65	0.32 ± 0.03[b]	35.35	0.14 ± 0.017[a]	78.46	0.18 ± 0.016[a]	78.82
3	BTT-1	25	0.15 ± 0.01	11.76	0.39 ± 0.04[c]	21.21	0.49 ± 0.02[c]	24.62	0.63 ± 0.025[c]	25.88
4	BTT-1	50	0.148 ± 0.01	12.94	0.346 ± 0.01[b]	30.10	0.435 ± 0.02[b]	33.08	0.540 ± 0.010[b]	36.47
5	BTT-2	25	0.162 ± 0.01	4.71	0.438 ± 0.06	11.52	0.56 ± 0.03	13.84	0.72 ± 0.03 [c]	15.29
6	BTT-2	50	0.158 ± 0.02	7.06	0.434 ± 0.03	13.54	0.538 ± 0.02[c]	17.23	0.56 ± 0.020[b]	34.12
7	BTT-3	25	0.159 ± 0.01	6.47	0.385 ± 0.03[c]	22.22	0.405 ± 0.03[b]	37.69	0.515 ± 0.04[b]	39.41
8	BTT-3	50	0.154 ± 0.02	9.41	0.345 ± 0.02[b]	30.30	0.325 ± 0.02[a]	50.00	0.248 ± 0.04[a]	70.82
9	BTT-4	25	0.163 ± 0.01	4.12	0.467 ± 0.01	5.66	0.565 ± 0.02	13.08	0.75 ± 0.015	11.76
10	BTT-4	50	0.159 ± 0.01	6.47	0.43 ± 0.015	13.13	0.452 ± 0.01[b]	30.46	0.541 ± 0.019[b]	36.35

PV: Paw volume, **% RPV:** percentage reduction of paw edema volume, Values are expressed as mean ± SEM; n=6, [c]$p < 0.05$, [b]$p < 0.01$, [a]$p < 0.001$ compared with vehicle treated group using one-way ANOVA followed by Tukey- Kramer test.

Table 4. Effect of BTT series of compound on Freund's adjuvant induced arthritis paw thickness (in mm)

Treatment	Paw thickness in mm from 0^{th} to 21^{st} day						
	0^{th} day	1^{st} Day	2^{nd} Day	4^{th} Day	7^{th} Day	14^{th} Day	21^{st} Day
Vehicle control	0.49 ± 0.01	0.92 ± 0.01	1.08 ± 0.01	1.09 ± 0.03	1.10 ± 0.02	1.12 ± 0.02	1.19 ± 0.03
Diclofenac sodium(5mg/kg,i.p)	0.46 ± 0.01	0.82 ± 0.02*	0.75 ± 0.02**	0.71 ± 0.01***	0.67 ± 0.01***	0.64 ± 0.01***	0.62 ± 0.01***
BTT-1(25mg,i.p)	0.47 ± 0.01	0.89 ± 0.03	1.04 ± 0.01	1.03 ± 0.03	1.02 ± 0.03	0.94 ± 0.02**	0.87 ± 0.02***
BTT-1(50mg,i.p)	0.47 ± 0.01	0.90 ± 0.02	0.92 ± 0.02*	0.91 ± 0.01**	0.85 ± 0.01***	0.79 ± 0.01***	0.72 ± 0.01***
BTT-2(25mg,i.p)	0.48 ± 0.01	0.89 ± 0.02	1.02 ± 0.02	1.03 ± 0.02	1.01 ± 0.01	0.97 ± 0.03**	0.85 ± 0.02***
BTT-2(50mg,i.p)	0.47 ± 0.01	0.87 ± 0.01	0.96 ± 0.01*	0.95 ± 0.02**	0.88 ± 0.02***	0.82 ± 0.01***	0.74 ± 0.01***
BTT-3(25mg,i.p)	0.48 ± 0.01	0.91 ± 0.02	0.95 ± 0.03*	0.93 ± 0.01**	0.89 ± 0.01***	0.84 ± 0.01***	0.76 ± 0.01***
BTT-3(50mg,i.p)	0.48 ± 0.01	0.88 ± 0.02	0.89 ± 0.02**	0.87 ± 0.03***	0.78 ± 0.01***	0.73 ± 0.01***	0.65 ± 0.01***
BTT-4(25mg,i.p)	0.47 ± 0.01	0.89 ± 0.01	1.02 ± 0.03	1.03 ± 0.01	0.98 ± 0.01	0.89 ± 0.01**	0.78 ± 0.01***
BTT-4(50mg,i.p)	0.47 ± 0.01	0.87 ± 0.03	0.94 ± 0.02*	0.92 ± 0.02**	0.87 ± 0.02***	0.78 ± 0.02***	0.71 ± 0.01***

Values are expressed as Mean ± SEM for 6 animals, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with vehicle treated group using one-way ANOVA followed by Tukey- Kramer test.

Table 5. Percentage inhibition of Freund's adjuvant induced arthritis by BTT series of compounds

Treatment	Mean Percentage inhibition of paw thickness from 0^{th} to 21^{st} day						
	0th day	1st Day	2nd Day	4th Day	7th Day	14th Day	21st Day
Vehicle control	0	0	0	0	0	0	0
Diclofenac sodium(5mg/kg,i.p)	6.12	10.87 *	30.56**	34.86***	39.09***	42.86***	47.90***
BTT-1(25mg,i.p)	4.08	3.26	3.70	5.50	7.27	16.07**	26.89***
BTT-1(50mg,i.p)	4.08	2.17	14.81*	16.51**	22.73***	29.46***	39.50***
BTT-2(25mg,i.p)	2.04	3.26	5.56	5.50	8.18	13.39**	28.57***
BTT-2(50mg,i.p)	4.08	5.43	11.11*	12.84**	20.00***	26.79***	37.82***
BTT-3(25mg,i.p)	2.04	1.09	12.04*	14.68**	19.09***	25.00***	36.13***
BTT-3(50mg,i.p)	2.04	4.35	17.59**	20.18***	29.09***	34.82***	45.38***
BTT-4(25mg,i.p)	4.08	3.26	5.56	5.50	10.91	20.54**	34.45***
BTT-4(50mg,i.p)	4.08	5.43	12.96*	15.60**	20.91***	30.36***	40.34***

Values are expressed as mean for 6 animals, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with vehicle treated group using one-way ANOVA followed by Tukey- Kramer test.

mg/kg, i.p.) from the 2^{nd} day and the inhibition showed by the BTT-3 (200 mg/kg, i.p.) was found to be more than that of reference drug diclofenac sodium (Tables 4,5). Furthermore, upon treatment with BTT series of compounds, the body weight and body weight to organ weight ratio was maintained consistently and it was found to be significant ($p < 0.001$) when compared to control, where the slight increase in body weight and significantly high increase in organ weights (thymus and spleen) was observed and hence the organ weight (thymus and spleen) to body weight ratio was significantly more than the normal values (Table 6). After administration of FCA, it was observed that, there was decrease in RBC count, % Hb from normal levels and significant increase in total WBC count, ESR and CRP levels above the normal. Apart from these parameters, RF test was found to be positive, its serum levels was found to be very high. The animals treated with BTT series of compounds for 21 days have maintained all the hematologicals parameters within the normal range and the RF levels was found to be very less compared to control group. The results are shown in Table 7.

DISCUSSION

In present study, BTT series of compounds were evaluated for analgesic, anti-inflammatory and anti-arthritic activity using various animals' models. Analgesic activity was evaluated by using hot plate test and formalin-induced paw licking test. The hot plate test ias considered to be selective for opioid–like compounds, the centrally-acting analgesics [24] and the validity of this test has been shown even in presence of substantial impairment of motor performance. At high dose (50 mg/kg, i.p.), all the test drugs have significantly increased the response latency time and at low dose (25 mg/kg, i.p.), except BTT-3, none of the test drugs have showed significant increase in response latency time. The BTT-3 (50 mg/kg, i.p.) was found to be more potent than pentazocin (4 mg/kg i.p) in hot plate test. In motor coordination test using rotarod apparatus, BTT-3 (100 mg/kg, i.p.) exhibited a significant sedative effect that was evidenced by reduction in endurance time. This could be the possible explanation for its central analgesic activity observed in hot plate test (Unpublished data).

Formalin causes inflammatory pain by inducing capillary permeability and liberating endogenous substances that excite the pain nerve endings. Non-steroidal anti-inflammatory drugs (NSAIDs) can inhibit cyclo-oxygenae (COX) in peripheral tissues with the mechanism of transduction of primary afferent noociceptors. The mechanism of analgesic effect of BTT series of compounds could probably be due to blockade of the effect or the release of endogenous

Table 6. Effect of BTT series of compounds on body weight and organ weight in CFA induced Arthritis in rats

Group	¥Body weight in grams (g)		Change in Body weight	¥Body weight to Organ weights ratio (%)	
	Before induction	On 21st day		Thymus	Spleen
Vehicle control	184	195	11 ± 0.96	0.254	0.404
Diclofenac sodium (5mg/kg,i.p)	186	243	57 ± 4.86 ***	0.198**	0.250**
BTT-1(25mg,i.p)	186	201	20 ± 1.02	0.235	0.364
BTT-1(50mg,i.p)	180	217	46 ± 1.05**	0.208*	0.296*
BTT-2(25mg,i.p)	188	207	19 ± 0.72	0.238	0.326
BTT-2(50mg,i.p)	182	231	43 ± 1.20**	0.212*	0.279*
BTT-3(25mg,i.p)	184	219	35 ± 1.62*	0.222	0.285*
BTT-3(50mg,i.p)	188	239	51 ± 5.2***	0.190**	0.250**
BTT-4(25mg,i.p)	186	205	19 ± 1.42	0.245	0.362
BTT-4(50mg,i.p)	184	228	44 ± 3.12**	0.216*	0.281*

Values are expressed as ¥Mean, ²Mean ±SEM, *p<0.05, **P<0.01, compared with vehicle treated group using one way ANOVA followed by Tukey- Kramer test.

Table 7. Effect of BTT series of compounds on Haematological parameters in Fruend's adjuvant induced Arthritis in rats

Treatment	Parameter					
	RBC (x 10^6/mm^3)	WBC (x10^3/mm^3)	ESR (mm/hr)	Hb (g/dl)	CRP (mg/dl)	RF (IU/ml)
Vehicle control	6.9 ± 0.3	14 ± 0.3	17 ± 0.2	11 ± 0.4	9.2 ± 0.6	68 ± 5.4
Diclofenac sodium (5mg/kg,i.p)	9.1 ± 0.02**	5.4 ± 0.2***	9 ± 0.3***	16 ± 0.2**	1.6 ± 0.2***	26 ± 0.6***
BTT-1(25mg,i.p)	7.4 ± 0.2	9.4 ± 0.8*	15 ± 0.2	11 ± 0.3	7.4 ± 0.7	58 ± 4.8
BTT-1(50mg,i.p)	8.3 ± 0.6*	6.7 ± 0.8***	11 ± 0.1**	14 ± 0.3*	5.5 ± 0.2*	42 ± 4.1*
BTT-2(25mg,i.p)	7.2 ± 0.4	8.9 ± 0.6*	13 ± 0.3*	12 ± 0.2	7.6 ± 0.6	61 ± 5.5
BTT-2(50mg,i.p)	8.5 ± 0.8*	6.5 ± 0.7***	10 ± 0.2**	15 ± 0.3**	4.9 ± 0.8**	39 ± 2.6**
BTT-3(25mg,i.p)	7.9 ± 0.6*	7.3 ± 0.6**	13 ± 0.2*	13 ± 0.2*	5.6 ± 0.6*	37 ± 3.1**
BTT-3(50mg,i.p)	9.2 ± 0.4**	5.9 ± 0.8***	9 ± 0.3***	17 ± 0.4**	2.9 ± 0.1***	29 ± 1.6***
BTT-4(25mg,i.p)	7.6 ± 0.5	8.7 ± 0.7*	14 ± 0.4	12 ± 0.5	7.2 ± 0.9	56 ± 4.2
BTT-4(50mg,i.p)	8.8 ± 0.6**	7.1 ± 0.5**	12 ± 0.3**	13 ± 0.3*	5.1 ± 0.7*	37 ± 2.5*

Values are expressed as Mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.01compared with vehicle treated group using one-way ANOVA followed by Tukey- Kramer test.

substances that excite the pain nerve endings similar to that of pentazocin and other NSAIDs.

The formalin test is used to evaluate the mechanism by which an animal responds to moderate, continuous pain generated by the injured tissue. This test is characterized by two phases. The early phase (immediately after injection) seems to be caused by C-fibre activation due to the peripheral stimulus, the late phase (starting approximately 20 min after formalin injection) appears to depend on the combination of anti-inflammatory reaction, activation of N-methyl D-aspartate (NMDA) and non-NMDA receptors, and the Nitric oxide (NO) cascade in the peripheral tissue and functional changes in the dorsal horn on the spinal cord [25, 26]. These functional changes appears to be initiated by the C-fibre barrage during the early phase and to be related to excitatory amino acid (EAA) release in the spinal cord and activation of NMDA receptors subtypes. The spinal cord contains mechanisms that inhibit the activity of neurons that receive and transmit nociceptive information. Primary afferent fibers of the spinal cord utilize the EAAs like glutamate and aspartate as their neurotransmitters. There are evidences that selective EAAs receptor antagonists produce antinociception while EAAs receptor agonists elicit hyperalgesia [27-30]. The formalin test has been used to evaluate the antinociceptive effects of competitive and non-competitive NMDA receptor antagonists administered intrathecally and systemically [31]. In formalin-induced paw licking test in mice, all the test drugs have significantly decreased the number of paw lickings in both early and late phase at high dose (50 mg/kg, i.p.), and at low dose (25 mg/kg, i.p.). Except BTT-3, none of the test drugs have showed significant inhibition. The BTT-3 (50mg/kg, i.p.) was found to be more potent than diclofenac sodium (5 mg/kg, i.p) in inhibiting neurogenic (early phase) and inflammatory (late phase) pain stimuli caused by formalin. These results suggest that BTT series of compounds may be acting through both central and peripheral mechanisms.

Anti-inflammatory activity of the test drugs was evaluated using formalin-induced paw edema model. Formalin-induced inflammation involves three distinct phases based on the release of different inflammatory mediators, namely serotonin and histamine in the first phase (0-2 h), kinins like bradykinin in second phase(3 h) and prostaglandins in the third phase (>4 h) [32]. The second and third phase has been reported to be sensitive to both steroidal and non-steroidal anti-inflammatory agents [33]. In the present study, we have examined the effects of BTT series of compounds on these phases of inflammation. The results of this study indicate that all the test drugs at high dose (50 mg/kg, i.p.) show very good antiiflammatory property in the second and third

phases of inflammation, where as the BTT-1 (25 and 50 mg/kg, i.p.), BTT-3 (25 and 50 mg/kg, i.p.) and diclofenac sodium (5 mg/kg, i.p.) have offered significant inhibition of inflammation in all the three phases. Furthermore, the possible mechanism of action of BTT-2 and BTT-4 may be associated with the inhibition of release of kinins and prostaglandins; where as BTT-1 and BTT-3 may be inferring with the release of histamine, serotonin, kinins and prostaglandins.

Based on the observations in the analgesic and anti-inflammatory studies, the BTT series of compounds were evaluated for their effect on chronic inflammation in FCA-induced arthritis in rats. In the present study, complete Freund's adjuvant-induced arthritis in rats were selected to induce arthritis, because it is the best and most widely used experimental model for arthritis with clinical and laboratory features such as chronic swelling in multiple joints due to accumulation of inflammatory cells, erosion of joint cartilage and bone destruction and it has close similarities to human rheumatoid diseases [23]. Chronic inflammation involves the release of number of mediators like cytokines (IL-1B and TNF-α), GM-CSF, interferon's and Platelet-derived growth factor (PDGF). These mediators are responsible for the pain, swelling of the limbs and joints, destruction of bone and cartilage that can lead to severe disability [34]. In present study, the intra-plantar administration of CFA showed significant increase in paw thickness which is the indication of arthritis; it mimics the rheumatoid arthritis in humans. All the BTT series of compounds upon intra-peritoneal administration for 21 days showed significant inhibition of CFA-induced paw edema ($p < 0.001$), the BTT-3 has showed significant activity from the 4^{th} day onwards and it was comparable with diclofenac sodium (5 mg/kg, i.p.), while all the other test drugs have showed significant inhibition from the 7^{th} day onwards.

Changes in the body weight have also been used to access the course of the disease and the response to therapy of anti-inflammatory drugs [35]. As the incidence and severity of arthritis increased, the changes in the body weights of the rats also occurred during the course of the experimental period. Earlier findings suggest that absorption of ^{14}C-glucose and ^{14}C-leucine in rat's intestine was reduced in the case of inflamed rats [36]. But on the treatment with anti-inflammatory drugs, the decrease in absorption was nullified. This shows that the anti-inflammatory drugs correct the decreased/deranged absorption capacity of intestine during inflammation.

In present study, all the BTT series of compounds upon administration for 21 days showed consistent increase in body weight compared to control. CFA increased weights of thymus and spleen weights above the normal, which leads to increase in organ weight (thymus and spleen) to body weight ratios. Upon administration of test drugs, the organ weights and organ weight to body weight ration were maintained within the normal range. This was highly significant compared to CFA control ($p < 0.001$). CFA administration leads to rise in total WBC count due to the release of IL-IB inflammatory response. IL-IB increases the production of both granulocyte and macrophages colony stimulating factors [34, 37], decreases RBC count and hemoglobin concentration. ESR is an estimate of the suspension stability of RBC's in plasma. It is related to the number and size of the red cells and to the relative concentration of plasma proteins, especially fibrinogen, alpha and beta globulins. Increase in the ESR, is an indication of active disease processes. The acute phase proteins in ESR and CRP share the property of showing elevations in the concentration in response to stress or inflammations [37]. The ESR significantly increased in arthritic control group. Treatment with BTT series of compounds and diclofenac sodium for 21 days remarkably counteracted the total WBC count, RBC count and hemoglobin concentration. CFA-induced arthritis in rats is also associated with an increase in the plasma levels of CRP and RF [38, 39]. RF is the immunological expression of an individual's immune system reaction to the presence of an immunoglobulin molecule that is recognized as non-self. This response to the non-self immunoglobulin results in the presence of immune complexes, these in turn bind to the complement and may eventually lead to destruction of synovium, cartilage and bone. The higher the levels of serum RF, the higher the development of inflammation [40]. Upon treatment with BTT series of compounds (50 mg/kg. i.p.), these parameters were significantly decreased when compare to CFA control.

In conclusion, all the BTT series of compound were found to possess very good analgesic, anti-inflammatory and antiarthritic activity only at 50 mg/kg, while the BTT-3 had showed good efficacy in 25 and 50 mg/kg, i.p. and it was found to be more potent than other compounds.

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CONFLICT OF INTEREST

The author declares that there are no conflicts of interest.

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