

## RESEARCH ARTICLE

# Analgesic, Anti-Inflammatory and Anti-arthritis Activity of Newly-Synthesized Bicyclo thieno 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-arthritis 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 Freund's adjuvant (CFA)-induced arthritis model was used for anti-arthritis 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 4<sup>th</sup> day of the treatment, while all the other test drugs have showed significant inhibition of CFA-induced paw edema from the 7<sup>th</sup> 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-arthritis 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-arthritis activity, CFA-induced arthritis, Formalin-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], 5HT<sub>3</sub> 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-arthritis 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<sub>2</sub> (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

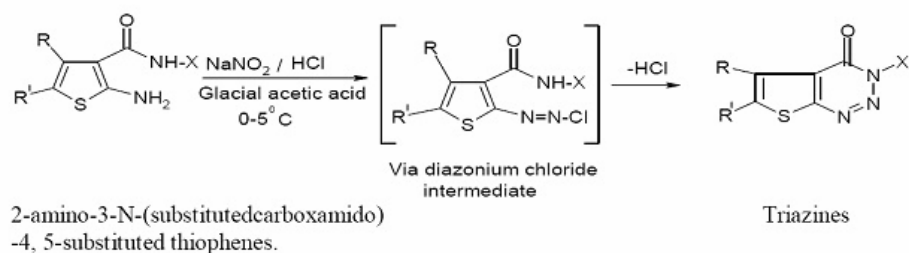
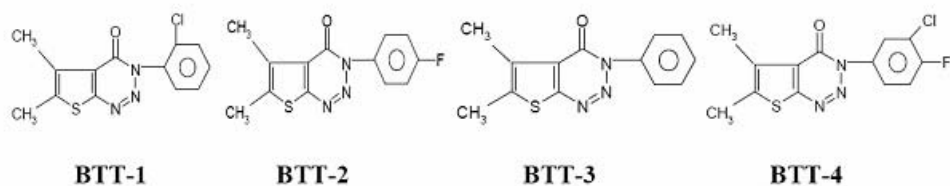


Fig 1. General experimental scheme for the synthesis of thienotriazines



#### IUPAC Names

BTT-1:- N<sub>3</sub> – (O-chlorophenyl) 4, 5-dimethyl thieno-1, 2, 3-triazin-4 -one.

BTT-2:- N<sub>3</sub> – (P-fluorophenyl) 4, 5-dimethyl thieno-1, 2, 3-triazin-4 -one.

BTT-3:- N<sub>3</sub> – (Phenyl) 4, 5-dimethyl thieno-1, 2, 3-triazin-4 -one.

BTT-4:- N<sub>3</sub> – (m-chloro-p-fluoro) 4, 5-dimethyl thieno-1, 2, 3-triazin-4 -one.

Fig 2. Structures of Synthesized thienotriazine derivatives.

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

66 In case of bicyclothieno triazines, the R and R1  
67 groups are replaced by methyl groups (-CH<sub>3</sub>) and the X  
68 group varies from one compound to other compound.  
69 The structures of synthesized thieno triazine derivatives  
70 namely BTT-1, BTT-2, BTT-3 and BTT-4 are given in  
71 Fig 2.

#### 72 Drugs and chemicals

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

#### 83 Experimental animals

84 Swiss albino mice of 18-25 g and Wistar rats of 180-  
85 200 g weight were procured from Bioneds limited,  
86 Nelamangala, Tumkur for experimental purpose. They  
87 were housed in separate room in animal facility of PES  
88 College of Pharmacy. Mice were maintained in  
89 polypropylene cages, while Guinea pigs were

90 maintained in stainless steel cages at a temperature of  
91 25 ± 1°C and relative humidity of 45 to 55% in clean  
92 environment under 12 h light-dark cycle. The animals  
93 had free access to food pellets (Pranav Agro Industry,  
94 Bangalore, India) and purified water *ad libitum*. All the  
95 experimental protocols were approved by Institutional  
96 Animal Ethics Committee (IAEC) of PES College of  
97 Pharmacy (No. PESCP/IAEC/04/2005-06) and were  
98 conducted according to the guidelines of CPCSEA,  
99 India.

#### 100 Experimental protocol

##### 101 Acute toxicity study

102 The acute intra-peritoneal toxicity for the test  
103 compounds was determined in female, nulliparous and  
104 non-pregnant Swiss Albino mice weighing 18-22 g.  
105 After administration of different doses of test  
106 compounds, the mortality with each dose was noted at  
107 48 h (acute) and 14 days (chronic) as per OECD  
108 guideline no. 425. LD<sub>50</sub> was calculated using AOT425  
109 stat program [16].

##### 110 Determination of Analgesic activity

##### 111 Hot plate method (INCO, Ambala, India)

112 Swiss Albino mice of either sex weighing 18 to 25 g  
113 were used for the study. The temperature of the hot  
114 plate was controlled between 55 to 56°C. The animals  
115 were placed into the perspex cylinder on the heated  
116 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 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:

Percentage reduction in edema was calculated using the following formula:

$$\% \text{ Inhibition of paw edema} = \left[ \frac{(\text{Control} - \text{Test})}{\text{Control}} \right] \times 100$$

#### Anti-arthritis 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 tuberculosis* 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 21<sup>st</sup> day. Paw thickness was measured on 1<sup>st</sup>, 2<sup>nd</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> days by using vernier 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:

$$\% \text{ Inhibition of paw edema} = \left[ \frac{(\text{Control} - \text{Test})}{\text{Control}} \right] \times 100$$

On 21<sup>st</sup> 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.

197

**RESULTS****198 Acute Toxicity**

199 Toxicity studies were carried out according to 200 OECD guideline no. 425. At 550 mg/kg i.p., no 201 mortality was observed and at 2000 mg/kg i.p., 100% 202 mortality was observed. LD<sub>50</sub> was calculated using AOT 203 425 Stat Programme. The LD<sub>50</sub> was found to be 1098 204 mg/kg for all the four test compounds.

**205 Analgesic activity:****206 Hot plate method**

207 Pretreatment with BTT series of compounds and 208 pentazocin (4 mg/kg, i.p.) increased the response 209 latency at various time points in the hot plate test. 210 Except BTT-3, none of the test drugs significantly 211 increased response latency, while at higher dose levels, 212 all the test drugs have showed significantly increase in 213 response latency time and the BTT-3 was found to be 214 more potent compared to all other test drugs. Results are 215 shown in Table 1.

**216 Formalin induced paw-licking test in mice**

217 In this test, all the test drugs have shown significant 218 inhibition of formalin-induced licking in both early- and

219 late-phase at 50 mg/kg, i.p. dose, while the BTT-3 has 220 shown significant inhibition in both 25 and 50 mg/kg 221 doses. The inhibition offered by the BTT-3 (50 mg/kg,

222 i.p.) was more than that of standard drug, diclofenac 223 sodium (5 mg/kg, i.p.). These results are shown in Table

**225 Anti-inflammatory Activity****226 Formalin-induced paw edema test in rats**

227 All the test drugs have offered significant inhibition 228 of formalin-induced paw edema at only high dose (50 229 mg/kg, i.p.), while the BTT-3 has shown significant 230 inhibition at both the dose levels (25 and 50 mg/kg, 231 i.p.). The results of given in the Table 3.

**232 Anti-arthritis activity****233 Freund's adjuvant induced arthritis**

234 In this model, all the BTT (25 and 50 mg/kg, i.p.) 235 series of compounds on chronic treatment for 21 days 236 showed significant inhibition (p < 0.001) of adjuvant- 237 induced increase in paw thickness. The inhibition 238 offered by the tests drugs was found to be significant 239 from 2<sup>nd</sup> day onwards (p < 0.05) at 50 mg/kg, i.p., 240 whereas at 25 mg/kg, i.p. the inhibition was significant 241 from 14<sup>th</sup> day onwards; exceptionally BTT-3 has 242 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 1 <sup>st</sup> hour		After 2 <sup>nd</sup> hour		After 3 <sup>rd</sup> hour		After 4 <sup>th</sup> 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 <sup>c</sup>	17.65	0.32 ± 0.03 <sup>b</sup>	35.35	0.14 ± 0.017 <sup>a</sup>	78.46	0.18 ± 0.016 <sup>a</sup>	78.82
3	BTT-1	25	0.15 ± 0.01	11.76	0.39 ± 0.04 <sup>c</sup>	21.21	0.49 ± 0.02 <sup>c</sup>	24.62	0.63 ± 0.025 <sup>c</sup>	25.88
4	BTT-1	50	0.148 ± 0.01	12.94	0.346 ± 0.01 <sup>b</sup>	30.10	0.435 ± 0.02 <sup>b</sup>	33.08	0.540 ± 0.010 <sup>b</sup>	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 <sup>c</sup>	15.29
6	BTT-2	50	0.158 ± 0.02	7.06	0.434 ± 0.03	13.54	0.538 ± 0.02 <sup>c</sup>	17.23	0.56 ± 0.020 <sup>b</sup>	34.12
7	BTT-3	25	0.159 ± 0.01	6.47	0.385 ± 0.03 <sup>c</sup>	22.22	0.405 ± 0.03 <sup>b</sup>	37.69	0.515 ± 0.04 <sup>b</sup>	39.41
8	BTT-3	50	0.154 ± 0.02	9.41	0.345 ± 0.02 <sup>b</sup>	30.30	0.325 ± 0.02 <sup>a</sup>	50.00	0.248 ± 0.04 <sup>a</sup>	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 <sup>b</sup>	30.46	0.541 ± 0.019 <sup>b</sup>	36.35

**PV:** Paw volume, **% RPV:** percentage reduction of paw edema volume, Values are expressed as mean ± SEM; n=6, <sup>c</sup>p < 0.05, <sup>b</sup>p < 0.01, <sup>a</sup>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 <sup>th</sup> to 21 <sup>st</sup> day						
	0 <sup>th</sup> day	1 <sup>st</sup> Day	2 <sup>nd</sup> Day	4 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day	21 <sup>st</sup> 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 <sup>th</sup> to 21 <sup>st</sup> 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.

243mg/kg, i.p.) from the 2<sup>nd</sup> day and the inhibition showed 269 arthritic activity using various animals' models. 244 by the BTT-3 (200 mg/kg, i.p.) was found to be more 270 Analgesic activity was evaluated by using hot plate test 245 than that of reference drug diclofenac sodium (Tables 271 and formalin-induced paw licking test. The hot plate test 246 4,5). Furthermore, upon treatment with BTT series of 272 ias considered to be selective for opioid-like 247 compounds, the body weight and body weight to organ 273 compounds, the centrally-acting analgesics [24] and the 248 weight ratio was maintained consistently and it was 274 validity of this test has been shown even in presence of 249 found to be significant ( $p < 0.001$ ) when compared to 275 substantial impairment of motor performance. At high 250 control, where the slight increase in body weight and 276 dose (50 mg/kg, i.p.), all the test drugs have 251 significantly high increase in organ weights (thymus 277 significantly increased the response latency time and at 252 and spleen) was observed and hence the organ weight 278 low dose (25 mg/kg, i.p.), except BTT-3, none of the 253 (thymus and spleen) to body weight ratio was 279 test drugs have showed significant increase in response 254 significantly more than the normal values (Table 6). 280 latency time. The BTT-3 (50 mg/kg, i.p.) was found to 255 After administration of FCA, it was observed that, there 281 be more potent than pentazocin (4 mg/kg i.p) in hot 256 was decrease in RBC count, % Hb from normal levels 282 plate test. In motor coordination test using rotarod 257 and significant increase in total WBC count, ESR and 283 apparatus, BTT-3 (100 mg/kg, i.p.) exhibited a 258 CRP levels above the normal. Apart from these 284 significant sedative effect that was evidenced by 259 parameters, RF test was found to be positive, its serum 285 reduction in endurance time. This could be the possible 260 levels was found to be very high. The animals treated 286 explanation for its central analgesic activity observed in 261 with BTT series of compounds for 21 days have 287 hot plate test (Unpublished data). 262 maintained all the hematologicals parameters within the 288 Formalin causes inflammatory pain by inducing 263 normal range and the RF levels was found to be very 289 capillary permeability and liberating endogenous 264 less compared to control group. The results are shown in 290 substances that excite the pain nerve endings. Non- 265 Table 7. 291 steroidal anti-inflammatory drugs (NSAIDs) can inhibit 292 cyclo-oxygenase (COX) in peripheral tissues with the 293 mechanism of transduction of primary afferent 294 nociceptors. The mechanism of analgesic effect of

266

## DISCUSSION

267 In present study, BTT series of compounds were 295 BTT series of compounds could probably be due to 268 evaluated for analgesic, anti-inflammatory and anti- 296 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	<sup>y</sup> Body weight in grams (g)		Change in Body weight	<sup>y</sup> 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 <sup>y</sup>Mean, <sup>z</sup>Mean ± 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 Freund's adjuvant induced Arthritis in rats

Treatment	Parameter					
	RBC (x 10 <sup>6</sup> /mm <sup>3</sup> )	WBC (x10 <sup>3</sup> /mm <sup>3</sup> )	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* < 0.01 compared with vehicle treated group using one-way ANOVA followed by Tukey- Kramer test.

substances that excite the pain nerve endings similar to non-competitive NMDA receptor antagonists that of pentazocin and other NSAIDs. administered intrathecally and systemically [31]. In The formalin test is used to evaluate the mechanism formalin-induced paw licking test in mice, all the test by which an animal responds to moderate, continuous drugs have significantly decreased the number of paw pain generated by the injured tissue. This test is licks in both early and late phase at high dose (50 characterized by two phases. The early phase mg/kg, i.p.), and at low dose (25 mg/kg, i.p.). Except (immediately after injection) seems to be caused by C-3 BTT-3, none of the test drugs have showed significant fibre activation due to the peripheral stimulus, the late inhibition. The BTT-3 (50mg/kg, i.p.) was found to be phase (starting approximately 20 min after formalin more potent than diclofenac sodium (5 mg/kg, i.p) in injection) appears to depend on the combination of anti-inhibiting neurogenic (early phase) and inflammatory inflammatory reaction, activation of N-methyl D-3 (late phase) pain stimuli caused by formalin. These aspartate (NMDA) and non-NMDA receptors, and the results suggest that BTT series of compounds may be Nitric oxide (NO) cascade in the peripheral tissue and acting through both central and peripheral mechanisms. functional changes in the dorsal horn on the spinal cord Anti-inflammatory activity of the test drugs was [25, 26]. These functional changes appears to be evaluated using formalin-induced paw edema model. initiated by the C-fibre barrage during the early phase Formalin-induced inflammation involves three distinct and to be related to excitatory amino acid (EAA) release phases based on the release of different inflammatory in the spinal cord and activation of NMDA receptors mediators, namely serotonin and histamine in the first subtypes. The spinal cord contains mechanisms that phase (0-2 h), kinins like bradykinin in second phase (3 inhibit the activity of neurons that receive and transmit 4h) and prostaglandins in the third phase (>4 h) [32]. The nociceptive information. Primary afferent fibers of the second and third phase has been reported to be sensitive spinal cord utilize the EAAs like glutamate and to both steroidal and non-steroidal anti-inflammatory aspartate as their neurotransmitters. There are evidences agents [33]. In the present study, we have examined the that selective EAAs receptor antagonists produce effects of BTT series of compounds on these phases of antinociception while EAAs receptor agonists elicit inflammation. The results of this study indicate that all hyperalgesia [27-30]. The formalin test has been used to the test drugs at high dose (50 mg/kg, i.p.) show very evaluate the antinociceptive effects of competitive and good anti-inflammatory 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- $\alpha$ ), GM-CSF, interferon- $\gamma$  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<sup>th</sup> 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<sup>th</sup> 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 <sup>14</sup>C-glucose and <sup>14</sup>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-1B inflammatory response. IL-1B 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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