The novel compound 2-(2-methyl-4-oxoquinazolin-3(4H)-yl)-N-p-tolylacetamide (P-TOL) (T-02) attenuates inflammatory and nociceptive transmission in experimental animal models of pain and inflammation

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Abstract— Quinazoline-4(3H)-ones and its derivatives are versatile nitrogen heterocyclic compounds which have long been known as a promising class of biologically active compounds. Here we have investigated anti-inflammatory and anti-nociceptive potentials of some of the newly synthesized Quinazolines derivatives. Pharmacological activity was evaluated in the animal models of inflammation (Carrageenan induced paw edema, CFA induced inflammatory pain model) and Pain (formalin induced nociception) in male Sprague Dawley rats. We have evaluated T-02 compound and showed notably effective in reducing the not only carrageenan induced paw edema and CFA induced inflammatory pain but also formalin induced nociception. In carrageenan induced paw edema, CFA induced inflammatory pain and formalin induced nociception. When given orally, at similar doses produced significant reduction in paw volume, mechanical hyperalgesia (PWT -paw withdrawal threshold) and inhibition of nocifensive behavior (duration of paw licking and bitting) induced by subplanatar formalin injection. T-02 showed most prominent activity in both the animal models. T-02 at 1 mg/kg, 3 mg/kg, and 10 mg/kg significantly lowered nocifensive score in rats. The paw licking behavior in formalin test was more potently suppressed during the late phase (20–40 min, inflammatory) than in early phase (0–5 min, neurogenic) for T-02. Also; in CFA induced inflammatory pain model (T-02) at a dose of 1, 3 and 10 mg/kg significantly reduced the mechanical hyperalgesia (PWT -paw withdrawal threshold) show signs of anti-hyperalgesic activity and in carrageenan induce paw edema model 3 mg/kg, and 10 mg/kg (T-02) significantly reduced the edema reveal anti-inflammatory activity.

These results showed that Quinazoline derivatives produces antinociception possibly involving ion channels, which advantage further studies on its efficacy in more specific models of neuropathic pain such as Bennett and chung 's model of neuropathic pain

Key words--- Quinazoline, Pain and Inflammation, Carrageenan, Formalin, Paw edema, nociceptive pain, Nociception.

1 Introduction

Heterocyclic compounds are among the most frequently encountered scaffolds in drug discovery and pharceutically industry. A heterocyclic core is propitious for variations of substitution pattern during Structure Activity Relationship (SAR). Quinazolinone are tremendous reservoir for the synthesis of new chemical entities. The stability of the Quinazoline nucleus has inspired medicinal chemists to introduce many bioactive moieties to this nucleus to synthesize new potential medicinal agents. Quinazoline and quinazolinone derivatives have continued to attract a widespread interest for a long time due to their diverse pharmacological activities such as antibacterial (1, 2), antitubecular (3), antifungal (4), antihyperglysemic (5), anti tumor (6).

In our research program we found that quinazolines and some of condensed quinazolines exhibit potent activities like analgesic, anti-inflammatory (7) and anticonvulsant (8). Quinazolin-4(3H) - ones with 2, 3-disubstitution is reported to possess significant analgesic, anti-inflammatory and anticonvulsant activities (9, 10). Many diseases like arthritis, tempomadibular joint disorder, lower back pain, post-operative pain are typically associated with the pain such as hyperalgesia and allodynia (11-14). It has been proved in many research publications that inflammatory pain can mark the changes in the neuronal plasticity due to peripheral sensitization of primary sensory neuron in the dorsal root ganglion and subsequently central sensitization in spinal cord (11).

Many Phlogistic agents like carrageenan, complete Freund's adjuvant, formalin, Zymosan, kaolin can cause tissue injury inflammation and pain in rodents. Tissue injury due to any agent (noxious or non-noxious stimulus) domino effect of inflammation and subsequently pain. Inflammatory mediators such as bradykinin, prostaglandins, prostacyclin, and nerve growth factor also some of the pro-inflammatory cytokines TNFα, IL-1β, IL-6 play important role in the peripheral sensitization of nociceptors. Most of the peripheral terminals of nociceptors express the receptors for almost all above mentioned inflammatory mediators and activation of these recep-

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tors can cause hyperactivity of some of ion channels such as TRPA1 or TRPV1 or sodium, calcium ion channels via activation of various kinases for instance protein kinase A (PKA), protein kinase C (PKC) and mitogen- activated protein kinases (MAPKs) (15).

Inflammatory diseases like rheumatoid arthritis (RA), osteoarthritis (OA) inflammatory bowel disease (IBD), hepatitis, asthma are major cause of morbidity in humans. Early inflammation is implicated in variety of disease such as cardiovascular complication, diabetics, cancer (16). The treatment of inflammatory disease includes use of non steroidal anti-inflammatory drugs (NSAIDs).Non steroidal anti-inflammatory drug's usually act by blocking the arachidonic acid metabolisms via cyclooxygenase and finally prostaglandins production. As Inflammation is protective phenomenon and synthesis of prostaglandin is key for the cytoprotection, but long -term synthesis of prostaglandin in human body can cause bleeding and ulcers and further renal complication as some of NSAIDs inhibit both the isoform COX-1 and COX-2 (17, 18).

Therefore, there is need to develop newer agents with more potent analgesic and anti-inflammatory activities and with lesser side effects and this is unmet therapeutic need.

Quinazolines and condensed quinazolines exhibit diverse pharmacological activities. 2-phenyl-3-substituted quinazoline series has shown good analgesic and anti-inflammatory activities (19), in the present study we aimed to synthesize some 2-methyl -3-substituted oxoquinazolin series for their analgesic and anti-inflammatory activities. The title compounds 2-(2-methyl-4-oxoquinazolin-3(4H)-yl)-N-p-tolylacetamide were prepared from an anthranilic acid or its derivative. Formation of 2-alkyl-4(3H) quinazolinone by condensation of anthranilic acid or substituted anthranilic acid and amides as designated in the Niementowski reaction (20).

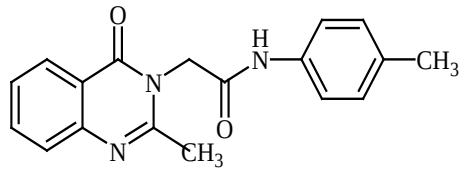

Fig. 1 T-02 (P-Tol) 2-(2-methyl-4-oxoquinazolin-3(4H)-yl)-N-p-tolylacetamide

There are no promising quinazolines which are in the market in these NSAIDs criteria except few drugs like Proquazone Afloqualone and Diproqualone etc (21, 22), fluproquazone (23) has potent analgesic and anti inflammatory actions with less gastric ulceration. So, present study was undertaken to observe pharmacological effect of newly synthesized Quinazolinone derivative in the animal models of inflammation (Carrageenan induced paw edema, CFA induced inflammatory pain) and Pain (formalin induced nociception) in male Sprague Dawley rats.

So, the present study to the best of our search is the foot step to report anti-inflammatory and anti-nociceptive potentials of some of the newly synthesized Quinazolines derivatives.

2. MATERIALS AND METHODS

2.1 Chemicals

Diclofenac Sodium (Sigma), Gabapentin (Fluorochem, Derbyshire, United Kingdom), Dexamethasone (Sigma), Carrageenan (Sigma), CFA (Sigma).

2.2 Compounds

The test compound and standard were dissolved in 0.5% Tween-80+0.5% carboxyl methyl cellulose solution.

2.3 Animals and experimental design

2.3.1 Animals

Male S.D Rats: 7-9 Weeks of age and body weight (210-250g) were procured from Laxmi Biofarms Pvt. Ltd. Ale Phata, Pune. Animals were maintained at a constant temperature and had free access to food and drinking water in a 12 hr light/dark cycle with lights on from 06:30 to 18:30 h. Food and water were available ad libitum. All experimental procedures using animals were performed under the guidelines of our Institutional Animal Ethical Committee.

2.3.2 In vivo pharmacokinetic profile of T-02

Male S.D Rats: 7-9 Weeks of age and body weight (210-250g) fasted for overnight were administered T-02 intravenously at 1 mg/kg dose and orally at 3 mg/kg dose. Blood samples following intravenous dosing were collected at 0.12 (7 min), 0.25 (15 min), 0.5 (30 min), 1, 2, 4, 8 and 24 h post dose (8 time points). Following oral dosing, blood samples were collected at 0.25 (15 min), 0.5 (30 min), 1, 2, 4, 8 and 24 h post dose (7 time points). Plasma samples were analyzed by LC-MS/MS following protein precipitation with acetonitrile containing internal standard.

2.3.3 In vivo efficacy of T-02 in inflammation (Carrageenan induced paw edema)

The initial hind paw volume of the Sprague–Dawley rats was determined volumetrically, 1% solution of carrageenan in saline (0.1 mL per rat) was injected s.c. into the plantar surface of the left hind paw 1 h after the test sample (1, 3, 10 mg/kg) had been administered orally. The paw volume was measured by plethysmometer (# 7140) from Ugo Basile, Varese, Italy after 3hr of carrageenan injection. Dexamethasone (30 mg/kg), an anti-inflammatory drug, was used as a positive control (24).

2.3.4 In vivo efficacy of T-02 in inflammatory pain (CFA Induced inflammatory pain)

CFA was injected Intra-plantar at concentration (75µg/150µL). CFA induced mechanical hyperalgesia was quantified by Von Frey test on day 2 (48h post injection of CFA). A cut off of <5.0 g was considered for selection of animals before treatment. The effect of test drug (1, 3, 10 mg/kg) and standard Gabapentin (150 mg/kg) administered orally on mechanical hyperalgesia was measured at 60 min post treatment (25, 26).

Behavioral tests:

Measurement of mechanical hyperalgesia

Mechanical hyperalgesia was measured by using previously described up-down method (26) von Frey filaments (Bioseb, France) were used to assess mechanical hyperalgesia. The animals were placed in a plexiglass cage (16 x 24 x 14 cm) with a grid bottom and adapted for at least 10 minutes. Mechanical stimuli were generated by touching the plantar region of the

left hind paw of the rat with a continuous increasing/decreasing pressure changes by the pattern of (xoxoxo). For the paw withdrawal threshold the mean of two independent measurements was calculated. Filaments used in experiment were 3.61, 3.84, 4.08, 4.31, 4.56, 4.74, 4.93 and 5.18 g. The values of the paw withdrawal thresholds were manually recorded and noted in result sheet (27).

2.3.5 In vivo efficacy of T-02 in nociceptive pain (Formalin induced nociception)

Animals were randomized by dose groups. On day of experiment non fasted animals were weighed and dosed with test compounds (1, 3, 10 mg/kg) and standard Tramadol (40 mg/kg) orally 1hr. before formalin challenge. Formalin was injected s.c. into the dorsal lateral surface of the left hind paw and time spent licking and biting in seconds was recorded for each animal at 5 min interval for 40 min immediately after formalin injected (28).

2.4 Statistical analysis

One-way analysis of variance followed by Dunnett test and Two way analysis of variance followed by Bonferroni (ANOVA; Graph Pad PRISM®, Version 4.0, San Diego, CA, USA) was applied to determine significant differences between the groups. A value of $P<0.05$ was considered significant. The pharmacokinetic parameters were calculated by a non-compartmental method with Win Nolin professional Version 4.1.

3. RESULTS

3.1 In vivo pharmacokinetic profile of T-02

The pharmacokinetics of T-02 (P-Tol) was evaluated in SD rats The PK profile of Test Compound T-02 (P-TOL) following PO administration, maximum plasma concentration (Cmax) was observed at 0.58 ±0.38 h (tmax) and the terminal half-life (t1/2, ß) was found to be 2.52±0.09 h. Following IV administration, elimination half life (t1/2, ß) was found to be 2.52±0.09 h and clearance was ~ 115.69±13.04 mL/min/Kg. The absolute oral bioavailability was 84 %.

Compound	Route	Dose (mg/kf)	Tmax (h)	Cmax or Co (ng/ml)	AUC last (ng.h/ml)	AUC inf (ng.h/ml)	T1/2 (h)	Vd (L/kg)	CL (ml/min/Kg)	%F
T-02	IV	1	-	194.13 ± 22.50	139.14 ± 15.71	145.65 ± 16.75	2.52 ± 0.09	11 ± 0.75	115.69 ± 13.04	84
	PO	3	0.58 ± 0.38	199.65 ± 33.71	351.32 ± 57.87	356.02 ± 56.90	-	-	-	

Table 1 Pharmacokinetic Parameters of T-02 (P-TOL) in Sprague Dawley Rat

3.2 Effect of T-02 on inflammation (Carrageenan induced paw edema)

The anti-inflammatory effects of tests compound T-02 (P-Tol) were initially evaluated in rats with the goal of proving the anti-inflammatory property of test compound; we found that the oral administration of compound reduced significantly the carrageenan-induced paw edema. The results in (Fig 2) indicate that the administration of T-02 (P-Tol) (1, 3 and 10 mpk P.O.) 60 min before carrageenan reduced significantly the oedema at 3h after the carrageenan injection (Table 2). This result reinforces the idea that the test compound possesses peripheral action, may relate to the arachidonic acid cascade.

Groups	% anti-inflammatory activity
Vehicle	0
T-02_1 mpk	6
T-02_3 mpk	22
T-02_10 mpk	52
Dexamethasone	62

Table 2 Effect of T-02 on Carrageenan induced paw edema

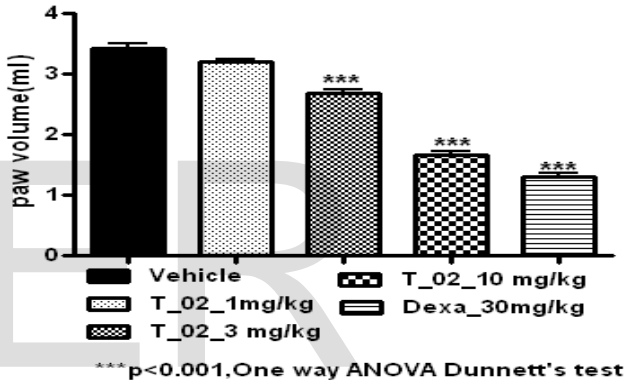

Fig. 2 Effect of T-02 on Carrageenan induced paw edema

3.3 Effect of T-02 on inflammatory pain (CFA Induced inflammatory pain)

In the present study, it has been demonstrated that acute oral administration of the T-02 (P-Tol) compound, reduces the mechanical hyperalgesia associated with an inflammatory pain model. In similar Gabapentin also reduced hyperalgesia. The results in (Fig 3) indicate that the administration of T-02 (P-Tol) (1, 3 and 10 mpk P.O.) reduced significantly CFA Induced Mechanical hyperalgesia (Table 3).

Treatment	% MPE@ 60 min			
	Mean	SEM	SD	N
Vehicle	0	5	12	6
T-02_1 mpk	20	5	12	6
T-02_3 mpk	41	3	8	6
T-02_10 mpk	53	3	7	6
Gabapentin_150 mpk	72	3	8	6

Table 3 Effect of T-02 on CFA induced mechanical hyperalgesia

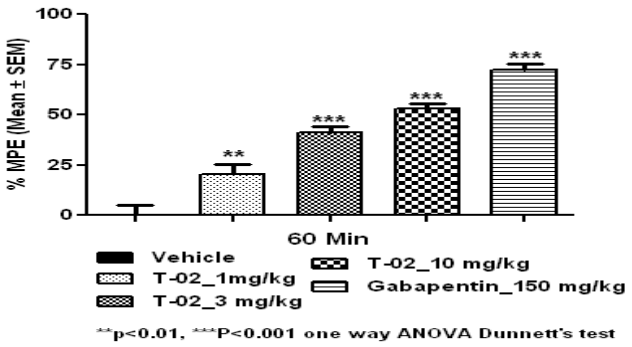

Fig. 3 Effect of T-02 on CFA induced mechanical hyperalgesia

3.4 Effect of T-02 on nociceptive pain (Formalin induced nociception)

Oral administration of tests compound T-02 (P-Tol) prior formalin injection decreased significantly the duration of nocifensive behaviors (licking and biting of injected paw) observed in phase II (Fig 4) in a dose-dependent fashion. Similarly, tramadol, which is widely used to treat nociceptive pain, had significant effect in Phase II. The results presented here support the hypothesis that formalin elicits pain-related behaviors by activating on primary afferent nociceptors. Additionally, these findings suggest that T-02 (P-Tol) blocks formalin-evoked responses in vivo through its inhibitory actions (Table 4).

Test Compound	% Reversal	
	Ist Phase	2nd Phase
Tramadol-40mpk	58±9	82±8
T 02 - 1mpk	55±5	34±9
T 02 - 3mpk	41±10	57±3
T 02 -10mpk	65±4	66±3

Table 4 Effect of T-02 on formalin induced nociception

Test Compound	Total paw licking & biting(Sec)	
	Ist Phase	2nd Phase
Vehicle	61±8	215±15
Tramadol-40mpk	26±5	39±17
T 02 - 1mpk	27±3	143±19
T 02 - 3mpk	36±6	93±6
T 02 -10mpk	21±3	74±7

Table 5 Total paw licking and biting duration in sec.

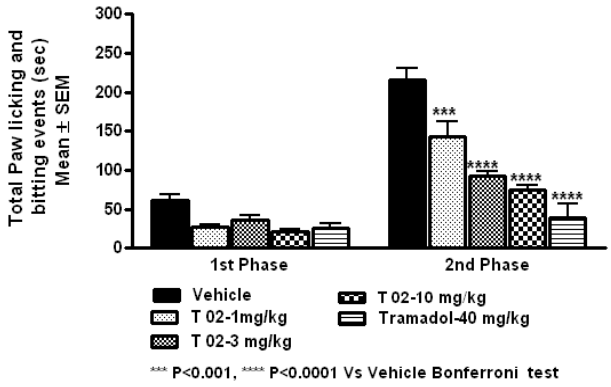

Fig. 4 Total paw licking and biting duration in sec.

4. DISCUSSION

The present study strongly stated that the anti-inflammatory and anti-nociceptive activity of new derivative of Quinazoline 2-(2-methyl-4-oxoquinazolin-3(4H)-yl)-N-p-tolylacetamide.

In general, quinazoline derivatives are known to possess wide range of activities. A specific activity depends on the substituent present at an appropriate position of quinazoline (29).

2-methyl -3-substituted oxoquinazolin derivatives with substitution on benzene ring at position-3 possess remarkable anti-inflammatory activity in carrageenan induced paw edema in dose dependant manner. This edema depends on the participation of kinins and polymorphonuclear leucocytes with their pro-inflammatory factors including prostaglandins. Development of edema in the paw of rat after carrageenan injection is a biphasic event. Initial phase observed during the first hour is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosome. Carrageenan-induced edema is characterized by the presence of PGs and other compounds of slow reaction. COX-2 is an inducible isoform found in activated inflammatory cells that generates prostanoid mediators of inflammation. The result of the present study indicates that 2-methyl -3-substituted oxoquinazolin derivatives and Diclofenac play a crucial role as protective factors against the carrageenan-induced acute inflammation (30). Anti-inflammatory effect of 2-methyl -3-substituted oxoquinazolin could be due to the inhibition of COX-2 inflammatory mediator. This could be one of the possible mechanisms of 2-methyl -3-substituted oxoquinazolin derivative.

The further study was used to assess the effect of compound on inflammatory pain. Injection of complete Freund's adjuvant (CFA) into a rat's hind-paw provides a very good model in order to study the mechanism of inflammatory pain and to screen for anti-hyperalgesic drugs. CFA-induced mechanical hyperalgesia is mediated via peripheral activation of NMDA receptors and release of inflammatory mediator cycloxygenase (COX) after the injection of CFA. A 2-methyl -3- substituted oxoquinazolin derivative shows the good activity on inflammatory pain in dose dependant manner. The exact mechanisms are not clear. The possible explanations might be due to the inhibition of inflammatory mediator as we have stated in earlier study or an antagonist of NMDA receptor (31).

The anti-nociceptive effect of quinazoline derivative has been demonstrated by using Formalin induced nociception model. Oral administration of compound produced a dose-related antinociceptive effect when assessed in formalin induced nociception model. Formalin injection into the rat hindpaw initiates triphasic spontaneous nociceptive behaviors consisting of flinching, and licking and/or biting of the injected paw. The response to formalin is typically biphasic. The early phase of intense pain, named non-inflammatory pain which starts immediately after formalin injection, seems to be caused predominantly by activation of C-fibers subsequent to peripheral stimulation. The late phase of moderate pain, named inflammatory pain which starts about 20 min after formalin injection and lasts about 40 to 60 mins, appears to be caused by tissue and functional changes in the dorsal horn of the spinal cord and by local inflammation with a release of inflammatory mediators (32). The antinociceptive effect of 2-methyl -3- substituted oxoquinazolin derivative might be due the inhibitory action on inflammatory mediators.

To summarize the present work, we would like to state that newly synthesized 2-methyl -3- substituted oxoquinazolin derivative was found to be potent anti-inflammatory and antinociceptive agent. The further studies should be initiated to know exact mechanism of action in different pain or diseased pain condition.

5. ACKNOWLEDGMENT

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