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Original Research Article

Screening of novel 2-4methylphenylimino-3-carboxamide substituted thiophene compound for peripheral analgesic activity

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ABSTRACT

Background: Pain is an unpleasant sensation that can range from mild, localized discomfort to agony. The mechanism of pain perception involves dedicated subsets of peripheral and central neurons. For management of pain, currently, available treatment regimens are opioid analgesics and NSAIDs which are easily available over the counter drugs. Long term use of analgesics can lead to significant impact on human renal system, cardiovascular system and analgesic abuse etc. So, there is a need for novel, safe and cost effective analgesic compound. Hence a study on 2-4methylphenylimino-3-carboxamide substituted thiophene compound, a novel thiophene compound has been carried out in different experimental animal models.
Methods: Two methods were used to evaluate the peripheral analgesic activity of 2-4methylphenylimino-3-carboxamide substituted thiophene compound. First method was Acetic acid induced writhing and compared to standard drug aspirin. Second method was late phase of formalin induced paw licking in mice and compared to a standard drug, aspirin.
Results: With Acetic acid induced writhing, 40mg/kg dose of 2-4methylphenylimino-3-carboxamide substituted thiophene compound has shown maximum Pain Inhibition Percentage (PIP) of 78 % compared to 91% by Aspirin. Whereas with late phase of Formalin test, 40mg/kg dose of 2-4methylphenylimino-3-carboxamide substituted thiophene compound has shown maximum PIP of 58% in late phase compared to 86% by aspirin. The results were statistically significant with $p<0.05$.
Conclusions: It can be concluded that 2-4methylphenylimino-3-carboxamide substituted thiophene compound found to have moderate peripheral analgesic activity as evident in acetic acid induced writhing and late phase of formalin tests.

Keywords: Analgesic, Acetic acid induced writhing, Formalin test, Thiophene, 2-4 Methylphenylimino-3-carboxamide substituted thiophene compound

INTRODUCTION

Despite an enhanced recognition of the molecular mechanisms of nociception, existing analgesic drugs continue to remain restricted in terms of efficacy since several mechanisms act in tandem to produce pain. Drugs acting either on the opioid receptor system or inflammatory cascade have been the only successful molecules over the past few decades. At this time, the marketed analgesic drugs are at best modestly effective and many of them are known to cause unacceptable side effects or have been linked to longterm safety issues.[1] Among the various marked oral analgesic drugs ibuprofen has emerged as the safest and is available as an OTC product in many countries.[2] No one likes the pain but it is one of the most important defensive mechanisms in our body, which provide us signal about the abnormality. Depending upon the severity and intensity of threshold, pain has classified into two main classes either acute or

chronic. When pain occurs in quick succession and disappears after few hours or day with or without medication then it is acute type and one the other hand if it develops after long time and goes slowly or incompletely than it is a chronic type. From the point or origin to the point of receiving, sensation of pain, involve central as well as peripheral nervous system. Alleviation of pain depends on several factors like its type, its origin point, and causes behind that pain. Neurogenic pain may be arise due to anxiety, depression, mania, epilepsy, seizer, phobia and many more, so their treatment need application of neurotherapeutic agents which act on serotonin/nor epinephrine reuptake inhibitor while the normal pain such as, body ach, arthritic pain, inflammatory pain, traumatic pain need normal analgesic medication like non-steroidal anti-inflammatory agents.[3] The chronic pain state is characterized by changes in somatosensory processing, affect, and motivation.[4] Human imaging studies suggest that alterations in mesolimbic circuitry of the brain accompany establishment of this state.[5] The nucleus accumbens (NAc) is a key hub in themesolimbic circuitry, and the release of dopamine (DA) in the NAc by neurons in the ventral tegmental area (VTA) modulates its functional connectivity with other regions implicated in chronic pain, such as the prefrontal cortex, amygdala, and hippocampus.[6,7]

In today's competitive world, people are always in race to surge ahead of each other. In doing so, we compromise with our health and suffer with pain viz. headache, back pain etc. Many of these pains are commonly due to our lifestyle and occupational hazards. In order to relieve ourselves from the pain, we rely on pain killer, but there is a great need for the development of better drugs for the alleviation and control of both acute (immediate) and chronic (long-term, pathological) pain.[8] Long term use of available analgesics causes disturbances in the body system. Opioids cause multiple adverse effects involving respiratory system, central nervous system, gastrointestinal system such as sedation, mental clouding, blurring of vision, respiratory depression, constipation and urinary retention. NSAIDs cause adverse effects involving several systems of the body such as gastrointestinal tract, kidneys, central nervous system, cardiovascular system, blood and skin. A search for new, safe & cost-effective analgesic continues. A study on 2-4methylphenylimino-3-carboxamide substituted thiophene compound has been carried out. The parent compound has been studied and shows predominant analgesic activity at 15 to 30 mg/kg.[9]

METHODS

Currently in this study, peripheral analgesic activity of test compound was evaluated using two experimental animal models. First method was Acetic acid induced writhing in mice and second method was Late phase of formalin induced paw licking in mice. The study was conducted in the Animal house affiliated to the People's Education Society Institute of Medical Sciences and Research, Kuppam, Chittoor district, Andhra Pradesh after obtaining approval from the Institutional Animal Ethics Committee (No: PES IMSR / Pharma / IAEC / 12 / 2012-13). All the animals were handled with gentle care as per the CPCSEA guidelines.

Details of experimental animals used

The current study was carried out in healthy male Swiss albino mice (Mus musculus) weighing between 20 to 25 g as they are the most widely used strain for assay of analgesics 15. Female mice were excluded to avoid the effect of estrous cycle. Also, the diseased mice and male mice weighing < 20g and >25g were also excluded. All the mice were in propylene cages in 12:12 hours light: dark cycle, under standard laboratory conditions and water was provided ad libitum. All the mice were kept in fasting state 12 hours prior to the conduction of experiment.

Test compound

2-4 methyl phenylimino-3-carboxamide substituted thiophene compound. Thiophene is a five-membered heterocyclic compound having Sulphur as the heteroatom. Over the past few years, research groups have conducted a comprehensive program me towards the synthesis of thiophene and their fused derivatives which have reported to possess wide range of activity. It has been reported that the parent compound, Thiophene and its derivatives possess analgesic, anti-inflammatory, local anesthetic, antimicrobial and antifungal activities.[10-14] In view of these findings, another novel synthesized derivative of thiophene namely 2-(4-Methylphenylimino)-N-(2-Chlorophenyl)-5-Isopropyl-4-Methyl Thiophene-3-Carboxamide substituted Thiophene was undertaken in the present study to evaluate the analgesic activity. It was synthesized in the P.E.S college of pharmacy, Bangalore and obtained by their kind courtesy.

Acetic acid induced writhing in mice

This experiment has been performed to verify the peripheral analgesic activity of 2-4methylphenylimino-3-carboxamide substituted thiophene compound. In this chemical method, acetic acid was injected as irritant into peritoneum to induce pain of peripheral origin in mice.[16,17] If the test compound has analgesic activity, then it can be inferred from decrease in frequency of writhings. Sigmund et al first described manifestations of abdominal writhings in mice as an arching of back, extension of hind limbs and contraction of abdominal musculature.[18]

The formalin test may allow dissociation between inflammatory and non-inflammatory pain, a rough classification of analgesics according to their site and their mechanism of action.

Animals

A total of 30 male Swiss albino mice were considered for this study.

Drugs and reagents

The test compound 2-4methylphenylimino-3-carboxamide substituted thiophene compound cannot be dissolved in water hence, drug suspending agent 10% Tween-80 or Polysorbate-80 (Merck Specialties private limited, Mumbai), was used. Acetic acid 0.6% was used. The standard drug utilized in this experiment was Aspirin, and test compound 2-4methylphenylimino-3-carboxamide substituted thiophene compound, both the drugs were administered orally using gavage.

Five groups of mice were created, and all the mice were selected randomly. Each group contains six mice. Group I received 0.5 ml of tween-80 per oral which was considered as control group. Group II received Aspirin which was considered as standard group. Groups III, IV and V received the test drug 2-4 methylphenylimino-3-carboxamide substituted thiophene compound per orally at 10mg/kg, 20mg/kg, 40mg/kg respectively and all these three groups were considered as test groups. All the grouping details were mentioned below in Table 1.

Table 1: Classification of mice into the groups in Acetic acid induced writhing in mice.

Group	Drug	Dose
I	Control (10% tween-80)	0.5 ml PO
II	Aspirin	100 mg/kg/PO/0.5 ml
III	2-4methylphenylimino-3-carboxamide substituted thiophene compound	10 mg/kg/PO/0.5 ml
IV	2-4methylphenylimino-3-carboxamide substituted thiophene compound	20 mg/kg/PO/0.5 ml
V	2-4methylphenylimino-3-carboxamide substituted thiophene compound	40 mg/kg/PO/0.5 ml

Mice were administered orally with respective drug. After 1 hour, 0.1 ml of 0.6% acetic acid is injected intra-peritoneally. Total number of writhes are recorded for a period of 20 minutes. For scoring purpose, writhe is indicated by characteristic abdominal stretching with simultaneous stretching of at least one hind limb.[19] Pain inhibition percentage (PIP) of the test group is calculated from the mean writhing count of control group.[20]

Formalin induced paw licking in mice - only late phase

Late phase of formalin induced paw licking method has been evaluated for peripheral analgesic activity of 2-4-methyl-phenylimino-3-carboxamide substituted thiophene compound. 30 male mice (20-25g) divided into 5 groups consisting of 6 animals in each group were randomly grouped as follows.

Animals

A total of 30 male Swiss albino mice were included for this study.

Drugs and reagents

Total 10% Tween-80 or Polysorbate-80 was used as a drug suspending agent, whereas Aspirin was used as a standard drug and 2-4methylphenylimino-3-carboxamide substituted thiophene compound as test compound. All drugs were administered orally using gavage. The procedure was done as per the standard method suggested by Murray et al and Hunskaar and Hole.[21-23] Five groups of mice were created, and all the mice were selected randomly. Each group contains six mice. Group I received 0.5 ml of tween-80 per oral which was considered as control group. Group II received aspirin per orally at the dose of 100 mg/kg/PO /0.5ml which was considered as standard group.[24] Groups III, IV and V received the test drug 2-4methylphenylimino-3-carboxamide substituted thiophene compound per orally at 10 mg/kg, 20 mg/kg, 40 mg/kg respectively and all these three groups were considered as test groups. All the grouping details were mentioned below in Table 2.

Table 2: Classification of mice into the groups in Formalin test.

Group	Drug	Dose
I	Control (10% tween-80)	0.5 ml PO
II	Aspirin	100 mg/kg/PO /0.5 ml24
III	2-4methylphenylimino-3-carboxamide substituted thiophene compound	10 mg/kg/PO/0.5 ml
IV	2-4methylphenylimino-3-carboxamide substituted thiophene compound	20 mg/kg/PO/0.5 ml
V	2-4methylphenylimino-3-carboxamide substituted thiophene compound	40 mg/kg/PO/0.5 ml

Procedure

Drugs were administered orally with the respective drugs. After 1 hour, 0.02 ml of 5% formalin is injected into the dorsum region of hind paw of mice. Pain response is indicated by the paw licking or biting of the paw. Number of paw lickings25 was recorded in late phase of 20-30 min. Analgesic response or protection was indicated if both paws were resting on the floor with no obvious favoring of the injected paw.

Evaluation

Pain inhibition percentage was calculated using the formula:

PIP = [No. of Licks (control–treated group) / No. of licks in control] × 100

RESULTS

Acetic acid induced writhing

Numbers of writhes were observed for 20 minutes after the intraperitoneal administration of 0.1 ml of 0.6% acetic acid in each mouse. Following observations were made and presented as mean number of writhes with±SD. The control group of mice (10% tween-80) produced 46.5±2.33 writhes. The standard group of mice (aspirin-100 mg/kg/PO) produced 3.83±1.16 writhes and has shown the pain inhibition percentage (PIP) of 91.76 when compared to control group. The test group of mice (test compound-10 mg/kg/PO) produced 26.66±2.16 writhes and has shown PIP of 42.66 when compared to control group.

Table 3: Mean no. of writhes and pain inhibition percentage produced by control, aspirin (100 mg/kg) and 2-chlorothiophene at 10, 20 and 40 mg/kg in Acetic acid induced writhing test in mice

S. no.	Group (n=6)	Mean no. of writhes±SD	Pain inhibition percentage (%)	P value
1	Control (10% tween-80)	46.5±2.33	-	-
2	Aspirin (100 mg/kg/PO)	3.83±1.16	91.76	< 0.0001
3	Test compound (10 mg/kg/PO)	26.66±2.16	42.66	< 0.0001
4	Test compound (20 mg/kg/PO)	18±2.60	61.29	< 0.0001
5	Test compound (40 mg/kg/PO)	10±2.60	78.49	< 0.0001

Table 4: Mean no. of paw licks and pain inhibition percentage (PIP) produced by control, aspirin (100 mg/kg) and Test compound at 10, 20 and 40 mg/kg in late phase of formalin induced paw licking in mice

S. no.	Group (n=6)	Mean No. of licks±SD	Pain Inhibition Percentage (%)	P value
1	Control (10% tween-80)	35.83±2.78	-	-
2	Aspirin (100 mg/kg/PO)	5±1.78	86.04	< 0.0001
3	Test compound (10 mg/kg/PO)	30.66±5.85	14.42	< 0.0001
4	Test compound (20 mg/kg/PO)	25.16±1.72	29.77	< 0.0001
5	Test compound (40 mg/kg/PO)	15±1.78	58.13	< 0.0001

Anova (P)<0.0001

The test group of mice (test compound-20 mg/kg/PO) produced 18±2.60 writhes and has shown PIP of 61.29 when compared to control group. The test group of mice (test compound - 40 mg/kg/PO) produced 10±2.60 writhes and has shown PIP of 78.49 when compared to control group. All the details were explained below in Table 3 and Figure 1.

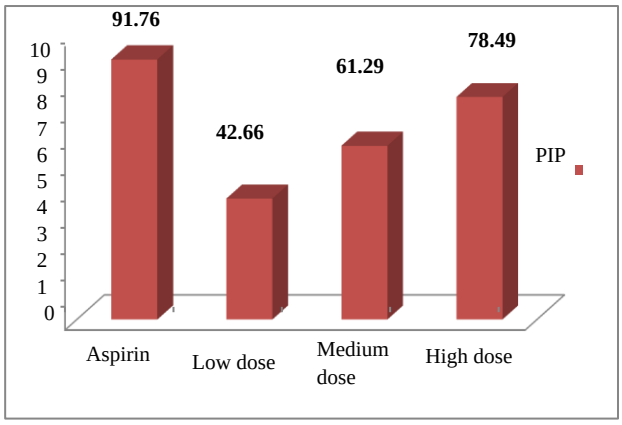

Figure 1: The pain inhibition percentage produced by aspirin (100 mg/kg) and test compound at 10, 20 and 40 mg/kg in acetic acid induced writhing test in mice.

Formalin induced paw licking in mice

Number of paw licks

Peripheral analgesic activity of test compound was determined by the late phase. Number of paw lickings were recorded in late phase of 20-30 minutes. Pain inhibition percentage was calculated by comparing the drug treated values to that of control group. Mean number of paw licks and PIP produced in late phase of formalin test shown in Table 4.

Number of paw licks

Number of paw licks were observed both in in late phase (20-30 min) after the injection of formalin. Following observations were made and presented as mean number of licks with±SD. The control group of mice (10% tween-80) has shown 35.83±2.78 licks in late phase. The standard group of mice (Aspirin-100 mg/kg/PO) has shown 05.00±1.78 licks in late phase. The test group of mice (Test compound-10 mg/kg/PO) has shown 30.66±5.85 licks in late phase. The test group of mice (Test compound -20 mg/kg/PO) has shown 25.16±1.72 licks in late phase. The test group of mice (test compound- 40 mg/kg/PO) has shown 15±1.78 licks in late phase. Pain inhibition

percentage details were explained below in Table 4, Figure 2.

Statistical analysis

All the continuous variables in this experiment were expressed as Mean and Standard deviation. Between group analyses was done with one-way ANOVA, followed by Dunnett post hoc test. Within group analyses was done with Repeated measure ANOVA test. All statistical calculations were performed using the software SPSS (statistical package for social service) for windows version 19.0. For statistical significance, two tailed probability<0.05 is considered.

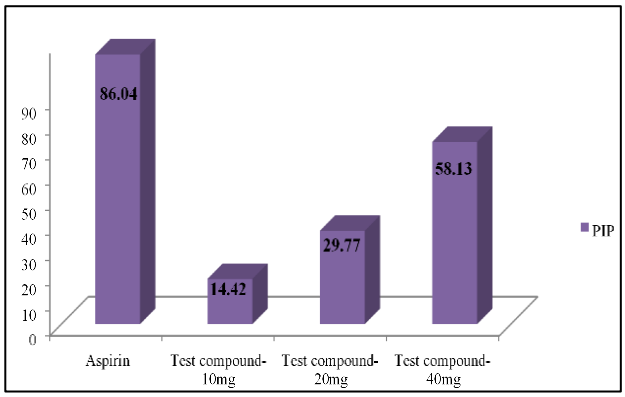

Figure 2: The pain inhibition percentage (PIP) shown by aspirin (100 mg/kg) and test drug at 10, 20 and 40 mg/kg in late phase of formalin induced paw licking in mice.

DISCUSSION

Late phase of formalin induced paw licking in mice

In late phase, The PIP produced by the Aspirin (100 mg/kg) is 86.04 whereas PIP values produced by 2-4 methylphenylimino-3-carboxamide substituted thiophene compound are 14.42, 29.77, 58.13 at the doses of 10, 20 & 40 mg/kg respectively. This indicates that the maximum PIP is produced by 40 mg/kg dose of test compound, however the activity is lesser compared to aspirin (100 mg/kg). Viswanatha et al evaluated analgesic activity of Newly synthesized bicyclothieno 1, 2, 3-triazinestest drugs (BTT-1, BTT-2, BTT-3, BTT-4) have showed significant analgesic activity by decreasing the number of lickings in formalin test ($p<0.01$, $p<0.05$, $p<0.01$, $p<0.01$), all the test drugs were found to be effective only in late phase of nociception.[27]

Acetic acid induced writhing test in mice

The PIP produced by the aspirin (100 mg/kg) is 91.76 whereas PIP values produced by the test compound i.e 2-4 methylphenylimino-3-carboxamide substituted thiophene compound are 42.66, 61.29, 78.49 at the doses of 10, 20 & 40 mg respectively. This indicates that maximum PIP is produced by 40 mg/kg dose of test compound, however the activity is lesser compared to Aspirin (100 mg/kg). Molvi et al evaluated the Sets of tetrasubstituted thiophene esters 4a-4g, 5a-5f and 6a-6e in vivo analgesic activity in acetic acid induced writhing response model at 10 mg/kg dose exhibited analgesic activity of 56% inhibition using ibuprofen as standard drug in mice.[26]

The current study infers that 2-4-methylphenylimino-3-carboxamide substituted thiophene compound has good peripheral analgesic activity at 40 mg/kg, but lesser than that of Aspirin (100 mg/kg).

The drug can be claimed for peripheral analgesic activity as the maximum PIP is>50% in both Formalin induced paw licking and Acetic acid induced writhing methods. If the study period had been continued, then the complete time course of action for all the drugs could have been recorded. Further investigation can throw light on the minimum effective dose and ceiling doses of all the drugs. This study is a simple screening test for the presence or absence of analgesic activity for 2-4 methylphenylimino-3-carboxamide substituted thiophene compound. The purpose of the study is served by demonstrating the analgesic activity in the doses employed. These results cannot be interpolated for human.

CONCLUSION

From this study, we can conclude that gentamicin is more the study was undertaken for screening of the synthetic compound 2-4-methylphenylimino-3-carboxamide substituted thiophene compound for analgesic activity. It has shown maximum peripheral analgesic activity (>50 %) at 40 mg/kg dose in both Formalin induced paw licking and Acetic acid induced writhing methods which cannot be interpreted as having significant peripheral analgesic activity when compared to aspirin (100 mg/kg). From this study, it can be concluded that the test drug, 2-4 methylphenylimino-3carboxamide substituted thiophene compound has good amount of peripheral analgesic action at 40 mg/kg dose in mice to a lesser extent. Further studies may be conducted in a greater number of animal species models to evaluate and confirm the findings.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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