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Research Article

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF 2-(4-FLUOROBENZYLTHIO)-N-(SUBSTITUTED PHENYL) PYRIMIDINE-4-AMINES

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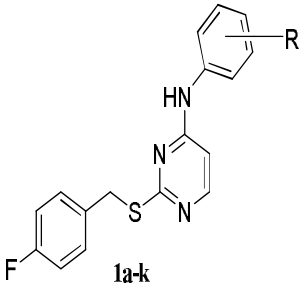
ABSTRACT

Pyrimidine scaffold being an integral part of DNA and RNA, occupy a unique and distinctive role in medicinal chemistry. In the past few years, fluorinated heterocyclic systems have been incorporated into drug discovery research to improve the drug physico-chemical properties. In view of this rational we have synthesized 2-(4-fluorobenzylthio)-N-(substituted phenyl) pyrimidine-4-amines **(1a-k)** and evaluated them for analgesic and anti-inflammatory activities. The analgesic and anti-inflammatory activities of the synthesized compounds 2-(4-fluorobenzylthio)-N-(substituted phenyl) pyrimidine-4-amines **(1a-k)** were carried out in *albino* mice and *spruge dawley* rats respectively. Compounds **1c** and **1f** showed good analgesic activity and remaining compounds exhibited moderate to poor analgesic activity when compared to the standard drug Pentazocin. Compounds **1a** and **1j** showed excellent anti-inflammatory activity and compounds **1g**, **1h, 1i** and **1k** showed good anti-inflammatory activity in comparison with the standard drug Diclofenac sodium. Remaining compounds exhibited moderate to poor anti-inflammatory activity. The present study showed that fluorine substituted heterocycles might serve as pharmacologically potent and biologically important drugs.

Keywords: Analgesic activity, Anti-inflammatory activity, Pyrimidines.

INTRODUCTION

Pyrimidine scaffold being an integral part of DNA and RNA, occupy a unique and distinctive role in medicinal chemistry. Pyrimidine derivatives have been reported to possess a variety of pharmacological activities, notable among are the antibacterial[1], antihypertensive[2], antihistaminic[3], antifungal[4], anti-inflammatory[5], antiviral[6] and anticancer drugs[7]. In the past few years fluorinated heterocyclic systems have been incorporated into drug discovery research[8-10] to improve the drug physico-chemical properties. Inflammation is a complex defensive mechanism of the body to any noxious stimulus; this process may vary from a localized to a generalized response characterized by the accumulation of fluids and leukocytes leading to edema and pain[11]. Inflammation plays an important role in various diseases with high prevalence within population such as rheumatoid arthritis, atherosclerosis and asthma. During our drug discovery program, we have synthesized 2-(4-fluorobenzylthio)-N-(substituted phenyl) pyrimidine-4-amines **(1a-k)**[12] (**Figure 1**) and herein report the analgesic and anti-inflammatory activities.



1a **1b** **1c** **1d** **1e** **1f** **1g** **1h** **1i** **1j** **1k**

Where R= 2-F, 4-Br,3-CF_3, 4-F, 4-Cl, 4-0CH_3, 3-F, H, 2-NO_2, 3-NO_2, 4-NO_2, 2-F,4-I

Fig. 1: Structure of Compounds (1a-k)

2. MATERIALS AND METHODS

Adult healthy *albino* mice (18-24 gm) of either sex were used for analgesic activity. Male or female spruge dawley rats (150-180 gm) were used for anti-inflammatory activity. All the animals were maintained under controlled standard animal house conditions with easy access to food and water. The institutional ethical committee for animal cares and use approved for the experimental procedure (Reg. No: 1046/a/07/CPCSEA).

2.1. Hot plate method

Eddy's hot plate method was used for the measurement of the analgesic activity by using Pentazocin as standard. In the hot plate method, albino mice were divided into ten groups with six animals in each group. One group served as a negative control (received 5% gum acacia 5 ml/kg), the second group received Pentazocin (5 mg/kg), while the third to tenth group received the compounds **1a**, **1b**, **1c**, **1d**, **1e**, **1f**, **1g** and **1j** respectively (200 mg/kg p.o.). Each mouse was placed on a hotplate (55°C) and the time lapse for the mouse to respond to the thermal pain (reaction time) was noted. Rubbing of palms or jumping was used as endpoint.

2.2. Mercury displacement method

Plethysmograph (mercury displacement method) was used for evaluation of anti-inflammatory activity against Diclofenac sodium as standard. For anti-inflammatory activity thirteen groups of six animals in each group were made serving for control (group-I), standard (group-II) and test compounds (group-III to XIII). Control groups received control, standard group received Diclofenac sodium 5 mg/kg p.o. and group-III to XIII received test compounds. Sixty minutes later rats were challenged with subcutaneous injection of 0.1 ml 1% formalin into the plantar region of left hind paw. The paw is marked at the level of lateral malleolus and immersed up to the mark. Paw volume is measured plethysmographically immediately after injection and again at 1, 2, 3 and 4 hour. The results were subjected for one way annova by using Graph Pad Prism software.

3. RESULT AND DISCUSSION

3.1. *In-vivo* analgesic activity

Analgesic activity was carried out by hot plate method[13] by using Pentazocin as standard. Compounds **1c** and **1f** showed good analgesic activity and remaining compounds exhibited moderate to poor analgesic activity when compared to the standard drug Pentazocin. The results were subjected for one way annova by using Graph Pad Prism software. The results are tabulated in (**Table 1**) and shown graphically in (**Figure 2**).

3.2. *In-vivo* anti-inflammatory activity

The anti-inflammatory activity was studied by formalin induced rat hind paw oedema model [14,15] measured by plethysmograph (mercury displacement method) using Diclofenac sodium as standard. Compounds **1a** and **1j** showed excellent anti-inflammatory activity, compounds **1g**, **1h, 1i** and **1k** showed good anti-inflammatory activity in comparison with the standard drug Diclofenac sodium. Remaining compounds exhibited moderate to poor anti-inflammatory activity. The results were subjected for one way annova by using graph pad prism software. The effect of drugs in percentage reduction in paw volume and mean increase in paw volume are tabulated in (**Table 2** & **3**) and shown graphically in (**Figure 3** & **4**).

4. STRUCTURE ACTIVITY RELATIONSHIP OF COMPOUNDS (1a-k)

SAR of the compounds 2-(4-fluorobenzylthio)-N-(substituted phenyl) pyrimidine-4-amines **(1a-k)** has been investigated. Compounds **1c** and **1f** have shown good analgesic activity which may be due to the presence of a fluoro substituent at its *para* and *meta* position on the aromatic nucleus. Introduction of a fluoro substitution on the phenyl ring at the *ortho* **(1a)** position resulted in loss of potency. This indicates that small atom like flouro which is similar to hydrogen in atomic radius is well tolerated in the *meta* and *para* positions, but substitution at the *ortho* position diminishes activity[16]. The anti-inflammatory activity of **1a** also may be due to the presence of fluoro substitution at its *ortho* position and the activity of **1j** may be due to the presence of electron withdrawing nitro group at its *para* position which inhibits COX-2, playing an important role in inflammation and wound healing[17]. The drug receptor interactions might be stronger through the hydrogen bonding between nitro group and receptors, while the activity decreased for the *ortho* **(1h)** and *meta* **(1i)** substituted nitro compounds due to intramolecular hydrogen bonding but activity was higher when compared to remaining compounds. Compound **1k** also showed good activity may be because of additional halogen viz iodo in addition to fluoro at its *para* and *ortho* position and remaining compounds showed moderate to poor activity.

5. CONCLUSION

The present pharmacological studies of 2-(4-fluorobenzylthio)-N-(substituted phenyl)pyrimidine-4-amines **(1a-k)** indicates

that introduction of electronegative fluorine on the aromatic nucleus led to the enhancement of biological activity. Further, preclinical studies on the most potent molecule are under investigation.

Table 1: Effect of compounds on reaction time of mice in Eddy's hot plate method of analgesia

S. No	Compound	0 hour reaction time mean±SEM (in sec)	1 hour Reaction time mean±SEM (in sec)
1	1a	42.00 ±05.40	59.00 ±05.40 ns
2	1b	44.75 ±09.20	99.75 ±10.16 ns
3	1c	52.75 ±09.00	107.8 ±17.90ns
4	1d	41.25 ±02.63	75.25 ±10.56 ns
5	1e	67.00 ±04.42	97.00 ±05.14 ns
6	1f	58.50 ±09.58	124.8 ±16.29 **
7	1g	37.50 ±04.57	78.00 ±18.06 ns
8	1j	48.75 ±10.47	71.50 ±09.56 ns
9	Control	63.75 ±08.38	65.75 ±08.71
10	Pentazocin	53.25 ±03.88	143.3 ±06.65***

***- potent activity, **- good activity, ns- less or no activity.

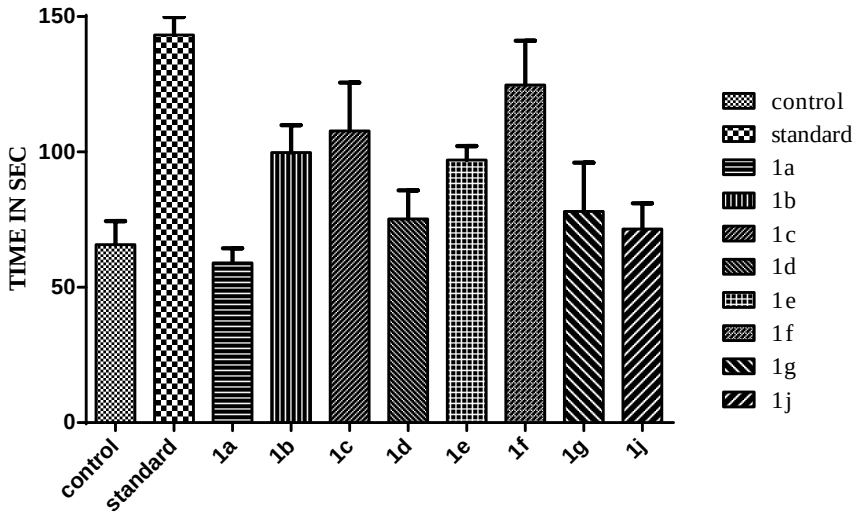


Fig. 2: Graph showing effect of compounds on reaction time

Table 2: Effect of compounds in percentage of reduction in Paw volume of rats

Compound	Effect of drugs in % of reduction in Paw volume of rats			
	1 hour	2 hour	3 hour	4 hour
1a	17.50	37.08	53.33	73.69
1b	8.33	10.00	35.00	36.07
1c	12.50	15.42	16.67	15.24
1d	10.00	24.17	28.33	39.64
1e	12.50	13.33	29.17	31.31
1f	8.33	15.42	18.33	20.71
1g	38.33	47.92	52.50	62.50
1h	37.50	42.08	48.33	65.36
1i	43.33	46.67	53.33	68.33
1j	43.33	60.00	65.00	82.26
1k	35.83	39.58	49.17	66.55
Diclofenac sodium	47.50	63.33	69.17	82.26

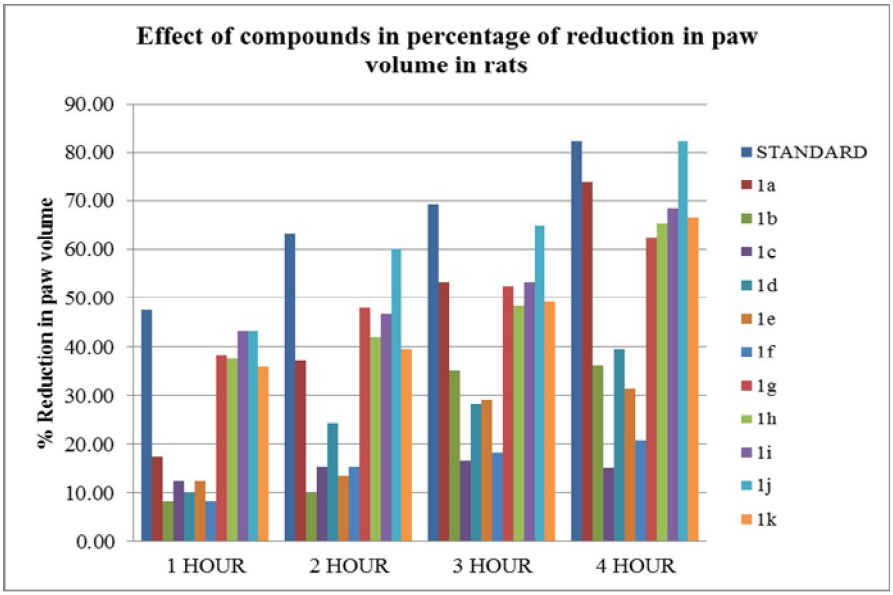


Fig. 3: Graph showing effect of compounds in percentage reduction in Paw volume of rats

Table 3: Mean increase in Paw volume of rats

Compound	Mean increase in paw volume of rats			
1a	0.48	0.33	0.18	0.15
1b	0.53	0.45	0.28	0.38
1c	0.50	0.43	0.38	0.48
1d	0.53	0.38	0.28	0.35
1e	0.50	0.43	0.30	0.40
1f	0.53	0.43	0.35	0.45
1g	0.35	0.25	0.18	0.20
1h	0.35	0.28	0.20	0.20
1i	0.33	0.25	0.15	0.18
1j	0.33	0.20	0.15	0.10
1k	0.38	0.30	0.20	0.20
Control	0.58	0.50	0.43	0.58
Diclofenac sodium	0.30	0.18	0.13	0.10

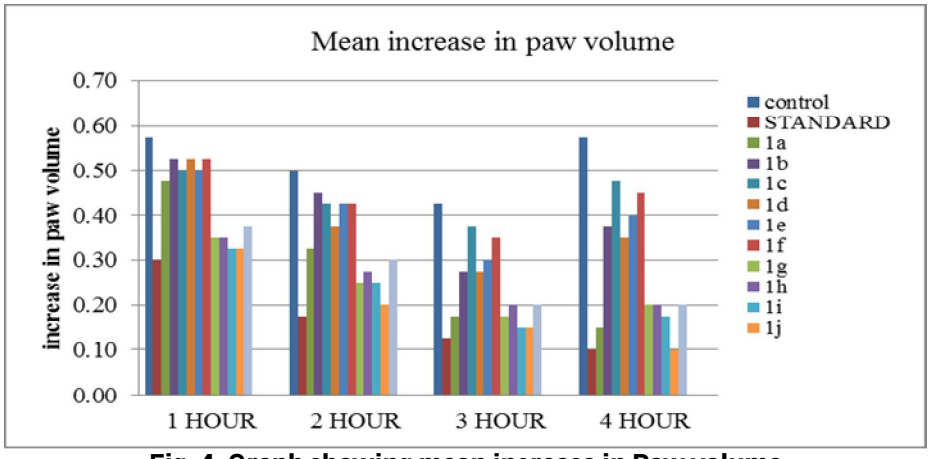


Fig. 4: Graph showing mean increase in Paw volume

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