



Original Research Article

Appraisal of analgesic and anti-inflammatory activity of some 2,5-disubstituted-1,3,4-thiadiazoles

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A B S T R A C T

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The purpose of this study was to explore the analgesic and anti-inflammatory activity of some synthesized 2-substituted acetamido-5-aryl-1,3,4-thiadiazoles (sixteen compounds). Analgesic activity was determined by using hot wire analgesiometer. The *in-vivo* anti-inflammatory effects of the thiadiazole compounds were studied in a carageenan induced rat paw edema model. None of the compounds showed any analgesic activity. Compound 9 showed very good anti-inflammatory activity (51 per cent paw oedema inhibition) comparable to that of standard drug. Compounds 1 showed good anti-inflammatory activity (~ 46 per cent paw oedema inhibition). Compounds 5, 2, and 10 showed moderate anti-inflammatory activity (22-37 per cent paw oedema inhibition). Rest of the compounds do not showed any activity or low activity. Results indicate the potential of these compounds as anti-inflammatory agents which are non-acidic and non steroidal.

Introduction

Inflammation is the response to an injurious stimulus such as infections, physical injuries or due to generation of antibodies. The ability to build up an inflammatory response is essential for survival in the face of environmental pathogens and injury; but in some situations and diseases, the inflammatory response may be overstated and unrelenting without perceptible benefit and even with severe adverse consequences. Irrespective of the initiating stimulus, the classic inflammatory response includes warmth, pain, redness, and swelling.

These responses are the consequence of augmented vascular permeability and vasodilatation which facilitates the admittance of leucocytes at the damaged region. The leucocytes then get rid of the stimulus followed by fibrosis. Chemical mediators such as prostaglandins, thromboxanes, prostacyclin and leukotrienes are postulated to play their roles in acute inflammatory reaction. They have been found basically in each section of body.

On the other hand the chronic inflammation involves the mediators like interleukins, interferon and tumor necrosis factor α (TNF- α), and a cytokine that plays a major role in this kind of inflammatory process and whose production is associated with some inflammatory diseases such as rheumatoid arthritis (Spirchez *et al.*, 2012; Mangge *et al.*, 1995).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used groups of therapeutic agents. NSAIDs are widely used in the treatment of rheumatoid arthritis and inflammatory diseases. However, long-term NSAID use has been associated with gastrointestinal (GI) ulceration, bleeding and nephrotoxicity (Kimmey, 1992). The tendency of many acidic drugs to accumulate in stomach walls soon after oral absorption, as evidenced by radioautography, has been considered as a contributing factor to GI irritation (Shen, 1981). In addition, cyclooxygenase (CO) inhibition, which is the principle mechanism for analgesic and antiinflammatory properties of NSAIDs (Vane, 1971), has also been associated with nephrotoxicity and GI side effects (Schoen and Vender, 1989).

However, their everyday and long term use is limited due to the presence of significant adverse side effects such as non-selective NSAIDs cause gastric injury which may result in gastric ulceration, thromboembolic problems and renal side effects.

1,3,4-thiadiazoles are an important class of heterocyclic compounds and from the in-depth literature review, it was observed that thiadiazole derivatives (Schenone *et al.*, 2006; Rostom *et al.*, 2009; Palaska *et al.*, 2002; Labanauskas *et al.*, 2001; Chawla *et al.*, 2012; Gilani *et al.*, 2010; Moise *et al.*, 2009; Sainy *et al.*, 2009; Amir *et al.*, 2008; Jain and Mishra, 2014a,b) have been

reported to have anti-inflammatory and analgesic activity with minimal gastrointestinal ulceration and other side effects. Therefore, it was thought useful to carry out the analgesics and anti-inflammatory activity on the synthesized compounds (Jain and Mishra, 2004, 2011; Jain, 2001) having the 1,3,4-thiadiazole nucleus with substitution on 2 and 5 position and in continuation of the earlier work (Jain and Mishra, 2014a,b) to find out their potential as analgesic and anti-inflammatory agents.

Materials and Methods

In the present study, albino rats (100-150 g) of either sex were used for determining the analgesic and anti-inflammatory activity of the synthesized compounds. The dose of the compound was 100 mg/kg body weight. Compounds were administered orally in all the studies. For the pharmacological evaluation, the compounds were suspended in water using Tween 80 as a suspending agent. For all the studies, animals were divided into groups of 4 animals. The control group received the calculated amount of normal saline. All the experiments were carried out at room temperature.

Analgesic activity

Analgesic activity was determined by using hot wire analgesiometer with an arrangement for flow of cold water to avoid over heating of the area surrounding the wire. The rats were then put into a rat holder individually with the tail protruding out of the hole. The tail was then kept on the hot wire of the instrument in such a way that it did not touch the wire. It is presumed that on feeling pain, the rat would withdraw its tail. The reaction time was noted before the administration of drug, which served as a

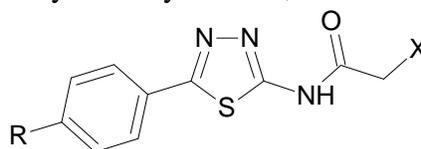
control reading. The animals of various groups were then given the synthesized compounds. The reaction time was noted after the administration of the drug at hourly intervals up to 4 hours (D'Amour and Smith, 1941).

Anti-inflammatory activity

Carageenan induced rat paw edema method was used in the present study. Initially right hind paw volume of different groups of rats was measured by the plethysmometer. The suspension was administered orally by means of a curved cannula. Albino rats weighing (100-150 grams) were divided into

the different groups, each having five animals. Each rat was weighed and the compounds were administered according to their body weight. After the lapse of 30 minutes, 0.1 ml of 1 % carageenan suspension was injected under the planter aponeurosis of the right hind paw of albino rats. The right hind paw volume was again measured after 3 hours by means of a plethysmometer. For the control group, only carageenan solution was injected into the right hind paw of each rat and the paw volume was measured by means of plethysmometer (Winter *et al.*, 1962). The results were reported in Table 1.

Table.1 Anti-inflammatory Activity of the 2,5-disubstituted-1,3,4-thiadiazoles



Compound No.	R	X	Change in paw volume	% inhibition	log P [#]
1	H	N-methyl-N- <i>n</i> -butylamino	0.19 ± 0.02768**	45.71	3.56
2	H	Dicyclohexylamino	0.25 ± 0.02113*	28.57	6.09
3	H	Morpholin-1-yl	0.33 ± 0.02352	5.71	1.91
4	H	4-Methyl piperidin-1-yl	0.38 ± 0.01712	-	3.72
5	CH ₃ O	N-methyl-N- <i>n</i> -butylamino	0.22 ± 0.02476*	37.14	3.73
6	CH ₃ O	Dicyclohexylamino	0.29 ± 0.01125	17.14	6.25
7	CH ₃ O	Morpholin-1-yl	0.37 ± 0.01980	5.71	2.08
8	CH ₃ O	4-Methyl piperidin-1-yl	0.42 ± 0.02129	-	3.89
9	CH ₃	N-methyl-N- <i>n</i> -butylamino	0.17 ± 0.01983***	51.42	4.02
10	CH ₃	Dicyclohexylamino	0.27 ± 0.02516	22.85	6.55
11	CH ₃	Morpholin-1-yl	0.30 ± 0.01366	14.28	2.37
12	CH ₃	4-Methyl piperidin-1-yl	0.42 ± 0.02191	-	4.18
13	Cl	N-methyl-N- <i>n</i> -butylamino	0.58 ± 0.01549	-	4.32
14	Cl	Dicyclohexylamino	0.32 ± 0.01788	8.57	6.85
15	Cl	Morpholin-1-yl	0.44 ± 0.01788	-	2.68
16	Cl	4-Methyl piperidin-1-yl	0.46 ± 0.01722	-	4.48
		Control#	0.35 ± 0.03087		
		Phenyl butazone (Standard drug)	0.16 ± 0.02390***	54.28	

* The difference is statistically significant (P<0.05) when compared with control; *** The difference is statistically significant (P<0.005) when compared with control; # Carragenan only; [#]Calculated by using ACD ChemSketch 12.0 (www.acdlabs.com)

Results and Discussion

Analgesic activity

None of the compounds gave any analgesic effect, this indicates that the synthesized compounds do not have any potential for analgesic activity.

Anti-inflammatory activity

Compound 9 showed very good anti-inflammatory activity (51 per cent paw oedema inhibition) comparable to that of standard drug. Compounds 1 showed good anti-inflammatory activity (~ 46 per cent paw oedema inhibition). Compounds 5, 2,

and 10 showed moderate anti-inflammatory activity (22-37 per cent paw oedema inhibition). Rest of the compounds do not showed any activity or low activity.

Results indicate that *p*-Chloro substitution in the aromatic ring (at position 5 of 1,3,4-thiadiazole ring) results in reduction (compound 14) or loss of anti-inflammatory activity (compounds 13, 15 and 16). Although compounds 13 and 16 have logP value comparable with that of most active compound 9 but devoid of biological activity, this may be due to the presence of electron withdrawing group in the aryl ring which is not favorable.

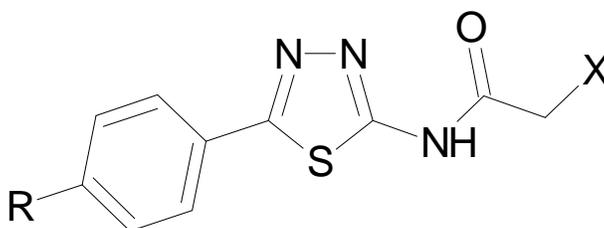


Fig.1 General structure of 2,5-disubstituted -1,3,4-thiadiazoles

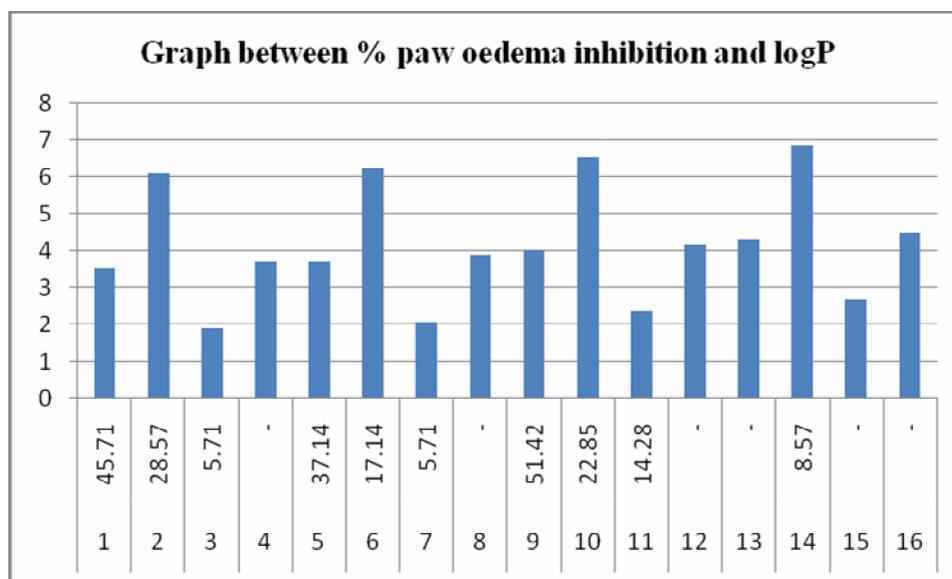


Fig.2a Graph between % paw oedema inhibition and logP for compounds 1-16 (3D column plot)

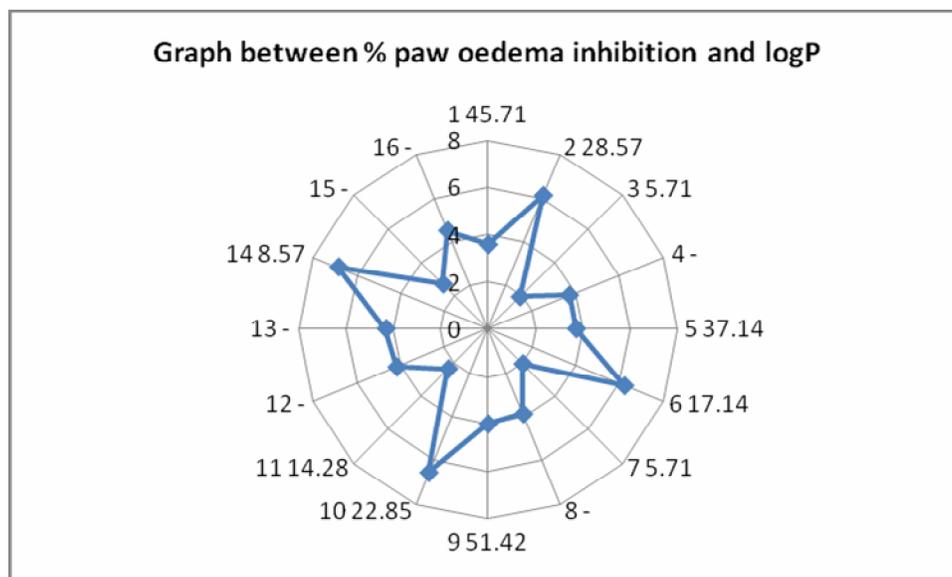


Fig. 2b Graph between % paw oedema inhibition and logP for compounds 1-16 (Radar plot)

Compound 2 has moderate activity that may be due to the presence of rigidity incorporated in the molecule (cyclohexyl ring; metabolic susceptibility decreased). It has higher lipophilicity and less active as compared to the most active compound 9, which may be due to less absorption and bioavailability. In compounds where X is N-methyl-N-*n*-butylamino group are more active than heterocyclic analogs [Morpholine and 4-Methyl piperidine] (Fig 1).

Morpholine and 4-Methyl piperidine substituted derivatives are not active or less active. Overall substitution in phenyl aromatic ring at 2 position of 1,3,4-thiadiazole ring by methoxy (CH₃O) result in slightly lesser activity, and chloro (Cl) result in loss of activity whereas by methyl group result in slightly more activity as compared to unsubstituted derivatives (compound 1-4). Among the unsubstituted derivatives (compound 1-4), compound 1 having N-methyl-N-*n*-butylamino group is highly active (\approx 46 per cent paw oedema inhibition), due its optimum lipophilicity. Compound 4 which is a rigid analog of N-

methyl-N-*n*-butylamino group is devoid of activity. Morpholine substituted derivative (less lipophilic) is less active whereas 4-methylpiperidine substituted derivatives (4,8,12 and 16) are inactive. Graph between % paw oedema inhibition and logP for compounds 1 to16 is shown in Fig 2a (3D column plot) and Fig 2b (Radar plot).

Assessment of analgesic and anti-inflammatory activity of some synthesized 2,5-disubstituted-1,3,4-thiadiazoles (sixteen compounds) were done. None of the compounds showed any analgesic activity. Compound 9 showed very good anti-inflammatory activity (51 per cent paw oedema inhibition) comparable to that of standard drug. Compound 1 showed good anti-inflammatory activity (\approx 46 per cent paw oedema inhibition). Compounds 5, 2, and 10 showed moderate anti-inflammatory activity (22-37 per cent paw oedema inhibition). Rest of the compounds do not showed any activity or low activity. Results indicate the potential of these compounds as anti-inflammatory agents which are non-acidic and non steroidal. Substitution in the aryl group at 5-position of 1,3,4-thiadiazole

nucleus result in reduction (CH₃O) or loss of activity (Cl) whereas methyl group result in slight increase in activity as compared to the unsubstituted derivative. 4-methylpiperidine derivatives are less active or devoid of biological activity. Further work may help in designing and developing more potent selective non acidic NSAIDs.

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