Analgesic and Anti-Inflammatory Activity Studies of Some New Aryl 4-Thiazolidinones in Experimental Mice

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Summary

Purpose: The core aim of the present study was to investigate analgesic antipyretic and anti-inflammatory activity studies of some synthesized new aryl substituted 4-thiazolidinone derivatives on experimental animals.

Methods: 1) analgesic activity: in the present study we have used eddy's hotplate method (thermal method in experimental animals to monitor whether the synthesized compounds have any analgesic action where by decreasing the pain sensation by increasing threshold to painful stimuli. The commonly used analgesics were aspirin, paracetamol, ibuprofen (non-narcotic type) and morphine (narcotic type).

Painful reaction in experimental animals can be produced by applying noxious (unpleasant) stimuli such as thermally and the extent of response time is studied.

2) Anti-inflammatory activity: the ability of a compound to reduce the local edema induced in a rat paw by various irritants is the most widely used method to screen the new anti-inflammatory agents. Compounds like formalin, carregenin, kaolin, yeast and dextran have been used as irritants to produce edema.

On the basis of this we have screened all the newly synthesized compounds during the present investigation for their anti inflammatory activity.

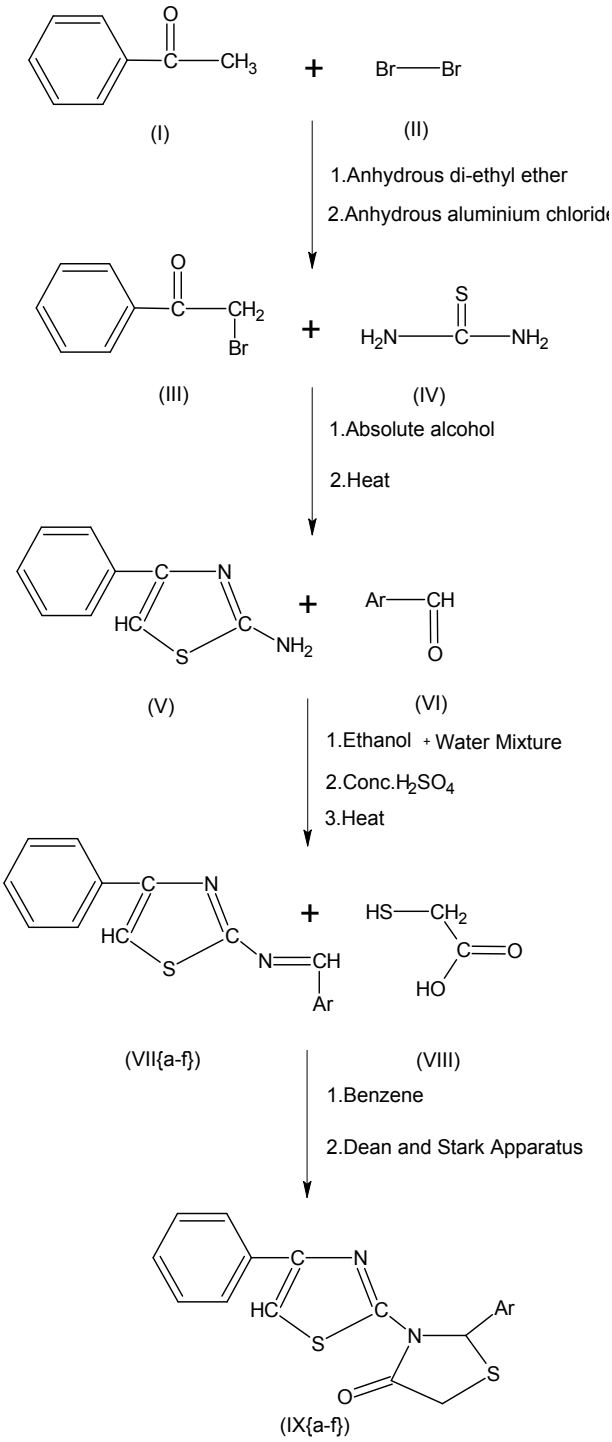
Results:1) analgesic activity: all the synthesized 4-thiazolidinones have shown analgesic activity. When compared to standard drug (ibuprofen) all the compounds were found to be slightly active among which NP3, NP4 AND NP8 showed good analgesic activity. However the activity was less than that of standard drug

2) Anti-inflammatory activity: all the synthesized 4-thiazolidinones have shown anti inflammatory activity in suppressing formalin induced edema in rats. When compared to standard drug (ibuprofen), all the compounds were found to be moderately active, among which NP3, NP4 AND NP8 showed good anti-inflammatory activity. However the activity was less than that of the standard drug.

Conclusion: analgesic and anti-inflammatory study of the synthesized compounds showed mild activity

Keywords: aryl -4-thiazolidinones, Eddy's hotplate method, Analgesic and antiinflammatory activity, Seizures, rats.

[Annexure - 1]


(I)
(II)
1.Anhydrous di-ethyl ether
2.Anhydrous aluminium chlorid
(III)
(IV)
1.Absolute alcohol
2.Heat
(V)
(VI)
1.Ethanol + Water Mixture
2.Conc.H2SO4
3.Heat
(VII{a-f})
(VIII)
1.Benzene
2.Dean and Stark Apparatus
(IX{a-f})

Introduction

Thiazolidinones are the derivatives of thiazolidine, which belong to an important group of heterocyclic compounds. Thiazolidinones with a carbonyl group at position 2, 4 or 5 have been subject of extensive study in the recent past. Numerous reports have been appeared in the literature which highlights their chemistry and use

Various activities such as bactericidal, pesticidal, fungicidal, insecticidal, anticonvulscent, tuberculostatic, antithyroidal, potentiation of Phenobarbital induced sleeping time etc have been found to be associated with 4-thiazolidinone derivatives

Thiazolidinones in the presence of various reagents, undergo different types of reactions

In the present note we have evaluated the newly synthesized aryl 4-thiazolidinone derivatives for their analgesic and anti-inflammatory activity

Materials and Methods

All the reactions were carried out under prescribed laboratory conditions. The products were purified by recrystallization and melting points were determined in open capillaries and are uncorrected. All the final compounds were characterized by their elementary analysis, IR spectra, mass spectra, and PMR spectra.

SHIMADZU PERKIN-ELMER 8201 PC, IR Spectrophotometer using a thin film supported on KBr pellets recorded FT-IR spectra. PMR spectra were recorded on BRUKER AC 300 (300 MHz), NMR Spectrometer using TMS as internal standard; FAB mass spectra were recorded on JEOL SX 102 (DA-6000 mass Spectrometer) Data system using Argon Xenon (6KV.10MA) as the FAB gas. The purity of the compounds was checked on silica gel coated plates (Merck).

Synthesis of Aryl-4- thiazolidinones involves four steps. In the first step acetophenone **(I)** in pure and anhydrous di-ethyl ether is treated with bromine **(II)** in presence of anhydrous aluminium chloride then phenacyl bromide **(III)** precipitates out. Second step consists of reaction of phenacyl bromide **(III)** in 100 ml of absolute alcohol and thiourea **(IV)**, which yields the desired 2-amino-4-phenyl thiazole **(V)**. Latter compound was then condensed with appropriate aldehydes **(VI)** in presence of ethanol water mixture, added two drops of conc. H2SO4 and refluxed for 4-5 hours in the third step and solid of Schiff's bases **(VII {a-f})** separates out. In the final step the resulting Schiff's bases in benzene were refluxed with thioglycollic acid (mercapto acetic acid) using Dean and Stark apparatus till the clear distillate is formed. Resulting mixture is cooled to 0^oC, solid separated out is washed with 5% NaOH solution to give aryl substituted -4-thiazolidinones **(IX {a-f})**. The molecules were synthesized as per the procedure and the outline is described in the scheme as given in annexure-1. Physical data of final products is given in table 1.

Synthesis of phenacyl bromide (III).

A solution of acetophenone (5gms, 0.042mol) in pure anhydrous diethyl ether (5ml) was placed in a three necked flask fitted with a mechanical stirrer and a thermometer. Solution was cooled to 0^oC and anhydrous $AlCl_3$ (50mg) was added to it.

When the temperature falls to 0^{o}C, bromine (6.72 Gms) was added to it gradually from the dropping funnel with stirring at the rate of about 1 ml per minute. The bromine colour disappears rapidly, although a very small amount of hydrogen bromide was evolved. Towards the end of reaction the solution turns pink.

After the addition is complete, ether and the dissolved hydrogen bromide was removed by applying suction with a slight current of air. Solid that formed was collected by filtration and shaken with 1 ml of petroleum ether. The crystals were washed with fresh portions of solvent and recrystallised from minimum qty of ethanol.

Synthesis of 2-amino-4-phenyl thiazole (V).

A mixture of phenacyl bromide (0.01 moles) in 100 ml of absolute alcohol and thiourea (0.02 moles) were taken in a round bottom flask and refluxed on a water bath for 3-4 hrs. Then the reaction mixture was cooled and decomposed with cold water. The solid separated was collected by filtration and the solvent was removed under vacuum. The residue obtained was dried, recrystallised from ethanol.

Synthesis of Schiff's base (VII {a-f}).

2-amino-4-phenyl thiazole (0.01 mol) was taken in a 250 ml round bottom flask and was dissolved in 20 ml of dry ethanol. A solution of appropriate aldehyde (0.2 mol) dissolved in ethanol was added. To this mixture 2-3 drops of conc.H_2SO_4 was added and the contents were refluxed on a water bath for 4-5 hrs. After refluxing excess of ethanol was distilled off and the reaction mixture was cooled to room temperature. The solid separated on cooling was filtered and washed with ice-cold water. After drying the product was recrystallised from ethanol to get pure compound.

Synthesis of aryl-4- thiazolidinones (IX **{a-f}).**

The resulting Schiff's base (0.02 mol) was taken in a 250 ml round bottom flask containing benzene (30ml). Thioglycollic acid (0.025 mol) was then added to flask. The mixture was then refluxed using Dean and Stark apparatus. At the end of reaction as indicated by a clear distillate, the excess benzene was distilled off. The mixture was then cooled and poured on to crushed ice, in portions, with constant stirring. Solid separated was then filtered and washed with cold water. This was then again washed with 5% sodium bi carbonate solution and filtered. After drying, it was then purified by recrystallisation from ethanol.

Results and Discussions

IR spectra of all synthesized aryl-4-thiazolidinones revealed the important functional groups. ^{1}H NMR spectra of the products indicate the formation of the 4-thiazolidinone derivatives having substituents at position 3 and having different aryl groups at position 2.

The mass spectral data indicated stable molecular ion peak for all the synthesized final products. All the synthesized compounds gave satisfactory elemental data

Table 1: Physical data of compounds prepared.

Sl.No.	Compound No.	Diff. Aldehyde used	Physical state	M.P. (^oC)	Yield (%)	Mol. Formula (Mol.Wt-gms)
01	**IX a**	3,4,5-Trimethoxy benzaldehyde	Dark red colour crystals	161	73	$C_{21}H_{20}N_2O_4S_2$ (428.527)
02	**IX b**	P-Dimethyl amino benzaldehyde	Red colour crystals	191	71	$C_{20}H_{19}N3OS_2$ (381.316)
03	**IX c**	Vanillin	Pale yellow colour crystals	174	68	$C_{19}H_{16}N_2O_2S_2$ (368.475)
04	**IX d**	4-Chloro benzaldehyde	Yellow colour crystals	184	70	$C_{18}H_{13}N_2OS_2$ (372.893)
05	**IX e**	4-Nitro benzaldehyde	Orange colour crystals	164	71	$C_{18}H_{13}N_3O_3S_2$ (383.0)
06	**IX f**	2,4-Dihydroxy benzaldehyde	Light orange colour crystals	172	72	$C_{18}H_{14}N_2O_3S_2$ (370.447)
07	**IX g**	4-Quinoline carboxaldehyde	Red colour crystals	151	69	$C_{21}H_{15}N_3OS_2$ (389.495)
08	**IX h**	Benzaldehyde	Yellow colour crystals	131	70	$C_{18}H_{14}N_2OS_2$ (338.449)

BIOLOGICAL ACTIVITY

Analgesic activity: Assessment of analgesic activity in laboratory animals is difficult specifically that of non narcotic drugs. Many methods are available for evaluation of drugs for analgesic effect. In all the methods one or the other type of stimulus is applied to produce a pain reaction. The various methods are Thermal stimulus, Mechanical stimulus, Chemical stimulus, Electrical stimulus. In the present study we have used Eddy's hot plate method (thermal Method). This method used in experimental animals to monitor whether the synthesized compounds have any analgesic action which by decrease the pain sensation by increasing threshold to painful stimuli. The commonly used analgesics were aspirin, paracetamol, ibuprofen (non-narcotic) and morphine (narcotic type)

Painful reaction in experimental animals can be produced by applying noxious (unpleasant) stimuli such as thermally and the extent of response time is studied.

Selected albino rats of either sex, were weighted accurately to the nearest gram and kept at room temperature in the laboratory. They were divided into six groups. One group serving us control, ibuprofen (standard drug), and all derivatives in saline- acacia vehicle (2%) was prepared. The compounds in vehicle were injected intraperitonially to the respective groups.

Before that 0 hr reaction time reading was determining 'this is by', the temperature hot plate was kept constant at 56.5C, rats were placed on the hot plate and the initial reaction time was measured for each rat. End point was taken as licking or blowing of paws or dancing on the hot plate. After injection, the reaction time was determined after 30 minutes of injection. These were compared with the control reaction time.

ANALGESIC ACTIVITY CHART

compounds	Before treatment	After treatment for 30mins	After treatment for 60mins	After treatment for 90mins
1	1.33 ± 0.09	2.33 ± 0.03**	2.70 ± 0.11	3.63 ± 0.09***
2	2.30 ± 0.1155	3.30 ± 0.1155**	4.57 ± 0.09***	5.47 ± 0.09***
3	1.77± 0.09	2.90±0.06**	3.73 ± 0.12**	4.60 ± 0.06**
4	1.87±0.03	2.90 ± 0.06***	3.70±0.06***	4.33±0.18**
5	2.30±0.06	4.67±0.09**	4.63±0.09***	4.400±0.06***
6	2.53±0.03	3.97±0.03**	4.73±0.22**	4.83±0.52*
7	2.13±0.09	3.80±0.11***	4.17±0.09**	4.50±0.17**
8	2.53±0.03	3.47±0.09**	4.77±0.14**	5.27±0.20**
Standard Pentazocin)	2.37±0.07	2.90±0.06*	5.60±0.06***	6.40±0.06***

All the synthesized 4-thiazolidinones have shown analgesic activity. When compared to standard drug (ibuprofen) all the compounds were found to be slightly active among which IXb, IXe &IXf showed good analgesic activity. However the activity was less than that of standard drug

ANTI-INFLAMMATORY ACTIVITY

Winters hind paw method was used in the present study for the evaluation of anti-inflammatory activity. Formalin (3.5%) was injected subcutaneously into the hind paw of the rat, to produce the edema. The different groups of animals were administered with a standard NSAID (ibuprofen), test samples. The increase in paw volume was measured before and after 3 hrs of administration and the results was compared.

General procedure:

Sixty healthy albino rats of body weight 100-200 gm were selected and made into 10 groups of six animals each. All the animals were kept on fasting for 18 hrs. One group served as control where equivolume of 2% W/V acacia mucilage was given intra peritonially.Another group received the standard drug ibuprofen 20 mg/kg body weight and the remaining groups received the test compounds. *The hind paw of the rat was marked at the level of malleolus, so that while measuring the volume of dipping was done to the same level. After 30 minutes of the administration of drugs.*

0.1 ml of formalin was administered by subcutaneous route. The volume was measured immediately and after 3 hrs using plethismograph. The experiments were performed under normal laboratory conditions. Animals were handled gently to avoid stress. The change in the paw volume was compared with in the vehicle treated control animals. The results obtained are shown in the table

ANTIINFLAMMATORY ACTIVITY CHART

Compound	Edema volume in ml			Percentage reduction		
	1/2h	1h	2h	1/2h	1h	2h
Control	0.743	0.755	0.796			
Standard (Indomethacin)	0.230	0.212	0.195	71.92**	71.92**	75.50**
IXa	0.368	0.358	0.372	50.47*	52.58*	53.27*
IXb	0.456	0.467	0.421	38.627 ^{ns}	38.15 ^{ns}	47.11 ^{ns}
IXc	0.238	0.312	0.298	61.52**	58.67*	62.56*
IXd	0.345	0.397	0.377	53.57*	47.42*	52.64*
IXe	0.541	0.567	0.538	27.19^{ns}	24.90 ^{ns}	32.41 ^{ns}
IXf	0.312	0.369	0.326	58.28*	51.13*	59.05*
IXg	0.282	0.317	0.291	62.04**	58.01*	63.44*
IXh	0.257	0.245	0.229	65.41*	67.54**	71.23**

*P<0.05, **P<0.01, ***P<0.001 and ns statistically not significant

All the synthesized 4-thiazolidinones have shown anti inflammatory activity in suppressing formalin induced edema in rats. When compared to standard drug (ibuprofen), all the compounds were found to be moderatively active, among which IXb, IXe &IXf showed good anti-inflammatory activity. However the activity was less than that of the standard drug.

Conclusion

Various 1, 3-thiazolidin-4ones having thiazole substituents at position 3 and different aryl substituents at position 2 were synthesized with a view of enhancing the biological activity. The structure of newly synthesized compounds was confirmed by IR, ^{1}H NMR, MASS spectra and elemental analysis. Further evaluation of analgesic and antiinflammatory activity was carried out. The synthesis of 1, 3-thiazolidin-4-ones by the described method resulted in products with good yield. Analgesic and antiinflammatory study of the synthesized compounds showed good to moderate activity.

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