

Pharmacological evaluation of oxazine sulfonamide derivatives for anti-inflammatory, analgesic and antipyretic activities

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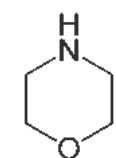
Abstract

Oxazine heterocyclic compound containing one oxygen and one nitrogen atom. They possess different pharmacological activities like anticancer, anti-ulcer, antifungal, antithrombotic, antihypertensive, analgesic, anti-inflammatory etc. Oxazine Sulphonamide derivatives were investigated for anti-inflammatory, analgesic and anti-pyretic activities. It was evaluated for anti-inflammatory activity by carrageenan induced paw edema in rats using Plethysmometer and it also shows significant analgesic effect in male Albino mice. Antipyretic activity was also investigated.

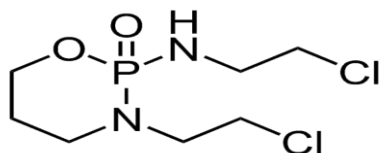
Keywords: Analgesic; Ant-Pyretic; Anti-Inflammatory; Oxazine Sulphonamide Derivatives; Yeast; Carrageenan; Plethysmometer.

1. Introduction

Oxazine heterocyclic compound containing one oxygen and one nitrogen atom. Many isomers exist depending on the relative position of the heteroatoms and relative position of the double bonds. The examples of Oxazine derivatives include Morphine-Tetrahydro-1,4-Oxazine, ifosfamide a nitrogen mustard alkylating agent used in the treatment of cancer.



Morphine-Tetrahydro-1,4-Oxazine



Ifosfamide

Benzoxazine is a molecule where an oxazine ring is attached to a benzene ring. There are several benzoxazines depending on the position of oxygen and nitrogen heteroatoms. Benzoxazine is the compound with double bond in the Oxazine ring. They possess different pharmacological activities like anticancer, anti ulcer, antifungal, antithrombotic, antihypertensive, analgesic, anti-inflammatory etc.

2. Materials and methods

2.1. Materials

For analgesic activity, pentazocine is used as standard drug and the animals were treated with Oxazine as test compound. For antipyretic activity, yeast is used to induce pyrexia and standard drug used was paracetamol. The animals are also treated with test drug.

For anti-inflammatory activity, carrageenan is used for inducing paw edema, Oxazine sulphonamide derivatives used as test and diclofenac is used as standard drug.

2.2. Methods

Analgesic activity was carried out with *Hot Plate Latency Test using Eddy's Hot plate method* and Antipyretic activity was done by using *Yeast inducing Hyper pyrexia method*. Anti-inflammatory activity is measured by using *Plethysmometer by carrageenan-induced hind paw edema in rats*.

2.3. Experimental animals

Healthy adult male Albino mice (25-35g) were selected for analgesic activity. They were maintained under proper conditions. Healthy adult male Wistar rats (150-250g) were used for antipyretic activity. They were fed with water and standard pellets and maintained under proper environment. For anti-inflammatory activity healthy adult male rats (150-250g) were used. They were housed in polypropylene cages and maintained under suitable nutritional and environmental conditions. Animals were fed with water and pellets.

3. Results and Discussions

The study showed that the Oxazine sulphonamide derivatives has potent anti-inflammatory and analgesic activities but not antipyretic action. This was confirmed by the observations from all the models of anti-inflammatory, antipyretic and analgesic activity used in this study.

3.1. Results

3.1.1. Acute toxicity study

The acute oral toxicity procedure was followed by using OECD 423 guidelines.

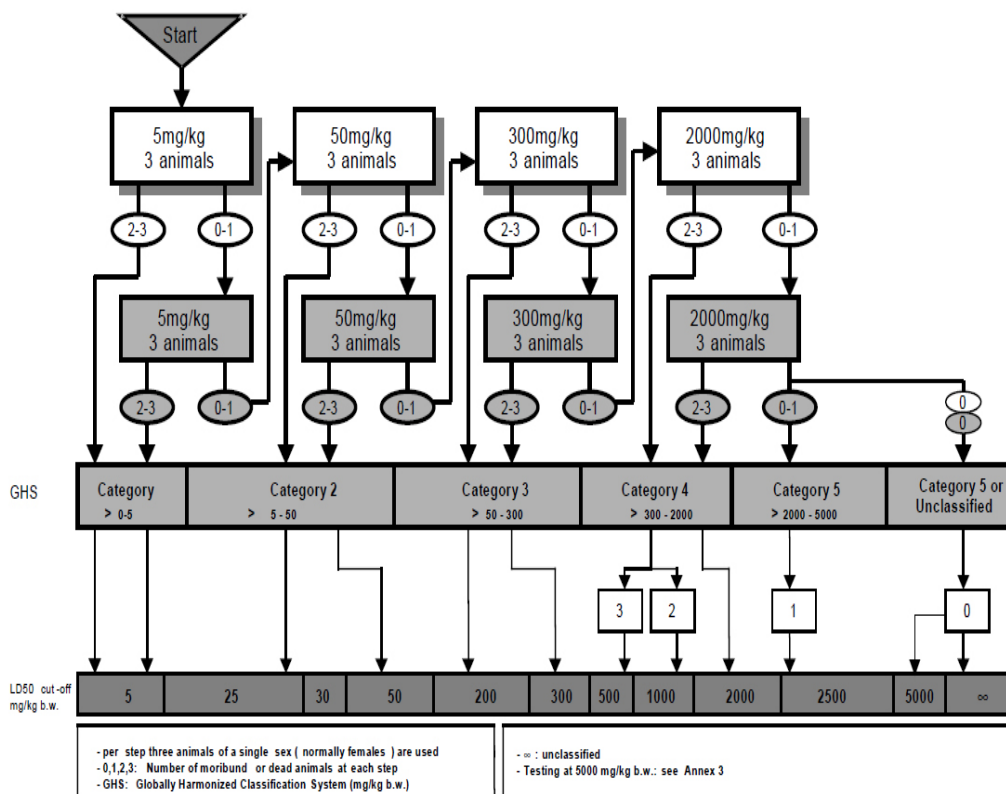


Fig.1: Acute Toxicity Design

3.1.2. Screening of analgesic activity

Hot plate latency test

The hot plate latency assay was carried out according to the method of Eddy et al.,1950.the rats used for this study were divided into 7 groups, 5groups received the test compound 1,test compound 2, test compound 3,test compound 4,test compound 5,while the remaining two groups was std and control. The test drugs, control and standard were administered orally to the animals after 12 hrs of fasting. The animals were each placed on a hot plate maintained at 55°C, 30 min after administration of test compounds, standard and saline. The time taken for the rats to respond to the thermal stimulus (usually by jumping) was noted as the latency (in second). The mean of the latency for each group was determined. The effects of the test drugs, standard and saline were also determined after 0, 30, 60, 120, 180 min of administration to rats.

Table 1: Animals and Treatment for Analgesic Activity

Group(n=6)	Treatment
1	Water(control)
2	Std drug (pentazocine 5mg/kg)
3	*Bvk.ks.01.14(10mg/kg)
4	*Bvk.ks.01.16(10mg/kg)
5	*Bvk.ks.01.17(10mg/kg)
6	*Bvk.ks.01.18(10mg/kg)
7	*Bvk.ks.01.20(10mg/kg)

Table 2: Analgesic Responses of Drugs with Time in Analgesic Activity

Group and dose	0min	30min	60min	120min	180min
Control	7+1.7	6+0.8	6+2.0	7+0.8	6.8+1.4
Std(5mg/kg)	5.6+1.	10.8+1.	12.6+2.	15.3+2.	17.5+2.
*Bvk.ks.01.14(10 mg/kg)	8	4**	1**	8**	1**
Bvk.ks.01.16(10 mg/kg)	7+1.2	10.5+1.	15+2.1	16.8+1.	17.3+0.
*Bvk.ks.01.17(10 mg/kg)	7.16+0.7	9.6+1.3	13.3+1.	15.6+1.	16.8+1.
*Bvk.ks.01.18(10 mg/kg)	7.5+1.	11.5+1.	14.6+1.	17.6+1.	16.6+1.
*Bvk.ks.01.20(10 mg/kg)	7.66+1.0	11.5+1.	14.8+2.	16.6+2.	15.3+0.
	0.7	8**	1**	0**	8**
	0	5**	5**	3**	2**
	7.16+0.7	9.5+0.8	14.1+1.	16+0.8*	16.5+1.
	0.7	**	4**	*	6**

Values are expressed in mean+SEM, significant P**<0.01; P*>0.05 compared to control.

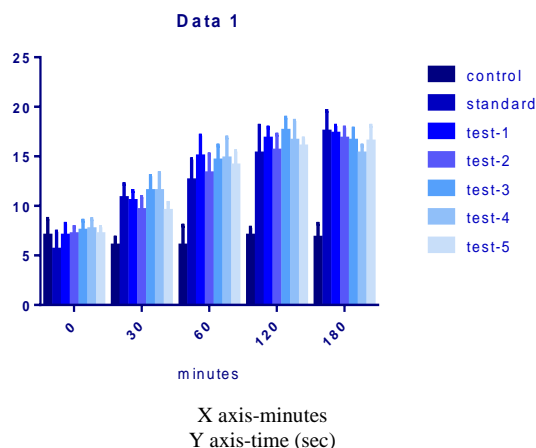


Fig. 2: Graph Representing the Analgesic Activity Results

3.1.3. Screening of anti pyretic activity

Yeast inducing hyperpyrexia method

The antipyretic activities of test compounds were evaluated by using digital thermometer. The antipyretic activity was screened by using yeast induced hyperpyrexia method. Rats were selected and divided into seven groups each having six animals. The animals were administered control, std and test drugs as shown in the table. They were maintained at standard at constant temperature of 97 to 98 °F for 24 hours before pyrexia was induced. Experimental pyrexia induced by 15% suspension of Brewer's yeast in 0.9% saline solution by giving 10 ml/kg. The rectal temperature before and after treatment was recorded with the help of digital clinical thermometer at every hour up to 3 hours and was compared with control. Before yeast injection the rectal temperature was recorded. The yeast injection was given to all the groups. Immediately after yeast injection increase in the rectal temperature is recorded after 30 min. The control, standard and test compounds 1-5 were administered orally. The rectal temperature was recorded using digital thermometer prod was inserted 3-4 cm deep into the rectum.

Table 3: Animals and Treatment for Pyretic Activity

Group(n=6)	Treatment
1(control)	Control(water)
2(std)	Paracetamol 10mg/kg
3 Test compound	*Bvk.ks.01.14(10mg/kg)
4 Test compound	*Bvk.ks.01.16(10mg/kg)
5 Test compound	*Bvk.ks.01.17(10mg/kg)
6 Test compound	*Bvk.ks.01.18(10mg/kg)
7 Test compound	*Bvk.ks.01.20(10mg/kg)

Table 4: Anti-pyretic Responses of Drugs with Time in Anti-pyretic Activity

Group and dose	Rectal temperature in °F at different time intervals				
	30 min	60min	90min	120min	180min
Control	99.4+0.6	99.4+0.4	99+0.4	99.2+0.6	99.2+0.5
Std(Paracetamol)	99.1+0.6	98.6+0.4	98.4+0.3	97.8+0.2	97.5+0.1
*Bvk.ks.01.14(10mg/kg)	100.5+1.2	101.2+0.2	100.8+0.1	101.8+0.1	100.9+0.4
*Bvk.ks.01.16(10mg/kg)	99.8+1.0	101.2+0.6	102+0.4	101.5+0.3	101.8+0.4
*Bvk.ks.01.17(10mg/kg)	100.9+1.1	100.5+0.27	100.9+0.2	101+0.8	101.8+0.4
*Bvk.ks.01.18(10mg/kg)	100.3+1.2	100.5+0.2	101.3+0.1	100.5+0.9	101.2+0.0
*Bvk.ks.01.20(10mg/kg)	99.9+0.9	100.7+0.65	101.4+0.2	101.3+0.68	101.4+0.6

Values are expressed in Mean+SEM, P* < 0.05 P** < 0.01 significant.

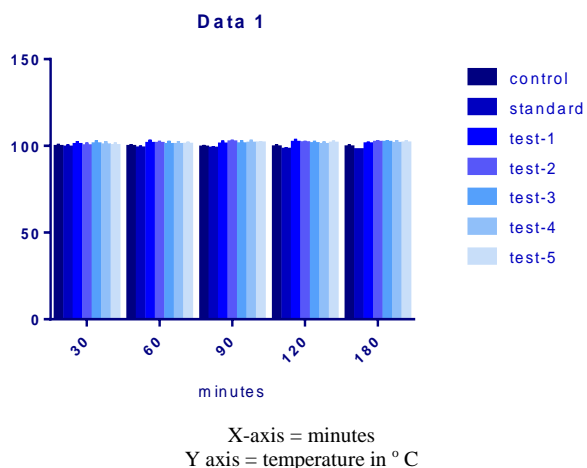


Figure 3: Graph Representing the Anti-pyretic Activity Results

3.1.4. Screening of anti inflammatory activity

Carrageenan-induced hind paw edema in rats

The acute anti-inflammatory effect was evaluated by carrageenan induced rat paw edema (Prabhakar et al., 2006). Edema was induced by injecting carrageenan (1% w/v, 0.1 ml) in the right hind paw of rats. The test compounds 1-5, diclofenac (10 mg/kg) and vehicle was administered orally one hour before injection of carrageenan. Paw volume was measured with digital plethysmometer after 30, 60, 120, 180 min of injection.

Percentage increase = $\frac{A-B}{A} \times 100$

- a) Paw volume of at different time intervals after injection.
- b) Paw volume before injection.

Table 5: Animals and Treatment for Anti-inflammatory Activity

Groups(n=6)	Treatment
1(control)	water+carrageenan
2(std)	Diclofenac 10mg/kg
3	*Bvk.ks.01.14(10mg/kg)
4	*Bvk.ks.01.16(10mg/kg)
5	*Bvk.ks.01.17(10mg/kg)
6	*Bvk.ks.01.18(10mg/kg)
7	*Bvk.ks.01.20(10mg/kg)

Table 6: Anti-inflammatory Responses of Drugs with Time in Anti-inflammatory Activity

Group & dose	0 min	30 min	60 min	120 min	180 min
Control	0.71+0.07	0.83+0.08	0.86+0.05	1.03+0.13	1.35+0.25
Std(10mg/kg)	0.72+0.09*	0.21+0.03**	0.23+0.04**	0.22+0.05**	0.30+0.05**
Bvk.ks.01.14(10mg/kg)	0.85+0.05	0.5+0.06**	0.37+0.04**	0.34+0.04**	0.28+0.07**
*Bvk.ks.01.16(10mg/kg)	0.8+0.08	0.42+0.04**	0.38+0.04**	0.31+0.04**	0.26+0.05**
*Bvk.ks.01.17(10mg/kg)	0.8+0.08	0.53+0.05**	0.43+0.07**	0.35+0.05**	0.28+0.04**
*Bvk.ks.01.18(10mg/kg)	0.75+0.10	0.48+0.07**	0.41+0.07**	0.38+0.07**	0.31+0.04**
*Bvk.ks.01.20(10mg/kg)	0.78+0.09	0.56+0.16**	0.48+0.04**	0.41+0.04**	0.35+0.08**

Values are expressed in mean+SEM, significant P** < 0.01; P* > 0.05 compared to control.

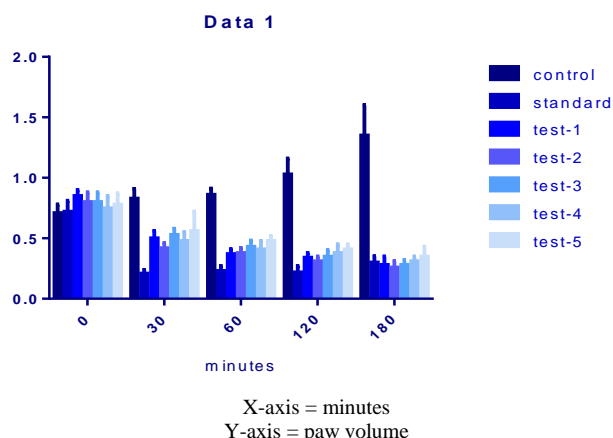


Figure 4: Graph Representing the Anti-inflammatory Activity Results

3.2. Discussions

3.2.1. Anti-pyretic activity

Fever is provoked by many exogenous substances in animal models, including bacteria, endotoxins and microbe infection. Exogenous pyrogen induced the production of pro-inflammatory cyto-

kinins such as IL-1 β , IL-6, interferons and Tumour Necrosis Factor which enter hypothalamic circulation and stimulate the release of local PG'S thereby resetting the hypothalamic thermal set point. In this study, Oxazine sulphonamide derivatives have not showed antipyretic action.

3.2.2. Analgesic activity

The test elucidates central and peripheral activities of nociception. The response pattern has two distinct periods of paw licking activity, an early response (0-5min after injection) and a late response (20-30mins after injection). Early phase is due to direct effect of drug on nociceptors (anti-inflammatory). The late phase response is develop due to an inflammatory response and release analgesic mediators which reflect inflammatory pain. Experimental results have indicated that substance p and Bradykinin participate in early phase and histamine, serotonin and PG's are involved in late phase. The study showed analgesic activity of Oxazine sulphonamide derivatives on both phases of this test. Suggesting both direct analgesic effect on the nociceptor and an inhibition of inflammatory pain. Thus these mode of actions probably involves the synthesis and release of PG's or other pain mediators.

3.2.3. Anti-inflammatory activity

Inflammation occurs in three distinct phases-Acute, Sub-acute and Chronic. The acute response to tissue injury occurs in the micro-circulation at the site of injury. First constriction occurs then after the release of chemical mediators causes relaxation of smooth muscles and vasodilation. The Sub-acute phase is characterised by movement of phagocytes to the site of injury. Blood clots are removed by fibrinolysis and damaged tissue are regenerated and replaced with fibroblasts, collagen or endothelial cells. However inflammation becomes Chronic, leading to further destruction and fibrosis. In this the tests compounds have showed maximum inhibition of 48% at 1 hour of drug treatment at a dose of 10mg/kg.

4. Conclusion

All the five test compounds are tested for anti inflammatory, analgesic and antipyretic activity and found that:

- 1) All the five compounds are showing anti inflammatory activity but *Bvk.ks.01.16, *Bvk.ks.01.17 and *Bvk.ks.01.18 are showing significant anti inflammatory activity.
- 2) All the five compounds are showing analgesic activity but Bvk.ks.01.17 and *Bvk.ks.01.18 are showing significant analgesic activity.
- 3) All the five compounds have no effect on anti pyretic activity.

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