Anti-inflammatory, Analgesic and Antiparkinsonism Activities of Some Novel Pyridazine Derivatives

Naif O. Al-Harbi [1], Saleh A. Bahashwan [2], Khalid A. Shadid [3]
[1.] Pharmacy Department, College of Health Sciences, King Saud University, Riyadh, Saudi Arabia
[2-3.] Pharmacy Department, College of Health Sciences, Taibah University, Medina Monawarah, Saudi Arabia
drnaifalharbi@yahoo.com

Abstract: Six compounds of pyridazine derivatives 1-6 have been pharmacological screening. The pharmacological screening showed that many of these compounds have a good anti-inflammatory, analgesic and antiparkinsonism activities comparable to reference drugs. The compounds 1a, 1b, 1c, 2a, 2b, and 5 were found more potent than Prednisolone (prednisolone)® and the inhibition of plasma PGE2 for these compounds were found more potent than prednisolone (prednisolone)®. The analgesic activities of all compounds were more potent than Valdecoxib (Bextra)®. Also, the compounds 1b, 2a, and 3b are the most potent antiparkinsonism agent comparable to Benzatropine (Benzotropene)®. The pharmacological properties are reported. [Journal of American Science 2010;6(7):353-357]. (ISSN: 1545-1003).

Keywords: Pyridazine; Anti-inflammatory; Analgesic; Antiparkinsonism.

1. Introduction

Pyridazine derivatives are currently being developed for the treatment of chronic inflammatory pain associated with osteoarthritis, rheumatoid arthritis and chronic lower back pain **[1,2]**, pyridazines retaing activity in the glia cell based screen for concentration dependent suppression of 1L/B production **[3,4]**. Pyridazine derivatives were found as anti-proliferative and anti-viral activity **[5]**, antinuclear and anti-bacterial action against *Helicobacter pylori* **[6]**, NMDA and AMPA receptor antagonistic action, anti-microbial and anti fungal activity **[7]**. In view of these observations used some novel pyridazine derivatives 1-6 which synthesized according Ref. **[8]**, tested their anti-inflammatory, analgesic and antiparkinsonism activities in comparison to some reference drugs.

2. Material and Methods

Determination of Acute Toxicity (LD_{50}): The LD_{50} for compounds were determined by injected different gradual increased doses of the tested compounds to adult male albino rats, then calculating the dose corresponding to 50 % animal death, according to Austen et al. **[9]**. (Table 1)

Anti-inflammatory Activity

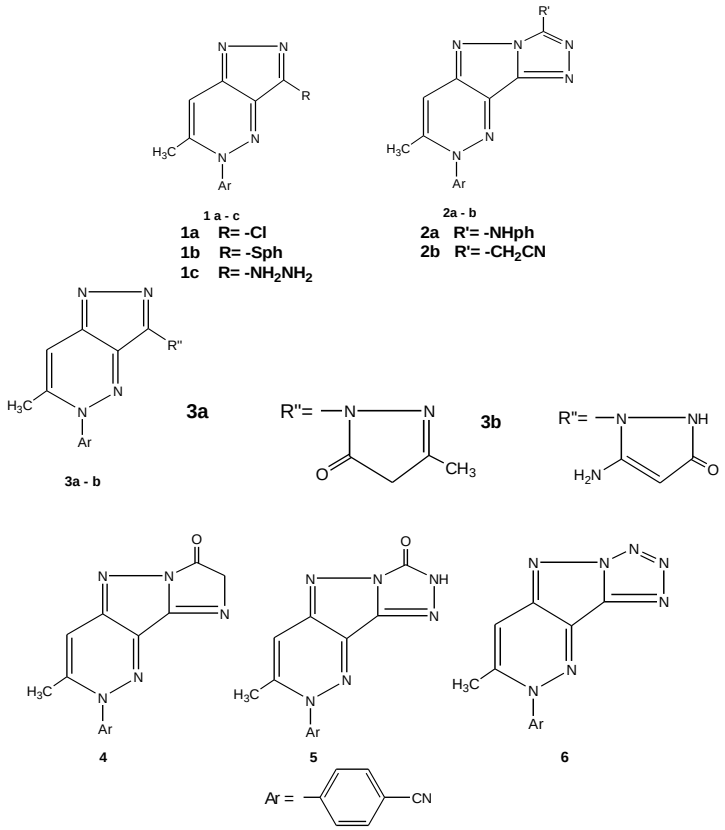
(Carrageenan)®-induced Edema (Rats Paw Test): Groups of adult male albino rats (150-180gm), each of eight animals were orally dosed with tested compounds at a dose level of 25–50 mg/kg one hour before (carrageenan)® challenge. Foot paw edema was induced by subplantar injection of 0.05 cm^3 of 1% suspension of (carrageenan)® in saline into the plantar tissue of one hind paw. An equal volume of saline was injection to the other hand paw and served as control. Four hours after drug administration the animals were decapitated, blood was collected and the paws were rapidly excised. The average weigh of edema was examined for the treated as well as the control group and the percentage inhibition of weight of edema was also evaluated. (Prednisolone)® (5mg/kg) was employed as standard reference against which the tested compounds were compared. (Table 2).

Table 1: Acute toxicity (LD_{50}) of the selected compounds

Compound No.	LD_{50} [mg/kg]
1a	2.068±0.012
1b	2.478±0.013
1c	2.100±0.012
2a	2.150±0.013
2b	1.820±0.011
3a	1.914±0.012
3b	2.561±0.011
4	2.705±0.011
5	2.681±0.012
6	1.185±0.011
Prednisolone® (ref.)	1.618±0.016

Estimation of plasma prostaglandin E2 (PGE2)

Heparinized blood samples were collected from rats (n=8),plasma was separated by centrifugation at 12,000g for 2 min. at 40 °C, immediately frozen and stored at 20 °C until use. The design correlate EIA prostaglandin E2 (PGE2) kit



(Aldrich ,Steinheim ,Germany) is a competitive immuno assay for the quantitative determination of PGE2 in biological fluids. The kit uses a monoclonal antibody to PGE2 to bind, in a competitive manner, the PGE2 in the sample after a simultaneous incubation at room temperature. The excess reagents were washed away and the substrate was added, after a short incubation time the enzyme reaction was stopped and the yellow color generated was read on a micro plate reader DYNATech, MR5000 at 405nm (Dynatech Industries Inc., Mclean,VA, USA).The intensity of the bound yellow color is inversely proportional to the concentration of PGE2 in either standard or samples.

Analgesic Activity

Sixty Webster mice of both sexes weighting 20-25 gm were divided into 10 groups. One group was kept as control (received saline), the second group received vehicle (Gum acacia) and the third one received Valdecoxib (Bextra)® as a reference drug, where as the other groups received the test compounds (SC administration). Mice were dropped gently in a dry glass beaker of 1dm^{3}capacity maintained at 55-55.5 °C. Normal reaction time in seconds for all animals was determined at time in seconds for all animals was determined at time intervals of 10, 20, 30, 45, 60, 90 and 120 minutes. This is the interval extending from the instant the mouse reaches the hot beaker till the animals licks its feet or jump out of the beaker (dose 5mg/kg) [10]. The relative potencies to Valdecoxib (Bextra)® were determined (Table 3)

Antiparkinsonism Activity

The muscarinic agonists Tremorine and Oxotremorine induce parkinsonisim like signs such as tremor, ataxia, spasticity, salivation, lacrimation and hypothermia. These signs are antagonized by antiparkinsonism agents. Groups of eight mail mice (18-20g) were used. They were dosed orally with the tested compounds (5mg/kg) or the standard Benzatropine [(Benzotropene)® mesilate,5mg/kg] [11] 1h.prior the administration of 0.5mg/kg of Oxotremerine S.C.Rectal temperature was measured before administration of the compounds and 1h. after (Oxotremerine) dosage. The scores for the recorded signs are zero (absent), one(slight), two(medium), and three (high). (Table 4) .

Table 2: Anti-inflammatory activities of some new synthesized compounds

Compound No.	Dose[mg / kg]	Protection against carrageenan-induced edema[%]*	Inhibition of plasma PGE2 [%]*
1a	25 50	87.16±0.058 98.41±0.072	84.61±0.0110 95.16±0.0120
1b	25 50	93.65±0.080 95,10±0.076	78.62±0.096 82 .66±0.087
1c	25 50	92.12±0.066 93.15±0.075	77.41±0.088 81.56±0.086
2a	25 50	91.58±0.090 97.16±0.082	95.55±0.110 92.33±0.087
2b	25 50	90.16±0.077 96.18±0.075	81.16±0.088 91.62±0.100
3a	25 50	63.86±0.065 84.10±0.067	53.11±0.088 73.82±0.079
3b	25 50	55.70±0.069 74.13±0.070	50.99±0.100 71.00±0.098
4	25 50	65.80±0.076 87.16±0.081	48.16±0.028 79.77±0.079
5	25 50	93.44±0.086 96.18±0.083	89.44±0.085 92.96±0.094
6	25 50	38.14±0.054 55.22±0.052	31.16±0.076 43.18±0.088
Prednisolone®(ref.)	25 50	81.00±0.100 93.01±0.082	77.00±0.084 91.00±0.087

Table 3: Analgesic Activities of some novel selected compounds

Compound No.	Comparative analgesic potency to valdecoxib after time in minutes			
	1 10min	30min	60min	120min
1a	0.46±0.01	0.51±0.04	0.68±0.06	1.30±0,08
1b	0.42±0.01	0.46±0.04	0.56±0.05	1.11±0.07
1c	0.74±0.03	0.98±0.09	1.23±0.11	2.20±0.20
2a	0.46±0.01	0.62±0.06	0.76±0.07	1.63±0.06
2b	0.44±0.01	0.51±0.05	0.60±0.06	1.28±0.16
3a	0.50±0.01	0.80±0.07	0.86±0.08	1.89±0.08
3b	0.46±0.02	0.58±0.05	0.73±0.07	1.58±0.04
4	0.46±0.01	0.96±0.08	1.01±0.01	2.15±0.18
5	0.54±0.01	0.85±0.05	0.96±0.08	2.22±0.21
6	0.65±0.02	0.80±0.07	1.12±0.14	2.40±0.14
Valdecoxib (ref.)	1.00	1.00	1.00	1.00

All results were significantly different from the standard and normal control value at p=0.05

Table 4: Antiparkinsonism activity of several compounds as compared with Benzatropine

Compound No.	Salivation and lacrimation score		Tremors score		% decrease from oxotremerine rectal temp.		Relative potency to Benzatropine	
Control	0	1	0	1	0	1	0	1
Benzatropine								
1a	2		2		28		0.45	
1b	2		2		28		0.83	
1c	1		1		13		0.61	
2a	2		2		19		0 .82	
2b	1		1		12		0.54	
3a	2		2		16		0.71	
3b	3		3		21		0.83	
4	3		3		18		0.42	
5	2		2		5		0.18	
6	3		3		4		0.15	

3. Results and Discussions

Three pharmacological activities namely, anti-inflammatory, analgesic and antiparkinsonism were tested despite their different biological receptors. Yet both are of neurological origin. Ten representative compounds 1-6 were studies with respect to their anti-inflammatory, analgesic and antiparkinsonism activities.

Anti-inflammatory Activity

Purpose and Rational: For the determination of the antiphogi-stic potency of the compounds, two standard tests were realized at 25 and 50 mg / kg rat body weight namely, the protection against (Carrageenan)® induced edema according Winter et al. [12] and the inhibition of plasma PGE2. The later is known as a good confirming indicator for the (Carrageenan)® induced rat paw edema [13]. Regarding the protection against (Carrageenan)® induced edema, six compounds namely 1a, 1b, 1c, 2a, 2b and 5 were found more potent than (Prednisolone)®.Where, their protection percentage against (Carrageenan)® induced edema at two dose levels 25 and 50mg/kg are 87.16±0.058/98.41±0.072, 93.65±0.080/95.10±0.076,92.12±0.066/ 93.15±0.075, 91.68±0.090/97.16±0.082,90.16±0.077/ 96.18±0.075, and 93.44±0.086/69.18±0.083 respectively [(Prednisolone)® 81.00-0.010/93.01±0.082]. On the other hand, the inhibition of plasma PGE2 for the compounds 1a, 1b, 1c, 2a, 2b and 5 were found more potent than (Prednisolne)® at two tested doses levels 25 and 50mg/kg. The inhibition percentage for the latter compounds were found as: 84.61±0.110/ 95.16±0.120,78.62±0.096/82.66±0.087, 77.41±0.088/ 81.56±0.086,95.55±0.110/92.33±0.087, 81.16±0.088/ 91.62±0.0100, and 89.44±0.085/92.96±0.094 respectively. (Table 1)

Analgesic Activity

All tested compounds exhibited analgesic activity in a hot plate assay. Interestingly, the analgesic activities of the all compounds were more potent than Valdecoxib (Bextra)® as a reference drug (Table3). Compounds 1a, 1b, 2a, 2b, 3a and 3b are arranged in descending order of analgesic potency. Compound 1c, 4, 5 and 6 showed more twice times the activity of Valdecoxib (Bextra)® after two hours .

Antiparkinsonism Activity

Compounds 1a, 1c, 2b, 3a and 4 showed moderate activity relative potencies to Benzatropine [(Benzotropene)® 0.45, 0.61, 0.54, 0.71 and 0.42]. Compounds 1b, 2a and 3b are the most potent antiparkinsonic agents (0.83, 0.82 and 0.83 relative potencies). But compounds 5 and 6 are weak activity (relative potencies 0.18 and 0.15) (Table 4).

Pharmacological screening

All animals were obtained from the Animal House Colony, Initially the acute toxicity of the compounds was assayedvia the determination of their LD50. All compounds were interestingly less toxic than the reference drug (Prednisolone)®. (Table1) Then the newly compounds were screened pharmacologically for their anti-inflammatory, analgesic and antiparkinsonism activities using male albino rats. (Tables 2,3 and 4).

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Corresponding Author:
Dr. Naif O. Al-Harbi
Pharmacy Department
College of Health Sciences
King Saud University 106150, Kingdom of Saudi Arabia
E-mail: drnaifalharbi@yahoo.com

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