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Synthesis and anti-inflammatory activity of some novel 1,2-pyrazoline derivatives

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ABSTRACT

Pyrazolines are well known, and important nitrogen containing five-membered heterocyclic compounds. It has been reported to show a broad spectrum of biological activities including antibacterial, antifungal, anti-inflammatory, and antidepressant activities and Its derivatives, possess a wide range of biological and physiological activities such as antitumor, antiarthritic, analgesic, immunosuppressive activities. In view of this various pyrazoline derivatives were synthesized and assessed for the anti-inflammatory activity. In this article new substituted several 1,2 Pyrazoline derivatives were synthesized by reacting Chalcones with hydrazine hydrate in dry benzene. The structures of the synthesized compounds were confirmed by spectral data. Compounds have been screened for anti-inflammatory activity by using Carrageenan induced rat hind paw oedema. Among the 7 compounds that were screened for anti-inflammatory activity, compounds SP1 to SP4 showed 30%, 34%, 30% and 32% inhibition of oedema volume, while the standard drug (Ibuprofen) showed inhibition of 51%.

Keywords: COX-2, 1, 2 Pyrazolines, Chalcones, Ibuprofen, Anti-inflammatory activity.

INTRODUCTION

Inflammation is a basic way in which the body reacts to infection, irritation or other injury, the key feature being redness, warmth, swelling and pain. In the early 1990's it was discovered that cyclooxygenase is the key enzyme for biosynthesis of prostaglandins, which catalyses the conversion of arachidonic acid to prostaglandins and thromboxanes. Cyclooxygenase enzymes exists as 2 isoforms i.e. COX-1 and COX-2. COX-1 produced in many tissues such as the kidney and the GIT, while COX-2 is inducible and is expressed during inflammation at a site of injury. Heterocycles are important components of biomolecules such as proteins, DNA, RNA and vitamins. Among the heterocyclic compounds, five membered heterocyclic moieties fused with aromatic ring systems containing various heteroatoms such as N, S and O, exhibited wide spectrum of pharmacological activities. [1-4]

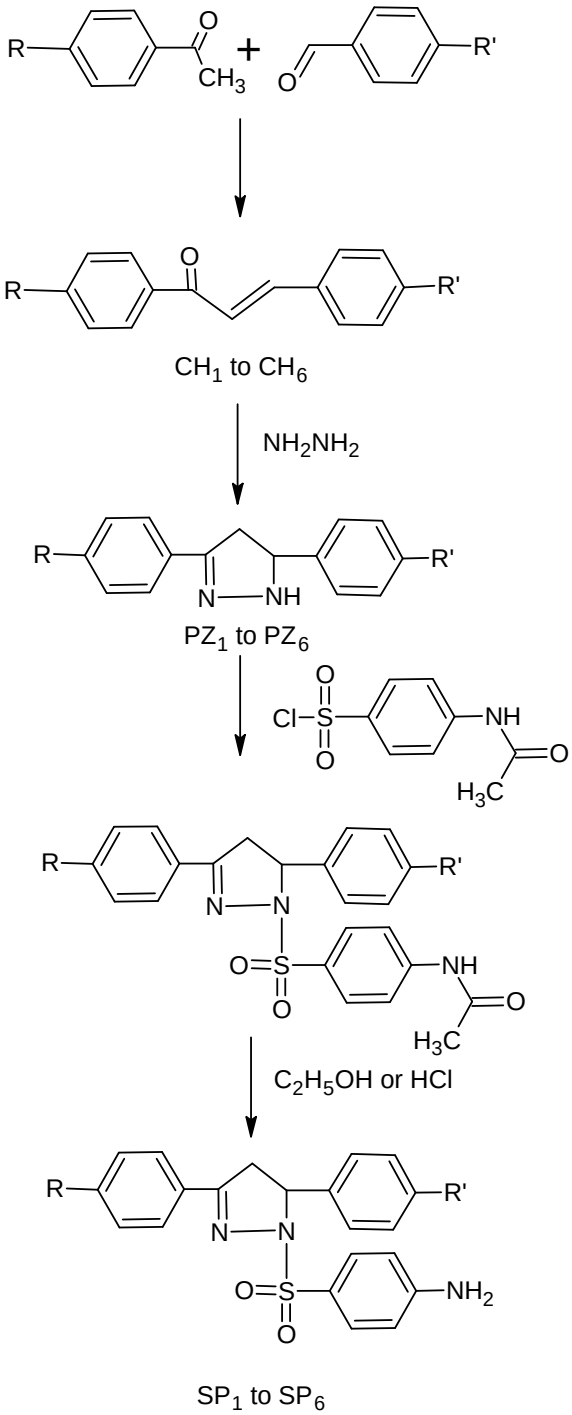
In view of this various pyrazoline derivatives were synthesized and assessed for the said biological activity. Pyrazolines are well known, and important nitrogen containing five-membered heterocyclic compounds. Various methods have been worked out for their synthesis. Synthesis and characterization of pyrazoline derivatives has been a developing field within the area of heterocyclic chemistry for the past several decades because of their ready accessibility through synthesis, wide range of chemical reactivity and broad spectrum of biological activity [5-7]

Pyrazolines have been reported to show a broad spectrum of biological activities including antibacterial, antifungal, anti-inflammatory, and antidepressant activities. Its derivatives, possess a wide range of biological and physiological

activities such as antitumor, antiarthritic, analgesic, anti diabetic, fungicidal, bactericidal, immunosuppressive activities.[6,8]

MATERIALS AND METHODS

All the solvents and reagents used were obtained from commercial sources and were used without further purification. The melting points of the organic compounds were determined by open capillary tube method and were uncorrected. FTIR were recorded on a Shimadzu Prestige 21. TLC was used to monitor the progress of all reactions. All the compounds showed satisfactory elemental analysis for C, H, and N.



Synthetic Route for the Synthesis of Pyrazolines

To perform biological activities, Oedema was produced using type IV lambada carrageenan from sigma laboratories. Foot volumes were measured in a plethysmograph by water displacement. The instrument was calibrated before

performing the experiment using standard calibrated probe number and standard drug used ibuprofen was procured from sun pharmaceutical industry.

All Experimental procedures were carried out in strict accordance with the guidelines prescribed by the committee for the Purpose of Control and Supervision on Experimentation on Animals (CPCSEA) and were approved by the Institutional Animal Ethical committee (Resolution no. 13).

Experimental:

General procedure for the synthesis of Chalcones (CH1 to CH6):

To the solution of sodium hydroxide (22g) in water (200 ml), ethanol (122.5 ml) was added and the flask was immersed in a bath of crushed ice. Equimolar quantity, 0.01M, of both aldehyde and p- phenoxy acetophenone were added to the above mixture with continuous stirring. Stirring was carried, for about 3-4 hr. The reaction mixture was kept in the ice chest for 24-hr and the solid obtained was filtered and washed using cold water until all the washings were neutral to litmus. The crude product obtained was crystallized from alcohol.[9,10,15]

General procedure for the synthesis of Pyrazolines (PZ1 to PZ6):

A mixture of chalcone (0.01 M) and hydrazine hydrate (0.24 M) in dry benzene (30 ml) was taken in an RBF. The reaction mixture was refluxed for 4-6 hrs using a Dean-stark water separator apparatus, until the theoretical volume of water was separated. The reaction mixture was monitored using TLC solvent system Benzene: Ethyl acetate – 9: 1 and purity was confirmed by single spot.

After the reaction was over, the RBF was placed on a magnetic stirrer and acetyl chloride (0.012 M) was added drop wise. The reaction mixture initially become viscous and on later stages it liquefies as addition of acetyl chloride was continued.

After the addition was over, an additional 2-3 hrs of stirring was carried out to complete the reaction. Then the solvent was evaporated in vacuum and the mass obtained was triturated in water to get the desired product, which was further crystallized, from Petroleum Ether.[11,16]

General procedure for the synthesis of P-amino benzene sulphonyl 1,2 pyrazoline (SP1 to SP6):

A mixture of Pyrazoline and p-acetamido benzene sulphonyl chloride (1:1) in benzene was taken in an RBF. The reaction mixture was refluxed for 4-6 hrs. After refluxing add triehylamine (2-3 drops) and reflux for 2 hrs. to evaporate benzene to form P- acetamido benzene sulphonyl 1,2 pyrazoline. Then this formed P- acetamido benzene sulphonyl 1,2 pyrazoline(2gm.) dissolved in 35 ml of boiling ethanol contained in a R.B.F. equipped with a reflux condenser. With the aid of a pressure dropping funnel add 4 ml of Conc. HCl down the condenser in small portions to the boiling solution. Reflux for 5-6 hrs until a test portion remains clear .then add ammonia solution dropwise with constant stirring until just alkaline. The reaction mixture was filtered **,** recrystallised from dilute alcohol.[17]

Table-1: List of synthesized compounds

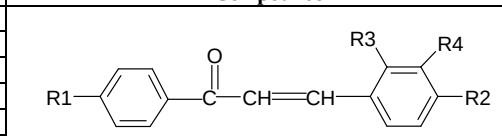
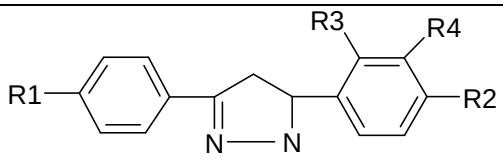
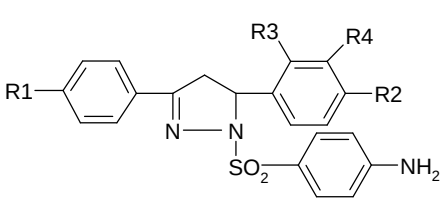
Sr. No.	Compounds	Comp code	R1	R2	R3	R4
1		**CH_1**	Cl	OCH_3	-	-
2		**CH2**	Cl	CH_3	-	-
3		**CH3**	OCH_3	CH_3	-	-
4		**CH4**	Cl	OCH_3	-	OCH_3
5		**CH5**	Cl	Cl	Cl	-
6		**CH6**	Cl	OH	-	-
7		**PZ1**	Cl	OCH_3	-	-
8		**PZ2**	Cl	CH_3	-	-
9		**PZ3**	OCH_3	CH_3	-	-
10		**PZ4**	Cl	OCH_3	-	OCH_3
11		**PZ5**	Cl	Cl	Cl	-
12		**PZ6**	Cl	OH	-	-
13		**SP1**	Cl	OCH_3	-	-
14		**SP2**	Cl	CH_3	-	-
15		**SP3**	OCH_3	CH_3	-	-
16		**SP4**	Cl	OCH_3	-	OCH_3
17		**SP5**	Cl	Cl	Cl	-
18		**SP6**	Cl	OH	-	-

Table 2: Analytical data of the synthesized compounds

Comp	Molecular Formula	Mol Wt.	M. P. ° C	Yield %	Elemental Analyses Calculated (Found)		
					C	H	N
CH1	$C_{16}H_{13}O_2Cl$	272.5	122-124	86.6	0.70	0.04	-
CH2	$C_{16}H_{13}OCl$	256.5	110-112	78.3	0.74	0.5	-
CH3	$C_{17}H_{16}O_2$	252.0	108-110	91.6	0.80	0.06	-
CH4	$C_{17}H_{15}O_3Cl$	302.5	128-130	90.0	0.67	0.04	-
CH5	$C_{15}H_9OCl_3$	311.5	134-138	86.6	0.57	0.02	-
CH6	$C_{15}H_{11}O_2Cl$	258.5	124-128	86.6	0.69	0.04	-
PZ1	$C_{16}H_{15}ON_2Cl$	286.5	180-182	74.4	0.67	0.05	0.09
PZ2	$C_{16}H_{15}N_2Cl$	242.5	194-198	69.5	0.79	0.06	0.11
PZ3	$C_{17}H_{18}ON_2$	266.0	144-146	75.8	0.76	0.06	0.10
PZ4	$C_{17}H_{17}O_2N_2Cl$	316.5	187-189	71.6	0.64	0.05	0.08
PZ5	$C_{15}H_{11}N_2Cl_3$	325.5	164-166	69.2	0.55	0.03	0.08
PZ6	$C_{15}H_{13}OCl$	244.5	154-156	76.5	0.73	0.05	-
SP1	$C_{22}H_{20}SO_3N_3Cl$	441.5	226-228	54.0	0.59	0.04	0.09
SP2	$C_{22}H_{20}SO_2N_3Cl$	487.5	236-238	55.6	0.54	0.04	0.08
SP3	$C_{23}H_{23}SO_3N_3$	389.0	205-207	49.6	0.70	0.05	0.10
SP4	$C_{23}H_{22}SO_4N_3Cl$	471.5	224-226	48.0	0.58	0.04	0.08
SP5	$C_{21}H_{16}SO_2N_3Cl_3$	479.5	186-188	52.3	0.52	0.03	0.08
SP6	$C_{21}H_{18}SO_3N_3Cl$	395.5	210-212	47.3	0.63	0.04	0.10

Table 3: IR Spectral Data of synthesized compound (SP1-SP6):

Compound Code	IR Bands(cm^{-1})	Types of Vibration
SP1	2850 1600 1400 1350 1100 755	-C-H Ar. Str. -C=C Ar. Str. -C= N Str. -C-N Str. -OCH_3 Str. -Cl Str.
SP2	2900 1600 1405 1300 2900 700	-C-H Ar. Str. -C=C Ar. Str. -C= N Str. -C-N Str. -CH_3 Str. -Cl Str.
SP3	2900 1600 1405 1350 2900 1100	-C-H Ar. Str. -C=C Ar. Str. -C= N Str. -C-N Str. -CH_3 Str. -OCH_3 Str.
SP4	2950 1605 1480 1300 1100,1170 700	-C-H Ar. Str. -C=C Ar. Str. -C= N Str. -C-N Str. -OCH_3 Str. -Cl Str.
SP5	2850 1600 1400 1380 730,740,755	-C-H Ar. Str. -C=C Ar. Str. -C= N Str. -C-N Str. -Cl Str.
SP6	2860 1600 1400 1380 730 3300	-C-H Ar. Str. -C=C Ar. Str. -C= N Str. -C-N Str. -Cl Str. -OH Str.

Biological Activity:

Acute anti-inflammatory method:

Carrageenan induced rat hind paw oedema:

The method of winter et al (Winter et al 1962) was used with slight modification. The animals were divided into 9 groups of 5 animals each one group served as control, another group served as a standard (ibuprofen) and the rest of the groups were used for the test drugs.[18,19]

The rats were dosed orally at 300mg/kg body weight, including the control and ibuprofen. Test compounds and standard drug were suspended 0.5% of sodium carboxyl methylcellulose mucilage, which was used as a vehicle. For the control group a solution of 1% of carrageenan was used as an inflammatory agent.

Food was withdrawn overnight with adequate water before the experiment. The drugs were dosed orally with the help of oral catheter. After thirty minutes drug administration, 0.1 ml of 1% carrageenan in normal saline was injected into the subplanter region of left hind paw. The volume of the injected paw was measured with a plethysmograph. By water displacement method at zero hour immediately after injecting carrageenan the same procedure was repeated at 1hr, 2hr and 4hr. The difference between 0 hour and subsequent readings was taken as actual oedema volume.[12-13]

RESULTS AND DISCUSSION

Synthesis of Pyrazoline:

We have reported various derivatives of 1,2 pyrazolines from Chalcones to achieve the promising and selective inhibition as far as anti-inflammatory activity is concerned. The synthesized 1,2 pyrazoline derivatives resemble to some of the COX-II inhibitory agents like celecoxib and rofocoxib. All the listed compounds (Table No. 01) were synthesized by above reported method. The characterization of intermediates was done by melting point and elemental analysis (Table No.02). While, the final derivatives were characterized by using IR also.(Table No.03) The Anti-inflammatory screening was performed by carrageenan induced paw oedema method by using water displacement plethysmography. As a result, the screened compounds have shown good Anti-inflammatory activity (Table No. 04).

Ibuprofen was used as the standard drug for Anti-inflammatory activity; synthesized derivatives have shown the maximum Anti-inflammatory activity when compared with control. The results were calculated with the help of ANNOVA.

Table No.04: Effect of synthesized compound, ibuprofen and carrageenan induced rat paw oedema by oral administration

Sr. No.	Drug (300mg/kg)	Mean Paw Oedema Volume (ml) ± SE						% inhibition after 4 hour
		O hr	1/2 hr	1 hr	2 hr	3 hr	4 hr	
1.	**Control**	0.133±0.0516	0.417±0.0753	2.02±0.172	2.60±0.261	2.57±0.301	1.97±0.327***	--
2.	**Std.**	0.0833±0.0753	0.600±0.228	1.03±0.242	1.68±0.182	1.15±0.243	0.967±0.197***	**51**
3.	**SP1**	0.117±0.0408	0.367±0.0816	1.60±0.424	1.98±0.145	1.47±0.314	1.37±0.258***	**30**
4.	**SP2**	0.133±0.0516	0.300±0.0632	1.48±0.214	1.62±0.138	1.35±0.315	1.30±0.126***	**34**
5.	**SP3**	0.100±0.0	0.333±0.0516	1.32±0.133	1.60±0.200	1.35±0.105	1.37±0.103***	**30**
6.	**SP4**	0.117±0.0408	0.267±0.103	1.38±0.194	1.82±0.232	1.45±0.442	1.33±0.207***	**32**

**** P < 0.001 when compared to control group (one way ANOVA followed by Dunnet's test).*
n=6. Values are expressed as Mean ± S.E.M.

Ibuprofen was used as the standard drug for Anti-inflammatory activity; compounds synthesized have shown the maximum Anti-inflammatory activity when compared with control. The active compounds could be taken as lead for structural and molecular modification was thought of in future.

The test compounds and standard drug were suspended 0.5% of sodium carboxyl methylcellulose mucilage, which was used as a vehicle. For the control group a solution of 1% of carrageenan was used as an inflammatory agent. Among 7 compounds that were screened for anti-inflammatory activity, compounds SP1 to SP4 showed 30% , 34%,30% and 32% inhibition of oedema volume, while the standard drug (Ibuprofen) showed inhibition of 51%.

CONCLUSION

Numerous derivatives of pyrazoline were synthesized and characterized. Spectral data confirms the structure of the synthesized derivative as expected. These derivatives later screened for their anti-inflammatory activity. From the results it can be concluded that the modified pyrazoline shows remarkable anti-inflammatory action.

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