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Evaluation of *In Vitro* Anti-inflammatory Activity of Azomethines of Aryl Oxazoles

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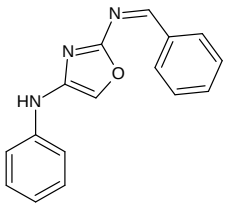
Abstract: Ability to inhibit erythrocyte hemolysis is often used as a characteristic of the membrane stabilising action of chemical compounds. Azomethines of aryl oxazoles were evaluated for anti-inflammatory by *in vitro* hemolytic membrane stabilising study. The effect of inflammation condition was studied on erythrocyte exposed to hypotonic solution. In this *in vitro* method the membrane stabilising action leads to anti-inflammatory activity and was compared with that produced by diclofenac sodium as the reference standard. Results of the evaluation indicate that the synthesised compounds found to exhibit membrane stabilising activity.

Key words: Erythrocyte, Hemolysis, Oxazoles azomethines

Introduction

Compounds with membrane stabilising properties are well known for their ability to interfere with the release of phospholipases that trigger the formation of inflammatory mediators likes prostaglandins and leukotrienes *etc.* through COX (cyclooxygenase) and LOX (lipoxygenase) pathway

Membrane stabilisers are chemical compounds which depress excitability in nerve and muscle without causing obvious changes in the resting membrane potential[1]. These compounds protect erythrocytes against hypotonic hemolysis and stabilise the lysosomes and cell organelles under *in vitro* conditions. The literature review suggests that the heterocyclic compounds are known to possess anti inflammatory activity[2-4], hence the anti-inflammatory potential of the synthesised compounds were evaluated by erythrocyte membrane stabilising method. The effect of inflammation condition was studied by erythrocyte exposing to hypotonic solution, the results were compared with that of the standard diclofenac sodium. The synthesis, experimental and spectral data are already sent for publication. The structure of the synthesised compounds are as follows.



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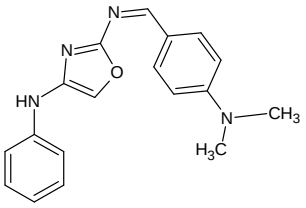

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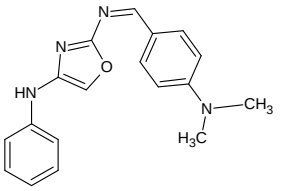

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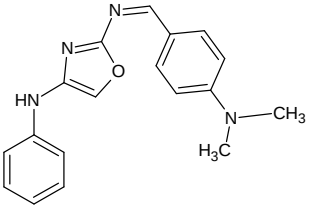

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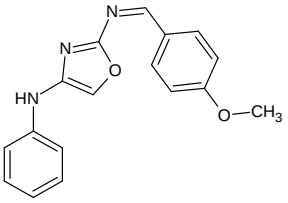

7e

(7a) *N^4-phenyl-N^2-[(Z)-phenylmethylidene]-1,3-oxazole-2,4-diamine,* ***(7b)*** *N^2-{(Z)-[4-(dimethylamino) phenyl]methylidene}-N^4-phenyl-1,3- oxazole-2,4-diamine,* ***(7c)*** *N^2-[(Z)-(4-chlorophenyl)methylidene]-N^4-phenyl-1,3-oxazole-2,4diamine,* ***(7d)*** *N^2-[(Z)-(3- nitrophenyl) methylidene]-N^4-phenyl-1,3-oxazole-2, 4-diamine,* ***(7e)*** *N^2-[(Z)-(4-methoxy phenyl) methylidene]-N^4-phenyl-1,3-oxazole-2,4-diamine*

Experimental

Erythrocyte suspension[5-8]

Blood was collected from median cubital vein of healthy volunteers. The blood was mixed with isosaline and centrifuged at 3000 rpm. The packed cells were further washed with isosaline and the sedimented erythrocytes were collected. A suspension of 2%v/v of erythrocyte in isosaline was prepared.

Procedure (hypotonic solution-induced haemolysis)

This test was done by the following method[9,10] with slight modification. Blood was collected from median cubital vein of healthy volunteers. The blood was mixed with isosaline and centrifuged at 3000 rpm. The packed cells were further washed with isosaline and the sedimented erythrocytes were collected. A suspension of 2%v/v in isosaline was prepared.

The assay mixture comprises of hyposaline (2 mL), sodium phosphate buffer at pH 7.4 (1 mL), varying volumes of drugs in the concentration ranging from 88.88-444.44 µg/mL and erythrocyte suspension (0.5 mL) were made up with isosaline to give a total assay volume of 4.5 mL. The control was prepared as above by omitting the drug sample.The reaction mixtures were incubated at 56 ^{0}C for 30 minutes. The tubes were cooled under running water followed by centrifugation at 5000 rpm. The supernatant was collected and the absorbance of the supernatant liquid at 560 nm. The results are given in the Table 1.

Calculation

The percentage of membrane stability was estimated using the expression:
Percentage inhibition of hemolysis = C-T/C*100,
Where, C - absorbance of control, T – absorbance of test sample

Table 1. Membrane stabilising activity

Conc. µg/mL of the test and std	% Inhibition of membrane Stabilizing Action					
	Standard (diclofenac sodium)	**7a**	**7b**	**7c**	**7d**	**7e**
88.88	43.07	45.78	44.87	41.86	36.74	48.49
177.77	58.73	56.32	55.12	47.89	52.10	50.90
266.66	78.01	75.60	73.19	57.34	57.22	55.12
355.55	82.52	76.20	74..09	72.20	61.74	62.04
444.44	85.24	81.62	78.31	75.0	64.45	78.01

Results and Discussion

The activity and the viability of the cell depend upon the integrity of the cell membrane. Exposure of erythrocyte cells to injurious substances such as hypotonic medium results in the lysis of membrane associated with hemolysis and oxidation of haemoglobin. The hemolysis effect of hypotonic solution is related to excessive accumulation of fluid within the cells and damaging of cell membranes. The damage of erythrocytes will further destruct the cell and make it vulnerable to secondary damage through free radical induced lipid per-oxidation. It is therefore expected that compounds with membrane stabilising properties should offer significant protection of cell membrane.

In the study of membrane stabilization by hemolytic method the synthesised compounds at concentration range of 88.88-444.44 µg/mL were found to protect significantly the erythrocyte membrane against lysis induced by hypotonic solution. The compounds **7a,7b, 7c** exhibited significant membrane significant activity when compared that of the reference; the other compounds **7d, 7e** had moderate activity. It was also observed that all the compounds showed dose dependent inhibition of hemolysis. Hence all the synthesised compounds are potential to anti inflammatory activity by erythrocyte membrane stabilisation method.

Acknowledgment

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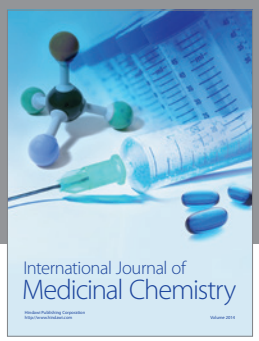

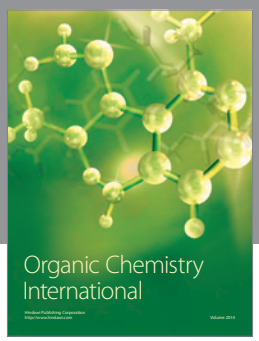

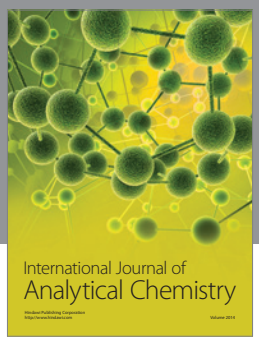

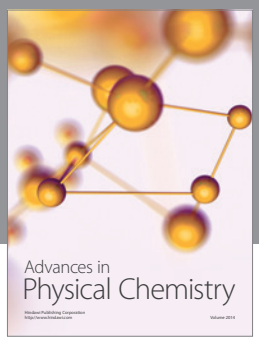

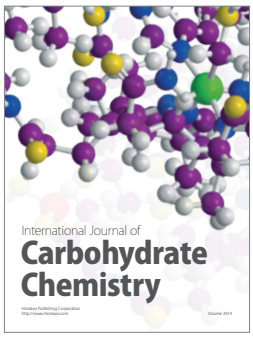

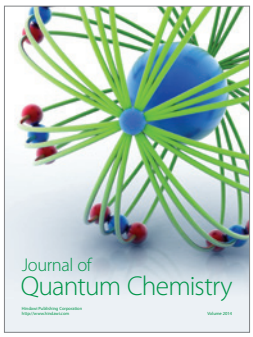

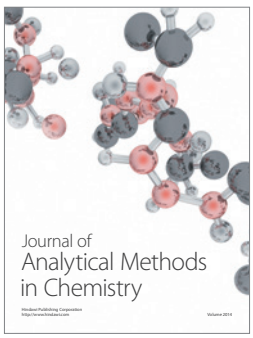

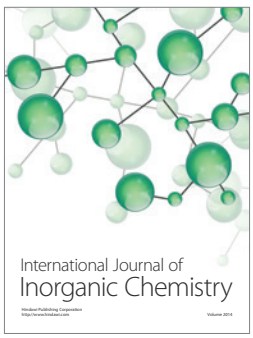

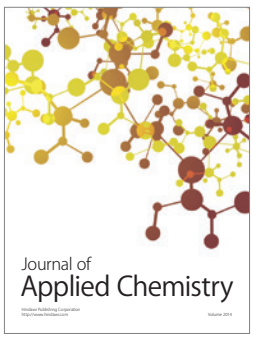

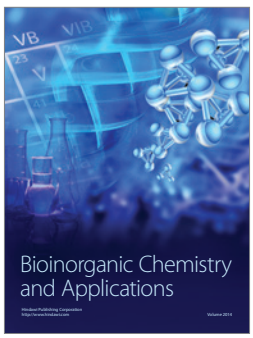

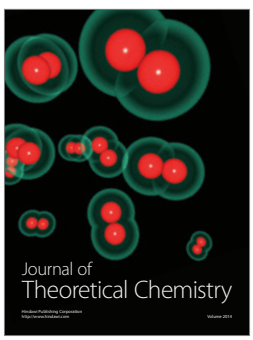

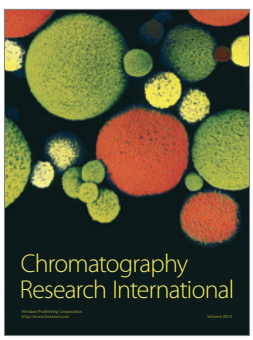

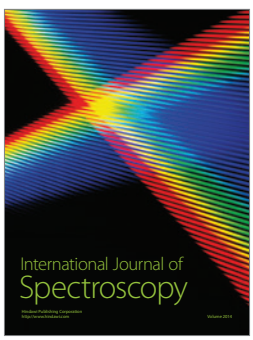

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