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Reserch Article

EVALUATION OF ANTIOXIDANT AND ANTI-ARTHRITIC ACTIVITIES OF SOME 2-ARYLAMINOTHIAZOLE DERIVATIVES

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ABSTRACT

Objective: A series of Arylaminothiazole were designed, synthesized and evaluated for their anti-arthritic activity and antioxidant activity.

Methods: Synthesized molecules were evaluated for their *in vitro* Anti-arthritic activity using protein denaturation method and antioxidant activity using DPPH scavenging assay.

Results: TTA50, TTA51, TTA54 and TTA55 showed significant anti-arthritic and antioxidant activity.

Conclusion: Thus, it may be concluded that anti-arthritic activity of TTA50, TTA51, TTA54 and TTA55 is associated with its antioxidant potential.

Keywords: Arylaminothiazole, Anti-arthritic action, Antioxidant action.

INTRODUCTION

Rheumatoid arthritis (RA) is a progressive, disabling, chronic multisystem disease of unknown cause characterized by pain, swelling and stiffness of synovial joints. An inflammatory reaction, increased cellularity of synovial tissue and joint damage are the pathological hallmarks of RA [1].

Though conventional treatment options for this condition have improved in terms of effectiveness, the use of non-steroidal anti-inflammatory drugs (NSAIDs) like etoricoxib, disease modifying anti-rheumatic drugs (DMARDs) like methotrexate, sulphasalazine, leflunomide, hydroxychloroquine, and corticosteroids like prednisolone, methylprednisolone have all been associated with adverse effects. Hence, recently researchers are directed towards the discovery of drugs that are long acting anti-inflammatory with minimum side effects [2].

It has been established that in many inflammatory disorders, there is excessive activation of phagocytes, production of reactive oxygen species, such as superoxide ($O2^{\cdot}$), hydroxyl ($OH^{\cdot}$) and peroxyl ($OOH^{\cdot}$, $ROO^{\cdot}$) radicals as well as non-free radical species (H_2O_2) which can harm surrounding tissue either by powerful direct oxidizing action or indirectly with hydrogen peroxide (H_2O_2) and $OH^{\cdot}$ radicals formed from O^{2-}, which initiates lipid peroxidation resulting in membrane destruction. Tissue damage then provokes inflammatory response by production of mediators and chemotactic factors. The reactive oxygen species are also known to activate matrix metalloproteinase (e.g. collagenase) causing increased destruction of tissues e.g. collagenase damage seen in various arthritic reactions [3].

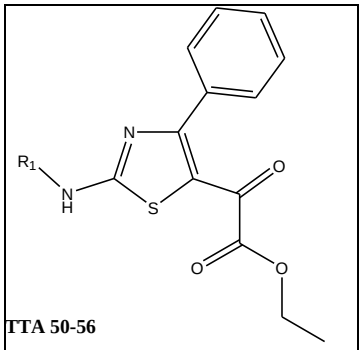
Hence, the agents that can scavenge these reactive oxygen species can be beneficial in the treatment of inflammatory disorders. Previous studies in our laboratory has already reported *in vitro* and *in vivo* anti-inflammatory activity of a series of 2-Aminothiaozoles using human red blood cell membrane stabilizing assay and carrageenan induced paw edema model of acute inflammation respectively. Hence, present study was designed to investigate anti-arthritic and antioxidant activity of 2-Arylaminothiaozoles [4].

MATERIALS AND METHODS

Drugs and chemicals: Diclofenac, Ascorbic acid, phosphate buffer, DPPH, methanol were purchased from S. D. Fine Chemicals, Mumbai, India.

Compounds

The novel 2-Arylaminothiaozoles were synthesized based on the previously described methods. The novel 2-Aminothiaozoles were divided on the basis of the substituents attached to the aryl ring attached to the amino group at 2 position of the thiazoles (figure 1). The substituents selected were ranging from electron donating and electron withdrawing groups at 4' and 2' positions of aryl ring to investigate the effect of the substituents and their positions on the activity [4].



TTA 50-56

R1=(p-OCH3)C6H4CO-, (p-OCH3)C6H4-, (o-CH3)C6H4-, C6H5-, (o-OCH3)C6H4-, (p-CH3)C6H4-, (p-Br)C6H4-

In vitro anti-arthritic activity using Protein denaturation assay

The reaction mixture (5 ml) consisted of 0.2 ml of bovine serum albumin, 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of synthesized compounds (100 and 200 µg/ml). Similar Volume of double-distilled water served as control. Then the mixtures were incubated at (37±2) ^{o}C in a BOD incubator (Labline Technologies) for 15 min and then heated at 70^{o}C for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank. Ascorbic acid was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times \frac{Vc - Vt}{Vc}$$

Where, Vt = absorbance of test sample, Vc = absorbance of control [5].

In vitro antioxidant activity using DPPH scavenging assay:

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH. 0.1mM solution of DPPH in methanol was prepared and 1.0ml of this solution was added to 3.0ml of extract solution of

varying concentrations of synthesized compounds (10, 25, 50, 100, 200, 500 µg/ml). The mixture was incubated for 45 min at room temperature and the absorbance was measured at 517nm against corresponding blank solution. Ascorbic acid was used as reference. Percentage inhibition of DPPH free radical was calculated based on the control reading, (which contained DPPH and distilled water without any extract) using the following equation:

$$\% \text{ DPPH Scavenged} = \frac{(Ac - At)}{Ac} \times 100$$

Where Ac is the absorbance of the control, and At is the absorbance of the extract or standard.

The antioxidant activity was expressed as IC50. The IC50 value was defined as the concentration in µg/ml of that extract that inhibits the formation of DPPH radicals by 50% [6].

RESULT AND DISCUSSION

Rheumatoid arthritis is a major ailment among rheumatic disorders. It is a chronic condition with multiple causation and affects the people in their most active period of life. The denatured bovine serum albumin expresses antigens associated to Type III hyper-sensitive reaction which are related to diseases such as serum sickness, glomerulonephritis etc. Heat denatured proteins are as effective as native proteins in provoking delayed hypersensitivity [7].

It was already proved that Conventional NSAID's like phenylbutazone and indomethacin act by the inhibition of endogenous prostaglandins production by blocking COX enzyme as well as by prevention of denaturation of proteins. Thus anti-denaturation assay is the convenient method to check the anti inflammatory activity. The production of auto antigens in certain arthritic diseases may be due to *in vivo* denaturation of proteins. Thus, anti-arthritic activity is associated with inhibition protein denaturation [8].

Hence, inhibition of protein denaturation is used as investigational tool to explore *in vitro* anti-arthritic activity of synthesized analogues. In this test TTA50, TTA51, TTA54 and TTA55 showed significant inhibition of protein denaturation as compared to control. Diclofenac at the concentration 100 µg/ml and 200 µg/ml was used as reference drug, which also showed significant inhibition of protein denaturation i.e. 55.25 % and 90.41 % respectively (*p<0.001). Thus, TTA50, TTA51, TTA54 and TTA55 showed significant anti arthritic activity as demonstrated by significant inhibition of heat induced protein denaturation while TTA52, TTA53 and TTA56 did not show inhibition of protein denaturation. The observations are given in table 1.

There is much evidence to suggest that oxygen free radicals and H_2O_2 are closely involved in the pathogenesis of RA. Granulocytes are strongly increased in number in Synovial fluid of rheumatoid arthritic patients and produce large amounts of O^{2-} and H_2O_2 during the phagocytosis of immune complexes and other materials [9]. Oxygen free radicals and other secondarily formed radicals are possibly responsible for at least part of the joint destruction. Various forms of antioxidant therapy have demonstrated promising results in experimental arthritis models [10]. Therefore, to elucidate mechanism involved in anti-arthritic action of TTA50, TTA51, TTA54 and TTA55, we studied *in vitro* antioxidant activity using DPPH scavenging assay. In this study, TTA50, TTA51, TTA54 and TTA55 showed a dose dependent DPPH scavenging activity with IC50 values comparable to reference compound ascorbic acid while TTA52, TTA53 and TTA56 showed comparatively less IC50 values. IC50 values of synthesized compounds (TTA50-TTA56) were summarized in table 2. Thus, it may be concluded that anti-arthritic activity of TTA50, TTA51, TTA54 and TTA55 may be associated with its antioxidant potential.

Table 1: Effect of Thiazole Analogs (TTA50 to TTA 56) on protein denaturation activity

Treatments	R_1	100µg/ml		200 µg/ml	
		OD	% Inhibition	OD	% Inhibition
Diclofenac	-	0.65±0.020**	55.25±1.39**	0.14±0.025**	90.41±1.72**
TTA 50	(p-OCH_3)C_6H_4CO-	0.86±0.026*	41.10±1.81*	0.69±0.018*	50.91±1.21*
TTA 51	(p-OCH_3)C_6H_4-	0.58±0.031**	60.27±2.09*	0.69±0.041**	64.16±2.78**
TTA 52	(o-CH_3)C_6H_4-	1.42±0.038	2.74±1.59	0.69±0.038	11.64±2.59
TTA 53	C_6H_5-	1.29±0.035	11.64±2.37	0.69±0.038	18.72±2.57
TTA 54	(o-OCH_3)C_6H_4-	0.61±0.035**	57.76±2.38**	0.69±0.020**	69.41±1.39**
TTA 55	(p-CH_3)C_6H_4-	0.62±0.027**	57.08±1.87**	0.69±0.032**	72.60±2.20**
TTA 56	(p-Br)C_6H_4-	1.29±0.035	11.64±2.37	0.69±0.038	18.72±2.57

n= 3, Data were analyzed by ANOVA followed by Dunnett's test; *p<0.001 compared to control

Table 2: IC50 value of synthesized Thiazole Analogs (TTA50 to TTA 56)

	IC50 value
Ascorbic acid	118.37
TTA 50	96.99
TTA 51	91.47
TTA 52	210.44
TTA 53	223.61
TTA 54	102.54
TTA 55	102.54
TTA 56	225.17

CONCLUSION

Rheumatoid arthritis is one of the major chronic inflammatory disorders. The role of oxidative free radicals in pathogenesis of rheumatoid arthritis is well established. The agents that can scavenge these reactive oxygen species can be beneficial in the treatment of inflammatory disorders. In the present study, we investigated anti-arthritic activity using protein denaturation assay and antioxidant activity using DPPH scavenging assay of 2Arylaminothiazoles. The results of current investigation conclude that TTA50, TTA51, TTA54 and TTA55 showed significant anti arthritic activity as demonstrated by significant inhibition of heat induced protein denaturation as well as showed DPPH scavenging activity. Thus, it may be concluded that anti-arthritic activity of TTA50, TTA51, TTA54 and TTA55 is associated with its antioxidant potential.

REFERENCES

1. Lipsky P. E., Rheumatoid arthritis. In: Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL, editors. Harrison's principles of internal medicine. 16th ed. New York: McGraw Hill; 2005, 1968-77

2. Barbier A., Novarro J., Brelliere J. C., Roncucci R.; Biochemical and clinical changes in rats with the developing arthritis.; Agents and Actions; 1984, 15:103-105.
3. Sakat S. S, Juvekar A. R., Gambhire M. N., *In Vitro* antioxidant and antiinflammatory activity of methanol extract of *Oxalis corniculata* Linn, International Journal of Pharmacy and Pharmaceutical Sciences., 2010, 2 (1), 146-155.
4. Ingale P. L., Kasture V. S., Ingale S. P., and Kasture S. B., Design, synthesis and characterization of novel oxo-[2-(arylamino)-4-phenylthiazol- 5-yl]-acetic acid ethyl esters as potential anti-inflammatory agents., International Journal of Pharmacy and Pharmaceutical Sciences, 2013, 5 (4) 534-537.
5. Chandra S., Chatterjee P., Dey P., Bhattacharya S.; Evaluation of *in vitro* anti-inflammatory activity of coffee against the denaturation of protein; Asian Pacific Journal of Tropical Biomedicine; 2012, S178-180.
6. Sheik S., Chandrashekar K. R.; *In vitro* antimicrobial, antioxidant, antiarthritic and phytochemical evaluation of Pscychotria flavida talbot, an endemic plant of western ghats., International Journal of Pharmacy and Pharmaceutical Sciences; 2013, 5 (1), 214-218
7. Gell P. G. H., Benacerraf B., et al.; Studies on hypersensitivity-II delayed hypersensitivity to denatured proteins in guinea pig.; Immunology; 1959, 2 (1) 64-70.
8. Grant N. H., Alburn H. E., Kryzanauskas C.; Stabilization of serum albumin by anti-inflammatory drugs.; Biochem Pharmacol; 1970; 19, 715-722.
9. Babior E. M. Kipnes R. S., Curnutte J. T.; Biological defense mechanism: the production by leucocytes of superoxide, a potential bactericidal agent.; J Clin Invest; 1973, 52 (3), 741-744.
10. Hitchon C. A. and El-Gabalawy H. S.; Oxidation in rheumatoid arthritis.; Arthritis Res & Ther; 2004 6 (6), 265-278.