Synthesis, Characterization, Anti-inflammatory, and Antioxidant Activities of Some New Thiazole Derivatives

Firas A. Hassan
Al-Nahrain University, College of science
Chemistry department, Al- Jadrya, Baghdad, Iraq.

Abstract

A series of novel thiazole derivatives (A, A_1, A_2) were synthesized starting from 1-(3-methoxy phenyl) ethanone and thiourea. The compound (A) was obtained by heating 1-(3-methoxy phenyl) ethanone with thiourea in iodine. Compound (A) on treatment with 4-nitrobenzaldehyde afforded (Z)-4-(3-methoxyphenyl)-N-(4-nitrobenzylidene) thiazole-2-amine (A_1). Acylation of compound (A) with 4-nitrobenzoyl chloride to obtain the corresponding N-[4-(3-methoxyphenyl) thiazole-2-yl]-4-nitrobenzamid (A_2). The structures of compounds have been established by means of FTIR and ^{1}H-NMR spectral analysis. All thiazole derivatives were evaluated for anti-inflammatory activity by carrageenan induced Rat hind paw method. Derivative A1 show maximum anti-inflammatory activity. All the derivatives were screened for their in vitro antioxidant properties, through total antioxidant capacity, (1, 1- diphenyl-2-picryl-hydrazyl) DPPH, Nitric oxide, lipid peroxide scavenging and reducing power. The highest activity was detected during the radicals scavenging, with A_1 and A_2 noticed as the most active.

Keywords: Thiazoles, anti-inflammatory, In vitro antioxidant activity, IC_{50} values, free radical scavenging activity (DPPH), Reducing power.

1. Introduction

Thiazole derivatives have possessed versatile biological activity [1]. Inflammation is defined as the local response to living mammalian tissues to injury due to any agent. Specifically it is a series of molecular and cellular responses acquired during evolution designed to eliminate foreign agent and promote repair of damaged tissues. There are two fundamental types of inflammation, Acute inflammation and Chronic inflammation. Acute inflammation is a rapid response to an injurious agent that serves to deliver mediators of host defense-leukocytes and plasma proteins to the site of injury. Chronic inflammation can be considered to be inflammation of prolonged duration (weeks to months to years) in which active inflammation, tissue injury and healing proceed simultaneously [2, 3].

Free radical is any species capable of independent existence that contains one or more unpaired electrons which reacts with other molecule by taking or giving electrons, and involved in many pathological conditions [4]. Free radicals can be described as chemical species that have an unpaired electron and play very important role in human health and beneficial in combating against several diseases like cardiovascular disorders, lung damage, inflammation [5, 6]. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. The most common reactive oxygen species (ROS) include superoxide (O_2^-) anion, hydrogen peroxide (H_2O_2), peroxyl (ROO^-) radicals, and reactive hydroxyl (OH^-) radicals. The nitrogen derived free radicals are nitric oxide (NO) and peroxy nitrite anion (ONOO) [7]. Under normal state of affairs, the ROS generated are detoxified by the antioxidant nearby in the body and there is symmetry between the ROS generated and the antioxidant present. However due to ROS over production and/or derisory antioxidant argument. The ROS readily attack and induce oxidation damage to various biomolecules including proteins, lipids, lipoproteins and DNA [8]. This oxidation damage is decisive etiological factor concerned in quite a lot of chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, and neurodegenerative diseases [9]. Many thiazoles were found to be associated with various biological activities, such as antimicrobial [10], antifungal [11], anti-inflammatory [12], antitubercular [13], antitumor [14], and antiprotozoal [15] properties. These findings prompted us to investigate a number of newly synthesized thiazole derivatives for nitric oxide radical scavenging, hydrogen peroxide scavenging and inhibition of lipid peroxidation and reducing power assays. Furthermore, the in vitro antioxidant effect of thiazole derivatives was compared with ascorbic acid and butylated hydroxyl toluene commonly used as antioxidant.

2. Materials and Methods

2.1. Chemistry

Melting points of the synthesized compounds were determined using the microcontroller based melting point apparatus and were found uncorrected. The IR spectra of the synthesized compounds were recorded using KBr pellets in a Fourier transform IR spectrometer (shimadzu 8700) and the frequencies were recorded in wave numbers. ^{1}H-NMR (400 MHz) spectra was recorded on a varian spectrometer in $CDCl_3$ solvent. Chemical shifts were reported in **σ** unit (ppm) with references to TMS as an internal standard.

2.1.1. Synthesis of (z)-4-(3-methoxyphenyl)-N-(4-nitrobenzylidene) thiazole-2-amine (A_1)

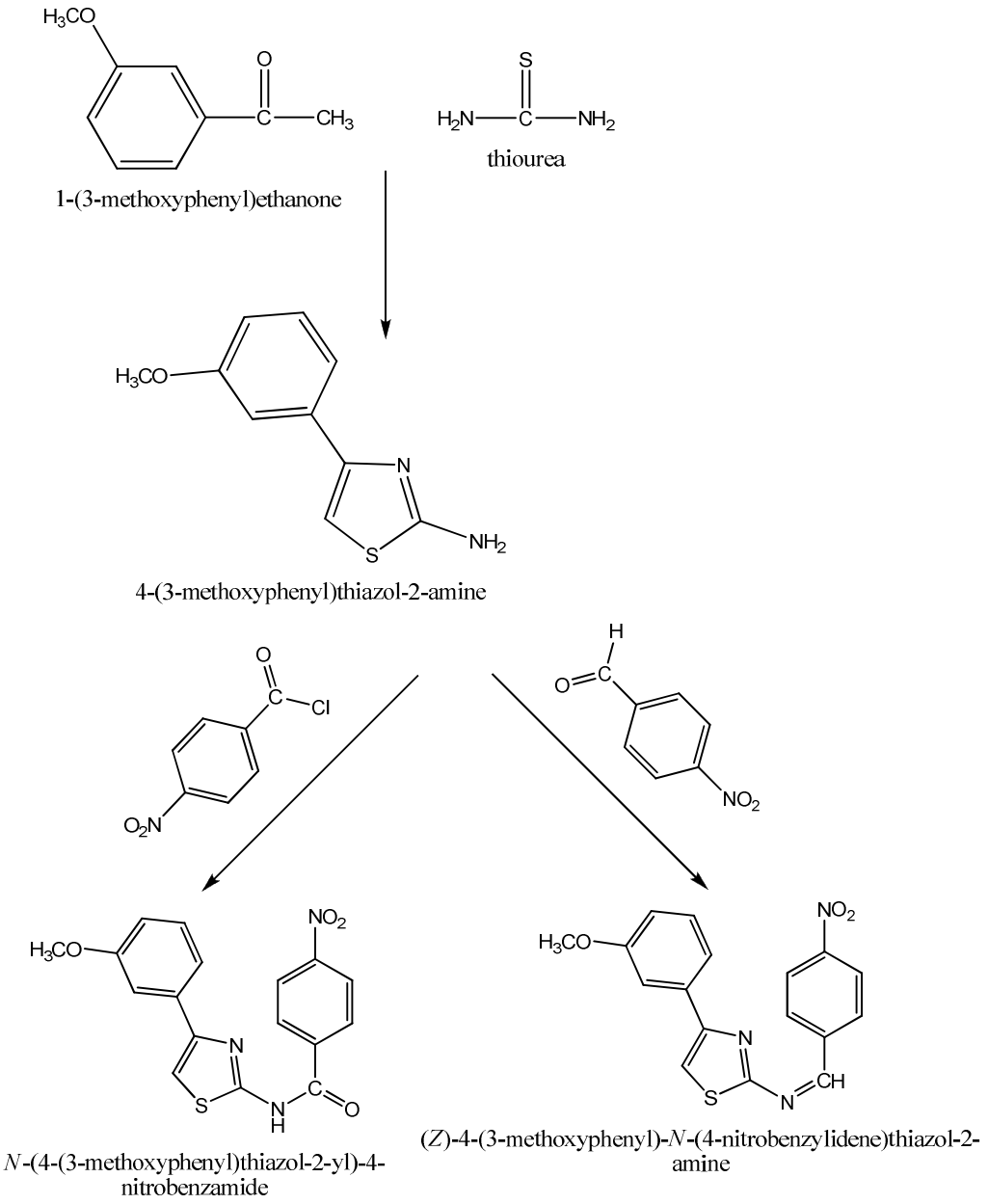
A mixture of compound (A) (Scheme.1) and 4-nitrobenzyaldehyde was dissolved in ethyl acetate: n-hexane, 1:5, v/v), the solvent was evaporated under reduced pressure. The obtained solid mass recrystallized from ethyl acetate and n-hexane solvent mixture.

*IR (KBr, cm-1): 3169 (C-H, aromatic), 1581 (C=N), 690 (C-S) stretching. ^{1}H-NMR ($CDCl_3$) **σ** (ppm): 8.22 (d, 2H, aromatic), 7.62 (s, 1H, = CH), 7.57 (s, 1H, -CH thiazole), 7.4-7.6 (m, 5H, ph), 4.25 (s, 3H, -OCH_3).*

2.1.2. Synthesis of N-[4-(3-methoxyphenyl) thiazole-2-yl]-4-nitrobenzamide (A_2)

A mixture of 4-(3-methoxyphenyl) thiazole-2-amine (A) (2 mmol) with 4-nitrobenzoyl chloride (2.1 mmol) was dissolved in dry pyridine (5ml). The reaction mixture was kept in an ice-bath for 6 hour with continuous stirring. After completion of the reaction, the mixture was poured into ice- water and extracted with CHCl3. The organic layer was washed with dil.NaHCO3 and the solvent was evaporated under reduced pressure. The obtained solid mass was recrystallized from ethyl acetate and n-hexane solvent mixture. The product was checked for purity on TLC.

*IR (KBr, cm-1): 3165 (N-H), 3023 (C-H, aromatic), 3065 (C=C), 1580 (C=N) for thiazole ring, 716 (C-S), 3072 (C-N). ^{1}H-NMR (CDCl3) **σ** (ppm): 8.50 (d, 2H, aromatic), 7.63 (s, 1H,-CH, thiazole), 7.35-7.21 (m, 5H, ph), 3.37 (s, 1H, NH), 3.72 (S, 3H, -OCH_3).*



Scheme.1. Synthetic Pathways for the Preparation of (z)-4-(3-methoxyphenyl)-N-(4-nitrobenzylidene) thiazole-2-amine (A_1) and N-[4-(3-methoxyphenyl)thiazole-2-yl]-4-nitrobenzamide (A_2).

2.2. Carrageenan Induced Rat hind Paw Edema

Anti-Inflammation activity was determined by carrageenan induced Rat hind edema method of Rats (120-140g) was used for the experiment. The drugs were prepared as a suspension by triturating with water and 0.5% sodium CMC. The standard group received 50 mg/Kg bodg weight of Nimesulide, test group received 200 mg/Kg body weight of synthesized compounds and the control group received 1% w/v of CMC. The difference between (zero hour) reading and one of the subsequent readings provides the actual edema volume at that time. The mean paw volume at different times was calculated and compared with the control of percentage inhibition of inflammation after 3 hour was then calculated by using the formula [16].

2.3. In vitro antioxidant activity of thiazole derivatives

2.3.1. Free radical scavenging activity (DPPH Assay) [17].
The anti-oxidant potential of any compound can be determined on the basis of its scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical [18, 19]. Hydrogen peroxide is generated in vivo by several oxidase enzyme. There is increasing evidence that hydrogen peroxide, either directly or indirectly via its reduction product hydroxyl radical (OH-) causes severe damage to biological systems. In this method, when a scavenger is incubated with hydrogen peroxide, the decay or loss of hydrogen peroxide can be measured spectrophotometrically at 230 nm [20]. A solution of hydrogen peroxide (25 Mm) was prepared in phosphate buffered saline (PBS, PH7.4). Various concentrations of 1 ml of the thiazole derivatives (A1 and A2) or standards in methanol were added to 2ml of hydrogen peroxide solution in PBS. The absorbance was measured at 230 nm after 10 min.

2.3.2. Scavenging of Nitric oxide radical
Sodium nitroprusside in aqueous solution at physiological PH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of modified Griess llosvay reaction with little modifications. Scavenging of nitric oxide complete with oxygen leading to reduce production of nitric oxide. The reaction mixture (6ml) containing sodium nitroprusside (10mM,4ml), phosphate buffer saline (PBS, PH 7.4, 1ml) and thiazole derivatives (A, A1, A2) or standard (1ml) in DMSO at various concentrations was incubated at 25 C0 for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min. for completion of diazotization. Then 1ml of NEDD was addad, mixed and allowed to stand for 30 min. in diffused light. A pink coloured chromophore was formed. The absorbance was measured at 540 nm [21].

2.3.3. Determination of Total Antioxidant Capacity by Phosphomolibdenium Assay.
The antioxidant activity of the tested compounds was evaluated by the phosphomolibdenium method according to the procedure of prieto [22]. An aliquot of 50 µl of the methanol solution of the tested compounds (10 – 60 µg/ml) was combined with 1ml of reagent solution (0.4M sulfuric acid, 25 mM sodium phosphate and 2Mm ammonium molybdate). The tubes containing the reaction solutions were incubated at 95 Co for 90 min. Then the absorbance of the solution was measured at 695 nm, using a Perkin- Elmer Lambda 25 UV/Vis spectrophotometer, against blank probe after cooling to room temperature. Methanol (50 µl) in the place of solution of the tested compound was used as the blank. The total antioxidant capacity of the tested samples was calculated according to the equation:

$$TAC\ \% = [(A_o - A_t)/A_o] \times 100$$

Where At is the absorbance value of the tested sample and A_o is the absorbance of the blank sample, in particular time. Ascorbic acid was used as reference standard. The results (TAC) are presented as the µg equivalents of the ascorbic acid per milliliter, obtained from the linear regression analysis. All the experiments were performed in triplicate and the average absorbance was noted for each concentration.

2.3.4. Inhibition of Lipid Peroxidation in a Linoleic Acid Emulsion Assay
The ability of thiazole derivatives to inhibit lipid chain peroxidation process was tested in a linoleic acid system [23]. Dilution (10 – 60 µg/ml) of the test compounds were prepared in methanol, to add to the linoleic acid emulsion. The linoleic acid emulsion was prepared by mixing 0.250g of linoleic acid, 0.250g oe tween 20 as emulsifier and 30 ml of phosphate buffer (0.2M, PH7) and the mixture was homogenized. A 0.5ml of thiazole methanol solution in different concentrations was mixed then in linoleic acid emulsion (2.5ml, 0.02M, PH7) and phosphate buffer (0.3M, PH7). The reaction mixture was incubated at 37 C^o in the dark to accelerate the peroxidation. The degree of oxidation was measured by sequentially adding ethanol (4.3ml, 75 %), ammonium thiocyanate sample solution (50µl, 30%) and Fecl2 (50µl, 0.03M in 3.5 % Hcl). The peroxide values were determined by reading the absorbance at 500 nm. Control was performed with linoleic acid but without the tested compounds. Percent inhibition of lipid peroxide generation was calculated using equation:

$$I\ \% = [(A_o - A_t)/A_o] \times 100$$

2.3.5. Reducing Power Assay
According to this method, the aliquot of various concentrations of the standard and tested sample of thiazole derivative (A_1) (10 – 100 µg/ml) in 1ml of deionized water were mixed with 2.5ml of (PH 6.7) phosphate buffer and 2.5ml of 1% potassium ferricyanide.

The mixture was incubated at 500 C^o in water bath for 20 min. after cooling, aliquots of 2.5ml of 10% trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10min. The upper layer of solution 2.5ml was mixed with 2.5ml distilled water and a freshly prepared 0.5ml of 0.1% ferric chloride solution. The absorbance was measured at 700nm in uv-vis. Spectrometer [24]. A blank was prepared without adding derivative. Ascorbic acid at various concentrations was used as standard. Stock solutions of compound (A_1) were prepared by dissolving 10mg of dried methanolic derivative in 10ml of methanol to give concentration of 1mg/ml, then prepares concentrations of 10, 25, 50, and 100 µg/ml thiazole derivative (A_1).

3. Results and Discussion

3.1. Chemistry

The synthetic route of the thiazole derivatives is outlined in (scheme. 1). 1-(3-methoxyphenyl)ethanone was heated with thiourea in presence of iodine to obtain 4-(3-methoxyphenyl)thiazole-2-amine (A). Refluxing of compound (A) with 4-nitrobenzaldehyde in ethanol afforded (z)-4-(3-methoxyphenyl)-N-(4-nitrobenzylidene)thiazole-2-amine (A1) in good yields. A cylation of compound (A) with 4-nitrobenzoyl chloride in dry pyridine gave N-[4-(3-methoxyphenyl)thiazole-2-yl]-4-nitrobenzamide (A2). The structure of the synthesized compounds was confirmed by IR and 1H-NMR spectral analysis.

3.2. Anti-Inflammation Activity

The compounds were subjected to anti-inflammation activities by paw edema method using Nimesulide drug as standard. All the thiazole derivatives (A, A_1, A_2) have shown promising anti-inflammation activities, as shown in table 1.

Table 1. Anti-inflammatory activity of thiazole derivatives (A, A_1, and A_2).

Compound No.	Mean Paw Edema Volume					
	0 hour	0.5 hour	1 hour	2 hour	3 hour	% inhibition after 3rd hour
Control	1.2±0.03	1.44±0.02	1.58±0.02	1.76±0.02	1.82±0.02	----------
Nimesulide	1.12±0.03	1.14±0.02**	1.22±0.02**	1.36±0.02**	1.24±0.02**	31.86
A	1.14±0.04	1.24±0.02*	1.30±0.04**	1.42±0.04**	1.34±0.02**	25.38
A_1	1.20±0.05	1.23±0.03*	1.32±0.04**	1.40±0.03**	1.28±0.03**	29.67
A_2	1.16±0.02	1.20±0.03*	1.36±0.02**	1.46±0.05**	1.34±0.02**	26.46

***P< 0.05, and **P< 0.01 – significant.**

The differences between (0 hour) reading and one of the subsequent readings (0.5, 1, 2, and 3 hour), respectively, provides the actual edema volume at that time. The mean paw volume at different times was calculated and compared with the control [25]. Derivatives (A, A_1, and A_2) show the percentage inhibition at 3rd hour (25.38, 29.67, 26.46), respectively, and the percentage inhibition of Nimesulide drug as (standard drug) at 3rd hour (31.86%). In comparison with Nimesulide as (standard drug) to anti- inflammatory activity, compounds A, A_1, and A_2 have shown significant anti-inflammatory activity. Compounds A_1 show excellent anti-inflammatory activity than A and A_2 compounds, by compared with standard drug.

3.3. In vitro Antioxidant Activity

All the compounds were tested for their in vitro antioxidant activity, such as hydrogen peroxide free radicals scavenging activities, nitric oxide, lipid peroxidation, and reducing power activity of the compounds. Compounds A_1 and A_2 possessed potent inhibitory activity against hydrogen peroxide scavenging free radical with IC_{50} value of 22±0.08µg/ml and 25±0.32µg/ml, respectively, when compared to the standard ascorbic acid 36±0.81µg/ml. Compound A showed mild inhibitory activity against hydrogen peroxide free radical scavenging activity with IC_{50} value of 42±0.01µg/ml, as shown in table 2. In nitric oxide free radical activity, compound A_2 showed strong inhibitory effect with IC_{50} value of 20±0.02µg/ml. The compound A_2 proved to be more active than the standard ascorbic acid IC_{50} (32±0.09µg/ml), as shown in table 2.

Table 2. IC_{50} values of 4-(3-methoxyphenyl)thiazole-2-amine(A), (z)-4-(3-methoxyphenyl)-N-(4-nitrobenzylidene)thiazole-2-amine(A_1) and N-[4-(3-methoxyphenyl)thiazole-2-yl]-4-nitrobenzamide(A_2).

Compound No.	IC_{50}±SD (µg/ml)	
	Hydrogen Peroxide	Nitric Oxide
A	42±0.01	61±0.10
A_1	22±0.08	28±0.02
A_2	25±0.32	20±0.09
Ascorbic acid (standard)	36±0.81	32±0.09

3.3.1. Determination of Total Antioxidant Capacity

The results of total Antioxidant Capacity (TAC) are presented as the µg equivalents of the ascorbic acid per milliliter, obtained from the linear regression analysis. All the experiments were performed in triplicate and the average absorbance was noted for each concentration. As the TAC value is higher, the better is the antioxidant activity. Also, the results of all tested are expressed as (TAC_{50}, IC_{50}) values presenting the concentration of the test thiazole derivatives that reduces 50% of the initial free reactive species concentration, for various concentrations of thiazole derivatives (10 – 60 µg/ml). A lower TAC_{50} and IC_{50} values indicates greater antioxidant activity. The in vitro activity of three thiazole derivatives (A, A_1, A_2) was comparable with the standard values of ascorbic acid and butylated hydroxytoluene (BHT) (30 minutes: ascorbic acid IC_{50} = 34µg/ml, BHT IC_{50} = 8.62µg/ml, 60 minutes: ascorbic acid 26µg/ml, BHT IC_{50} = 6.12µg/ml), as shown in table 3.

Table 3. Total antioxidant capacity and DPPH radical scavenging activity of synthesized thiazole derivatives *in vitro*.

Compound no.	TAC	TAC_{50}	IC_{50} (µg/ml)	
			30 min.	60 min.
A	121.46±0.42	97.45±0.31	39±0.01	29±0.01
A_1	742.6±0.38	17.25±0.34	19±0.08	15±0.05
A_2	324±0.28	35.69±0.17	23±0.32	18±0.35
Ascorbic acid	_	_	34±0.81	26±0.42
BHT	_	_	8.64±0.02	6.12±0.01

From this table 3. Above, compound A1 showed strong inhibitory activity against hydrogen peroxide free radical scavenging activity, by determined the total antioxidant capacity with TAC value 742.67µg/ml and TAC_{50} value 17.25µg/ml.

3.3.2. Inhibition of Lipid Peroxidation in Linoleic acid Emulsion

During the four days long inhibition of lipid peroxidation by thiazole derivatives, the absorbance of the control sample at 500 nm has been increasing up to the maximal value in 72 hour, and then, on the 96 hour the absorbance decreased due to the decomposition of linoleic acid hydroperoxides generated during the peroxidation [26]. Consequently, the results of thiazole derivatives induced inhibition of lipid peroxidation until the 72 hour, table 4. BHT was excellent standard for this measurement with the I50 < 7.82µg/ml.

Table 4. Lipid peroxide of thiazole derivatives *in vitro*.

Compound No.	I_{50}, (µg/ml)		
	24 hour	48 hour	72 hour
A	18.96±0.04	37.89±0.28	80.45±0.24
A_1	5.72±0.37	10.08±0.34	12.23±0.34
A_2	< 3.54	10.42±0.16	10.76±0.16
BHT	< 7.82	< 7.82	< 7.82

During the observed 24 – 72 hour time interval, compounds A_1 and A_2 have presented significant lipid peroxide scavenging capacity, compared to compound A and BHT as (standard).

Regarding the results, some patterns in the activity among derivatives could be obtained. Thus, the compound A_2 (I_{50}= < 3.54µg/ml, 10.42µg/ml, 10.76µg/ml, for 24, 48, 72 hrs., respectively) and derivative A_1 (I_{50}= 5.72µg/ml, 10.08µg/ml, 12.23µg/ml, for 24, 48, 72 hrs., respectively) retained the level of activity after the second and third day. Compared to BHT, and compound A_2 expressed 50 % higher potential in the first 24 hours. Compound A showed slightly lower radical scavenging intention than previously mentioned derivatives with I_{50}= 18.96µg/ml, 37.89µg/ml, 80.45µg/ml, for 24, 48, 72 hrs. respectively. Compound A_1 showed higher activity of radical on the 48 hour.

3.3.3. Reducing Power Assay

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe^{+3}) to form potassium ferrocyanide (Fe^{+2}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. The reducing power of the thiazole derivatives (A_1 and A_2) and standard increases with increase in amount of this derivatives and ascorbic acid concentrations, as shown in table 5.

Table 5. Shows the absorbance of Ascorbic acid (standard) and thiazole derivatives at various concentrations (µg/ml) in ferric reducing power determination model.

Concentration (µg/ml)	Compound (A_1)	Compound (A_2)	Ascorbic acid
10	0.044±0.002	0.081±0.002	0.120±0.001
25	0.086±0.005	0.102±0.004	0.153±0.003
50	0.142±0.001	0.133±0.001	0.260±0.002
75	0.190±0.002	0.170±0.001	0.382±0.002
100	0.236±0.002	0.248±0.002	0.493±0.001

Values are mean ± SD of three parallel measurements.

This results indicates that increase in absorbance of the reaction mixture indicates increase in reducing power. This study provides that thiazole derivatives (A_1 and A_2) possessed interesting antioxidant properties, which was expressed by their inhibitory effect on the nitric oxide scavenging, hydrogen peroxide and lipid peroxidation. The reducing ability increases with increasing absorbance. It confirms derivatives (A_1 and A_2) are having capacity to reduce ferric to ferrous ion.

Conclusion

All the thiazole derivatives have shown promising anti-inflammatory activities. Compounds A_1 has shown excellent anti-inflammatory activities. The thiazole derivatives (A_1 and A_2) showed antioxidant activity by inhibiting DPPH, Nitric oxide, lipid peroxidation, and reducing power activities. The results of this study show that the thiazole derivatives (A_1 and A_2) can be used as easily accessible source of antioxidant and in pharmaceutical industry.

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