In-Silico Study of the Antioxidant Ability for 4-thiazolidinone Analogs Using DPPH assay

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Abstract

Oxidative stress and related diseases resulting from the overproduction of free radicals can be counteracted by designing and developing novel antioxidative agents that can protect the human body against the damage caused by free radicals. The present study, A series of new 4-thiazolidinone derivatives were synthesized by reacting (S, E)-4-(4-(2-(4-substituted benzylidene) hydrazinyl) benzyl) oxazolidin-2-one with thioglycollic acid. The intermediate (S, E)-4-(4-(2-(4-substitutedbenzylidene) hydrazinyl) benzyl) oxazolidin-2-one were synthesized by condensation of (S)-4-(4-hydrazinyl benzyl) oxazolidin-2-one with different aryl benzaldehydes in the presence of ethanol. All the newly synthesized compounds were assigned on the basis of IR, ^{1}H NMR and ^{13}C NMR spectral data. All the newly synthesized compounds were evaluated for in-vitro antioxidant activities by using DPPH radical scavenging assay models. Most of the synthesised compounds showed good antioxidant activity.

Keywords: Thiazolidinone, spectral, antioxidant activity, invitro, DPPH

1. Introduction

It has been demonstrated that oxidative stress (OS)-related disorders can be treated safely and effectively using antioxidants[1]. It has been demonstrated that consuming antioxidants lowers the risk of developing cancer as well as other diseases like neurological and cardiovascular disorders[2]. The delicate control of free radicals is crucial for maintaining the homeostasis of the organism. A number of diseases, as well as ageing, are linked to disturbances in an organism's red-ox equilibrium (the natural result of free radical overproduction and/or their insufficient sequestration by antioxidant defence mechanisms)[3-4]. Antioxidant defence mechanisms control the concentration of free radicals, such as reactive oxygen, nitrogen, sulphur, and carbon species (ROS, RNS, RSS, and RCS), under physiological conditions[5]. Oxidative, nitrosative, thilyl, or carbonyl stress is caused when the body's antioxidant defence mechanisms are unable to control the number of free radicals there. Free radicals willfully oxidise anything in their path, harming both endogenous and exogenous materials, including all classes of biomolecules (proteins, lipids, and DNA), destroying cellular energy stores, depleting reducing equivalents (reduced nicotinamide adenine dinucleotide and glutathione), and eventually killing cells by inducing apoptosis[6-7] .

A wide range of biological activities, including the neutralization/sequestration of ROS and RNS, are imposed by compounds having thiazolidinone structures. Thiazole compounds have lately been recognised for their antioxidant activity (AOA)[8]. As potential antioxidant agents, Kavitha et al. created some novel 4-(4-chlorophenyl)-2-aryl substituted metheniminothiazoles. Kachroo et al[9]. discovered that newly synthesised N-[(4E)-arylidene-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl] was both antibacterial and AOA.acetamide-2-(2-methyl-1,3-thiazol-4-yl). Gull et al[10]. produced 2- amino-6-arylbenzothiazoles and studied them, among other things, for their capacity to scavenge RNS. By substituting thiosemicarbazide and thiazolidinone at positions 7 and 4, Sarkanj et al[11]. created 4-methyl-7-hydroxycoumarin compounds and assessed their potential for AOA. Thiazolidinone is a core that has a variety of biological actions, including antioxidants. Thiazolidinone derivatives are heterocyclic compounds containing nitrogen, oxygen and sulphur atoms in their structure and are proved to be clinically useful agents against different kinds of disease[12 - 15]. During the past decade, combinatorial chemistry has provided access to chemical libraries based on privileged structures, with heterocyclic structures receiving special attention as they belong to a class of compounds with proven utility in medicinal chemistry[16-17]. There are numerous biologically active molecules with five member rings, containing three hetero atoms. Thiazolidinone derivatives have become a very important group of heterocyclic compounds in drug discovery[18-19].

The goal of the current work is to synthesis new heterocyclic compounds with the thiazole moiety in light of the significant significance of thiazolidinones, which are the primary structural components in a variety of pharmaceuticals with a

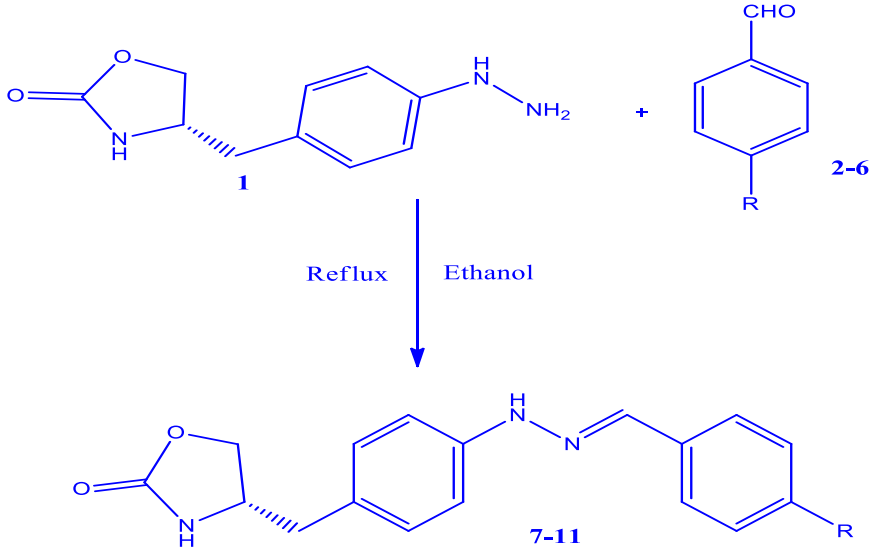
wide range of biological activity. The substitutions in the selected derivatives were either meant to contribute labile hydrogens from the 1,3-thiazolidinone system or stabilize the radicals produced from compounds via resonance. In order to create stable DPPH molecules, these hydrogens could be given to the DPPH radical.

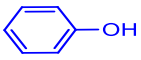
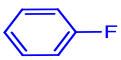
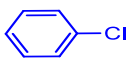
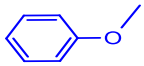
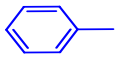
2. Eperimental

(S)-4-(4-hydrazinylbenzyl) oxazolidin-2-one, hydrazides, mercapto acetic acid , Zinc chloride and ethanol were purchased from sigma Hi-media. The melting points were identified using open capillary vessels in an electrically operated instrument and are uncorrected. Precoated plates (60 F254 silica gel) were used to track reaction progress in analytical thin-layer chromatography (TLC) and iodine chamber spots were visualized. Infrared (IR) spectrums (KBr discs) have been recorded with a Shimadzu spectrometer (FTIR-4100). The Bruker Advance II of 400 MHz spectrometers in $CDCL_3$ recorded nuclear magnetic resonance proton (^{1}H NMR) spectrum and nuclear magnetic resonance carbon (^{13}C NMR).

2.1 Synthesis and biological evaluation of (S, E)-4-(4-(2-(4-substitutedbenzylidene) hydrazinyl) benzyl) oxazolidin-2-one

About 10 mmol of (S)-4-(4-hydrazinylbenzyl) oxazolidin-2-one (1) and 10 mmol of the substituted aryl benzaldehydes (2-6) taken in a 100 ml round bottom flask, dissolved in 20 ml of ethanol and added a drop of a acidic acid. The reaction mixture was refluxed about five hrs. The TLC test was carried out to the reaction mixture to known the reaction status. After completion of the reaction the reaction mixture was poured in ice cold water. The slowly formed residue was filtered, washed with water and dried under the vacuum to get the final compounds (S, E)-4-(4-(2-(4-substitutedbenzylidene) hydrazinyl) benzyl) oxazolidin-2-one (7 - 11).

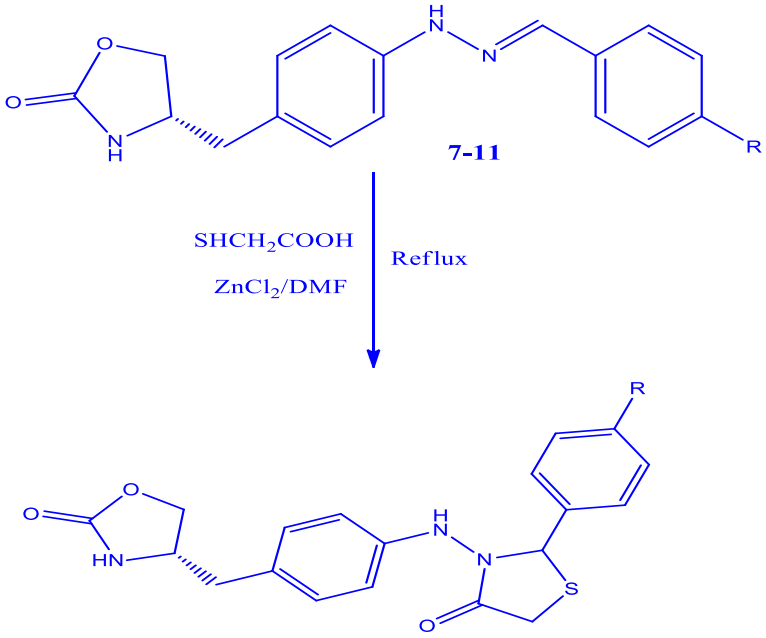


Compound	7	8	9	10	11
R					

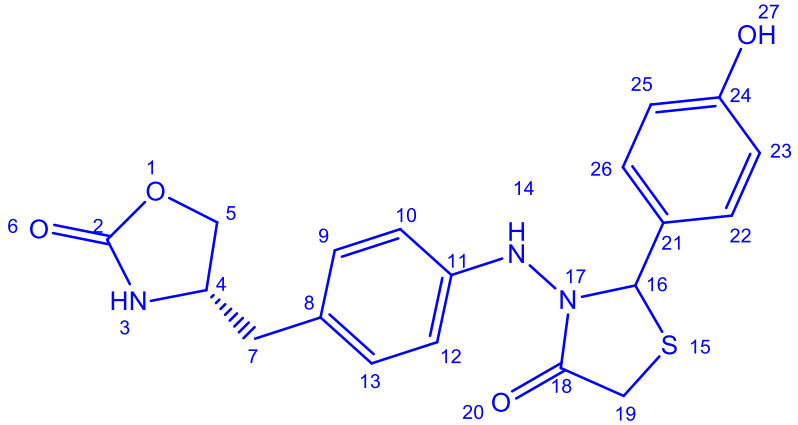
2.2 Synthesis and biological evaluation of (4S)-4-(4-((2-(4-substitutedphenyl)-4-oxothiazolidin-3-yl) amino) benzyl) oxazolidin-2-one (12-16)

Equimolar concentrations of (S, E)-4-(4-(2-(4-substitutedbenzylidene) hydrazinyl) benzyl) oxazolidin-2-one (7 - 11) (0.01 mol) and mercaptoacetic acid (0.01mol) in 30ml of DMF with anhydride $ZnCl_2$ was added at room temperature under stirring. The reaction mixture was refluxed under water bath for 5-6hrs. The resultant solution was cooled and poured into cold water. The precipitated

product was then purified by re-crystallization from hot ethanol to afford **(4S)-4-(4-((2-(4-substitutedphenyl)-4-oxothiazolidin-3-yl) amino) benzyl) oxazolidin-2-one** (12-16) derivative in high yield. The assigned structure and molecular formula of the newly synthesized compound was confirmed and characterized by melting point, ^{1}H NMR, ^{13}C NMR and IR data which were in full agreement with proposed structures.



Compound	12	13	14	15	16
R	OH	F	Cl	O	



2.2.1 Synthesis of (4S)-4-(4-((2-(4-hydroxyphenyl)-4-oxo thiazolidin-3-yl) amino) benzyl) oxazolidin-2-one (12)

Yield %: 78. Yellowish White crystals. m.p: 178-180 ^{0}C. FT-IR (KBr), ν (cm^{-1}): 1695 (C=O), 3295 (N-H), 2966 (Ali C-H), 3058 (Aro C-H), 3345 (O-H), 1126 (N-N). ^{1}H-NMR (25 °C, CDCl3): 8.16 (s, NH, 1H), 2.77 (dd, CH_a, 1H), 2.86 (dd, CH_b, 1H), 5.0 (s, OH,

1H), 4.07 (dd, CH_A (5), 1H), 4.29 (dd, CH_B (5), 1H), 4.54 (m, CH_X (4), 1H), 5.90 (s, CH (16), 1H), 4.52 (s, CH_2 (19), 2H), 6.77 – 7.28 (m, 8H, Ar-H). ^{13}C-NMR (25 °C, CDCl3): 166.8 (C=O), 40.2 (CH_2 (Thiazolidinone)), 54.4 (CH_2),65.9 (CH(Thiazolidinone), 86.5 (CH_2,(oxazolone)), 71.1 (CH (oxazolone)), 115.1 – 160.9 (ArC).

2.2.2 Synthesis of (4S)-4-(4-((2-(4-fluorophenyl)-4-oxo thiazolidin-3-yl)amino) benzyl) oxazolidin-2-one (13)

Yield %: 65. Yellowish White crystals. m.p: 162-164 ^{0}C. FT-IR (KBr), ν (cm^{-1}): 1695 (C=O), 3298 (N-H), 2934 (Ali C-H), 3060 (Aro C-H), 3346(O-H), 1176 (N-N). ^{1}H-NMR (25 °C, CDCl3): 7.39 (s, NH, 1H), 2.78 (dd, CH_a, 1H), 2.86 (dd, CH_b, 1H), 5.35 (s, OH, 1H), 3.05 (dd, CH_A (5), 1H), 3.08 (dd, CH_B (5), 1H), 3.97 (m, CH_X (4), 1H), 5.94 (s, CH (16), 1H), 4.36 (s, CH_2 (19), 2H), 6.87 – 7.38 (m, 8H, Ar-H). ^{13}C-NMR (25 °C, CDCl3): 176.0 (C=O), 34.8 (CH_2 (Thiazolidinone)), 36.9 (CH_2), 39.7 (CH(Thiazolidinone), 62.0 (CH_2,(oxazolone)), 59.3 (CH (oxazolone)), 115.1 – 163.8 (ArC).

2.2.3 Synthesis of (4S)-4-(4-((2-(4-chlorophenyl)-4-oxo thiazolidin-3-yl) amino) benzyl) oxazolidin-2-one (14)

Yield %: 70. Yellowish White crystals. m.p: 168-170 ^{0}C. FT-IR (KBr), ν (cm^{-1}): 1656 (C=O), 3174 (N-H), 2933 (Ali C-H), 3061 (Aro C-H), 3301 (O-H), 1140 (N-N). ^{1}H-NMR (25 °C, CDCl3): 7.35 (s, NH, 1H), 2.68 (dd, CH_a, 1H), 2.80 (dd, CH_b, 1H), 5.94 (s, OH, 1H), 2.99 (dd, CH_A (5), 1H), 3.82 (dd, CH_B (5), 1H), 3.86 (m, CH_X (4), 1H), 6.01 (s, CH (16), 1H), 4.36 (s, CH_2 (19), 2H), 6.85 – 7.35 (m, 8H, Ar-H). ^{13}C-NMR (25 °C, CDCl3): 169.1 (C=O), 32.6 (CH_2 (Thiazolidinone)), 34.8 (CH_2), 40.7 (CH(Thiazolidinone), 61.8 (CH_2,(oxazolone)), 52.1 (CH (oxazolone)), 115.2 – 146.2 (ArC).

2.2.4 Synthesis of (4S)-4-(4-((2-(4-methoxy phenyl)-4-oxo thiazolidin-3-yl)amino) benzyl) oxazolidin-2-one (15)

Yield %: 70. Yellowish White crystals. m.p: 156-158 ^{0}C. FT-IR (KBr), ν (cm^{-1}): 1620 (C=O), 3377 (N-H), 2927 (Ali C-H), 3026 (Aro C-H), 3464 (O-H), 1144 (N-N). ^{1}H-NMR (25 °C, CDCl3): 7.27 (s, NH, 1H), 2.75 (dd, CH_a, 1H), 2.84 (dd, CH_b, 1H), 5.94 (s, OH, 1H), 2.99 (dd, CH_A (5), 1H), 3.82 (dd, CH_B (5), 1H), 3.90 (m, CH_X (4), 1H), 6.03 (s, CH (16), 1H), 4.36 (s, CH_2 (19), 2H), 6.85 – 7.27 (m, 8H, Ar-H). ^{13}C-NMR (25 °C, CDCl3): 173.1 (C=O), 34.8 (CH_2 (Thiazolidinone)), 35.6 (CH_2), 40.9 (CH(Thiazolidinone), 62.1 (CH_2,(oxazolone)), 51.8 (CH (oxazolone)), 114.2 – 146.2 (ArC).

2.2.5 Synthesis of (4S)-4-(4-((4-oxo-2-(p-tolyl)thiazolidin-3-yl)amino) benzyl) oxazolidin-2-one (16)

Yield %: 65. Yellowish White crystals. m.p: 160-162 ^{0}C. FT-IR (KBr), ν (cm^{-1}): 1635 (C=O), 3370 (N-H), 2921 (Ali C-H), 3026 (Aro C-H), 3465 (O-H), 1133 (N-N). ^{1}H-NMR (25 °C, CDCl3): 7.29 (s, NH, 1H), 2.76 (dd, CH_a, 1H), 2.85 (dd, CH_b, 1H), 5.0 (s, OH, 1H), 3.90 (dd, CH_A (5), 1H), 4.07 (dd, CH_B (5), 1H), 4.29 (m, CH_X (4), 1H), 5.94 (s, CH (16), 1H), 4.36 (s, CH_2 (19), 2H), 6.87 – 7.29 (m, 8H, Ar-H). ^{13}C-NMR (25 °C, CDCl3): 169.1 (C=O), 34.8 (CH_2 (Thiazolidinone)), 40.2 (CH_2), 54.4 (CH(Thiazolidinone), 70.6 (CH_2,(oxazolone)), 62.1 (CH (oxazolone)), 115.1 – 146.2 (ArC).

2.3 Results and discussion

2.3.1 Antioxidant activity

The antioxidant activity of newly synthesized compounds was also screened by using DPPH method[20]. This technique is conventional, sensitive and very much acknowledged for screening of newer antioxidant agents. The action of compounds has been screened at the concentration of 100, 250 and 500 µg/mL oral dose and the same dose of standard drug ascorbic acid is used. The outcome is presented in Table 2. All tested compounds offered adequate protection in a dose-dependent manner. The activity was increased with increasing concentration

.

Table 1. IC50 Values of compounds 12-16 calculated based on DPPH method

Compounds	DPPH method IC50 Value (µg/ml)
Control	-
12	366.5
13	188.2
14	299.6

15	291.5
16	170.8
Ascorbic acid	84.7

At 250 µg/mL the compounds such as **13 and 16** showed more than half maximal (50%) inhibition, whereas compounds **12, 14** and **15** exhibits below 50%**.** When we increased concentration from 250 to 500 µg/mL, the inhibition follows the same trend. It is evidence from Table 2, All the test compounds exhibits excellent protection in 500 µg/mL. The standard ascorbic acid showed inhibition 42.20, 68.40 and 80.00% protection at 100,250 and 500µg/ml respectively. All the compounds showed excellent inhibition at a concentration of 500µg/ml.

Table 2. Anti-oxidant activity evaluation of compounds (12-16) and standard

Compound	**concentration (µg/ml)**	**Mean absorbance**	**% inhibition**	**% inhibition**	**ic50**
12	100	0.812	0.188	18.80	366.5
	250	0.554	0.446	44.60	
	500	0.388	0.612	61.20	
13	100	0.653	0.347	34.70	188.2
	250	0.334	0.666	66.60	
	500	0.209	0.791	79.10	
14	100	0.666	0.334	33.40	299.6
	250	0.559	0.441	44.10	
	500	0.311	0.689	68.90	
15	100	0.743	0.257	25.70	291.5
	250	0.531	0.469	46.90	
	500	0.252	0.748	74.80	
16	100	0.609	0.391	39.10	170.8
	250	0.356	0.644	64.40	
	500	0.202	0.798	79.80	
Ascorbic acid	100	0.578	0.422	42.20	84.7
	250	0.316	0.684	68.40	
	500	0.2	0.8	80.00	

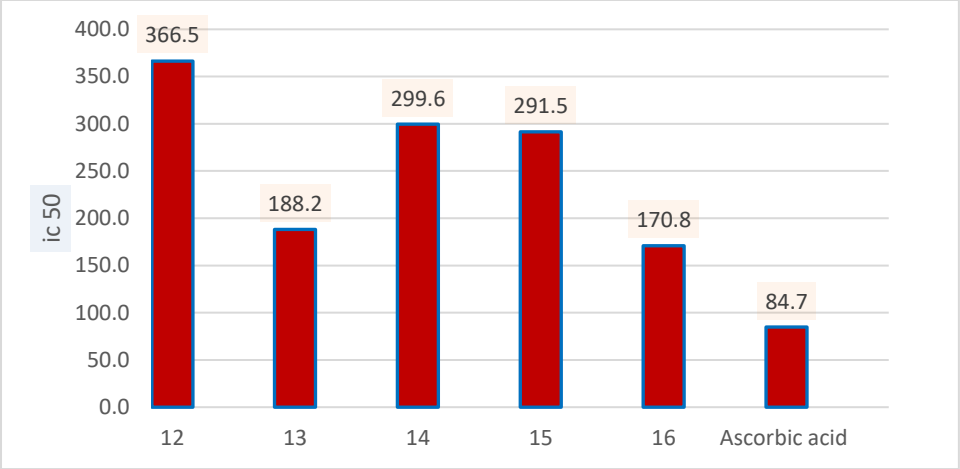


Figure 5.2. IC50 Values of compounds 12-16 calculated based on DPPH method

The compound **16,** methyl substituted showed good activity. It showed 79.8 % inhibition. The 4-fluoro substituted compound **13** showed 79.1 %. The increasing order of inhibition of compounds 12 – 16 are as follows **16< 13< 15< 14 <12.** The ic50 value of synthesized thiazolidinones (12-16) is presented in Table 2, Fig.1. According to these results all the compounds showed dose dependent inhibition of hemolysis. Compound 16 (IC50 = 170.8µg/ml), 13 (IC50 = 188.2 µg/ml) displayed very good activity among the series as compared to standard ascorbic acid (IC50 = 84.7 µg/ml). Other compound 15 (IC50 = 291.5µg/ml), 14 (IC50 = 299.6 µg/ml) also showed good antioxidant activity as compared to standard. The presence of electron withdrawing group like nitro, fluoro, chloro and electron releasing group like methyl resulted in increased antioxidant activity. K. Ishwar Bhat[21] synthesized coumarin incorporated thiazolidinone and evaluate its antioxidant activity, the results showed most of the compound with electron releasing and electron withdrawing groups has more activity

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Conclusion

A series of new 4-thiazolidinone derivatives were synthesized by reacting (S, E)-4-(4-(2-(4-substituted benzylidene) hydrazinyl) benzyl) oxazolidin-2-one with thioglycollic acid. All the newly synthesized compounds were assigned on the basis of IR, ^{1}H NMR and ^{13}C NMR spectral data. All the newly synthesized compounds were evaluated for in-vitro antioxidant activities by using DPPH radical scavenging assay models. Compound 16 with electron releasing group methyl has more activity than other compounds.

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