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RESEARCH ARTICLE

SYNTHESIS, CHARACTERIZATION OF 2,4 -THIAZOLIDINEDIONE DERIVATIVES AND EVALUATION OF THEIR ANTIOXIDANT ACTIVITY

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ABSTARCT:

The research work was carried out to evaluate the antioxidant activity of derivatives of 2, 4-thiazolidinedione (ISS-1, ISS-2, ISS-3, ISS-4, and ISS-5). Thiazolidinedione are synthetic agonists for the transcription factor peroxisome proliferators activated receptor gamma and are therapeutically used as insulin sensitizers. 2, 4-thiazolidinedione derivatives show variety of biological activities such as antibacterial, antifungal, anti-inflammatory, antitumor, anticonvulsant, and cardiotonic besides showing promising anti- diabetic activity. 2, 4-thiozolidinedione was synthesized by condensing thiourea with chloroacetic acid in the presence of concentrated hydrochloric acid and water. A series of derivatives of 2, 4-thiazolidinedione was prepared by mannish reaction by reacting various secondary amines with formaldehyde. In these mannish reaction derivatives of 2, 4-thiazolidinedione has been used as hydrogen active compounds. The synthesized compounds were characterized by IR, 1H NMR and chromatographic method (TLC). The mannish base derivatives of 2, 4-thiazolidinedione were tested for antioxidant activity using free radical scavenging activity by DPPH (1, 1-diphenyl-2-picryl-hydrazil) assay method and ascorbic acid was used as reference standard. All the tested mannish base derivatives show antioxidant activity but compound ISS-3 and ISS-5 show promising antioxidant activity concentration at 50µg /ml and can be further studied with modifications.

Keywords: 2, 4-thiazolidinedione, ascorbic acid, antioxidant activity.

INTRODUCTION:

The medication class of thiazolidinediones (TZDs; also called as "glitziness") was introduced in late 1990's as an adjunct therapy for type II diabetes mellitus and related diseases. Thiazolidinediones bind to the gamma form of the peroxisome proliferators activated receptor (PPAR$^{\gamma}$). This stimulates peripheral adiposities to increase their uptake of free fatty acids, which leads to reduction in the fat stored in muscles, liver and visceral fat deposits. The TZDs also leads to an increase in the secretion of adiponectin and a decrease in the production of resistin and tumor necrosis factor α (TNF-α). It is unknown if TZDs have direct effect on muscles or liver.[1, 9]

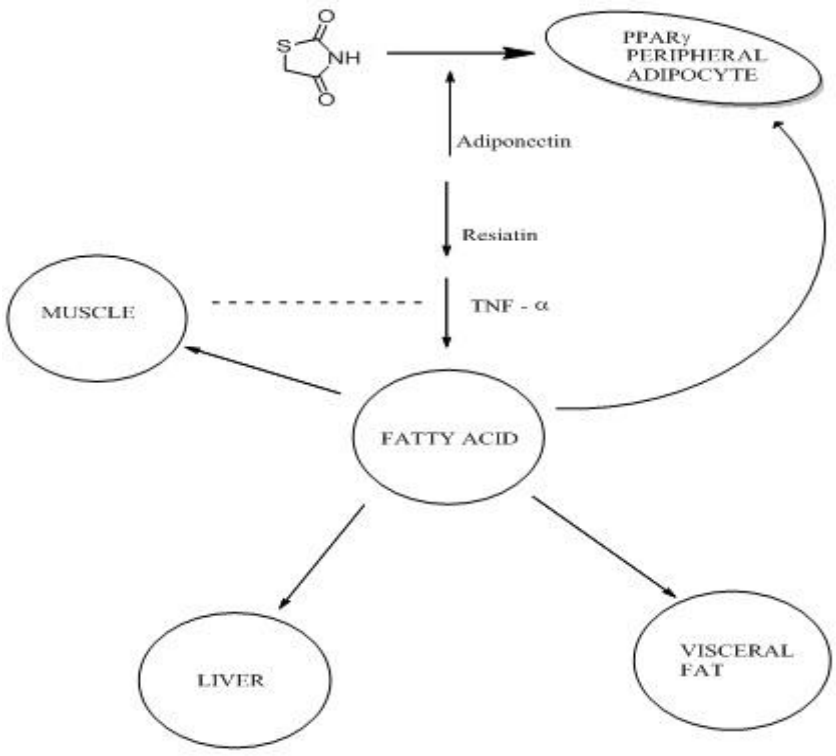
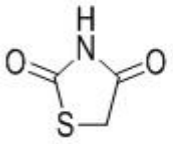


Figure.1: Mechanism of action of 2, 4-thiazolidinedione

CHEMISTRY:



2,4 THIAZOLIDINEDIONE

Thiazolidinediones are five member heterocyclic compounds having sulphur, nitrogen and oxygen atom in their ring structure and exhibiting potent as well as wide range of pharmacological activities. [10]

MATERIALS AND METHODS:

All the chemicals used for synthetic work were purchased from CDH and Hamada. Melting points were determined in an open capillary tubes and are uncorrected by using Veego microprocessor based programmable melting point apparatus. The completion of the reaction was routinely determined by thin layer chromatography on glass plates using silica gel G as absorbent and using chloroform: methanol (9:1) solvent system. Spots were visualized by iodine chamber. IR spectra were recorded in cm^{-1} using KBr pellets on PERKIN ELMER spectrophotometer. 1H NMR spectra (δ, ppm) was recorded on BRUKER AVANCE II 400 NMR spectrophotometer using DMSO-d_6 or $CDCl_3$ solvent (TMS as internal standard).

SCHEME OF SYNTHESIS:

Step1- synthesis of 2, 4-thiazolidinedione [11, 15]

THIOUREA CHLOROACETIC ACID 2,4 THIAZOLIDINEDIONE

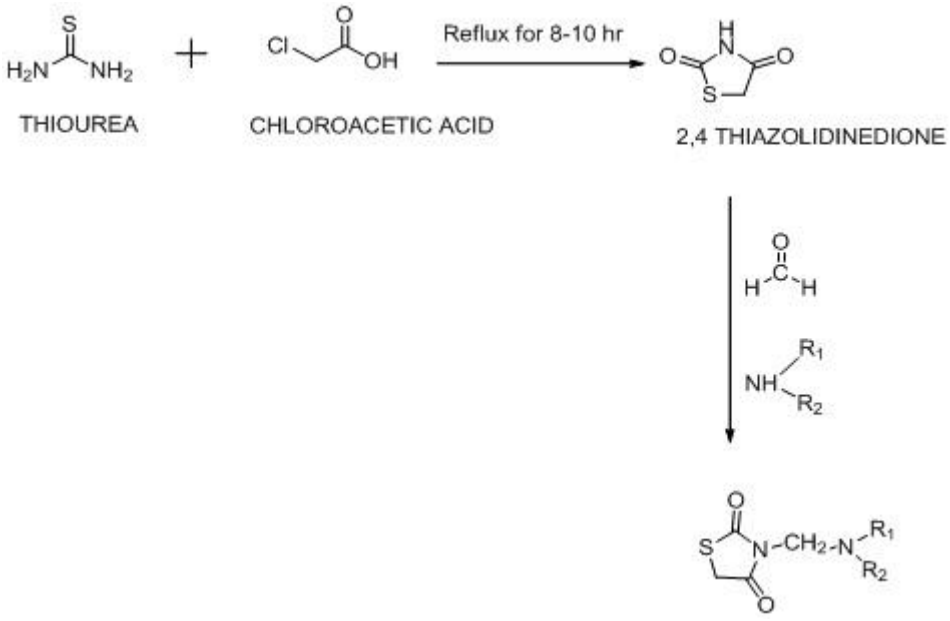
PROCEDURE:

In a 250ml three-necked flask, a solution containing 56.4g (0.6M) of chloroacetic acid in 60ml of water and 45.6g (0.6M) of thiourea was dissolved in 60ml of water. The mixture was stirred for 15minute till occurrence of white precipitates. To the contents of flask was now added slowly 60ml of conc. hydrochloric acid from dropping funnel to dissolve the precipitates, after which the reaction mixture was stirred and refluxed for 10-12hrs at 100-110˚C, on cooling the contents of flask were solidified to a mass of clusters of white needles. The product was filtered and washed with water to remove traces of hydrochloric acid and dried. It was recrystallised from ethanol, yield 80%, m.p. (123-125˚C).

TLC: chloroform: methanol (9:1)

RF: 0.62

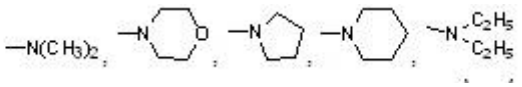
Step 2: Synthesis of various mannish bases derivatives of 2,4-thiazolidinedione



THIOUREA CHLOROACETIC ACID 2,4 THIAZOLIDINEDIONE

Where:

R_1=R_2:



PROCEDURE:

To a solution of 2, 4-thiazolidinedione (0.1M) in DMF, formaldehyde (0.2M) was added under stirring. The reaction mixture was stirred at room temperature for 0.5hrs to complete the reaction of formaldehyde. To the solution of secondary amine in DMF was added drop wise and reflux for several hrs to complete the reaction. The completion of reaction monitored by TLC using solvent system chloroform: methanol (9:1). After the completion of reaction was poured in an ice cold water and filtered off and wash with hot water. Finally it was recrystallised from chloroform, ethanol to give final compound.[16]

ANTIOXIDANT ACTIVITY:

Free radical scavenging activity by DPPH assays method:

DPPH (1, 1-diphenyl-2-picryl-hydrazil) is stable free radical. Methanol solution of DPPH is used to evaluate the antioxidant activity of several synthetic compounds. Antioxidant on interaction with DPPH, both transfer electron or hydrogen atom to DPPH, thus neutralizing its free radical character and convert it to1, 1-diphenyl-2-picryl hydrazine. The degree of discoloration indicates the scavenging activity of the drug .The change in absorbance produced at 517 nm has been used as measure of its antioxidant activity.

Chemicals used:

1,1-diphenyl-2-picryl-hydrazil (DPPH)-Sigma Ltd., Ascorbic Acid-Qualigens, Methanol-Qualigens.

Preparation of DPPH solution: It was prepared by dissolving 33 mg of DPPH in 1 lit. Of methanol just before use and kept in dark amber colored bottle to protect from sunlight.

Sample preparation:

Preparation of stock solution of ligands:

It was prepared by dissolving 50 mg of ligand in 100 ml of methanol.

Standard preparation:

Preparation of Ascorbic Acid solution:

It was prepared by dissolving 50 mg of ascorbic acid in 100 ml of methanol.

PROCEDURE:

A 10, 20,30,40,50 µg/ml concentrations of ligands and ascorbic acid were prepared. From this stock solution 1ml has been pipette out and 5ml methanol solution of DPPH was added ,shaken well and the mixture was incubated at 37^0C for 30 minute absorbance of all samples were measured against blank at 517 nm.The absorbance of DPPH reagent alone was taken as control. The % radical scavenging activity can be calculated following formula:

$$\%\text{free radical Scavenging activity} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

And calculated IC_{50} value.

TABLE 1: DPPH Radical scavenging assay of Compd ISS-1 and Ascorbic acid

Compd ISS-1			Ascorbic acid		
Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition	Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition
10	1.1233±0.0004	30.9	10	0.8240±0.0015	49.3
20	1.0063±0.0008	38.1	20	0.7620±0.0022	53.2
30	0.9116±0.0014	43.9	30	0.6830±0.0002	57.9
40	0.7110±0.0014	48.1	40	0.5540±0.005	65.8
50	0.5310±0.0002	56.4	50	0.4810±0.0002	70.2

TABLE 2: DPPH Radical scavenging assay of Compd ISS-2 and Ascorbic acid

Compd ISS-2			Ascorbic acid		
Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition	Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition
10	1.0200±0.0006	37.20	10	0.8240±0.0015	49.3
20	0.9510±0.0002	41.40	20	0.7620±0.0022	53.2
30	0.8900±0.0002	45.0	30	0.6830±0.0002	57.9
40	0.7500±0.0002	53.80	40	0.5540±0.005	65.8
50	0.6810±0.0002	58.10	50	0.4810±0.0002	70.2

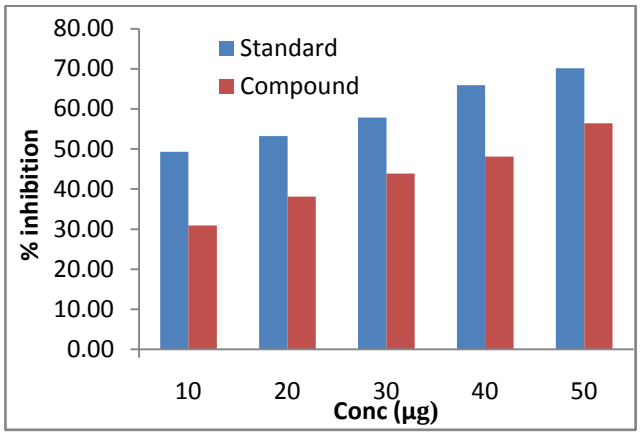


Figure 1: DPPH Radical scavenging assay of Compd ISS-1 and Ascorbic acid

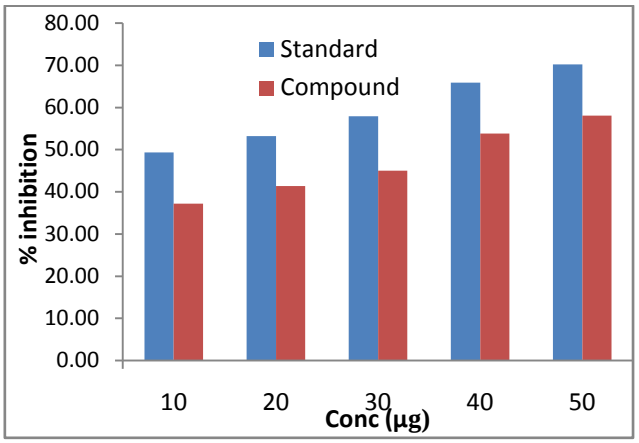


Fig 2: DPPH Radical scavenging assay of Compd ISS-2 and Ascorbic acid

TABLE 3: DPPH Radical scavenging assay of Compd ISS-3 and Ascorbic acid

Compd ISS-3			Ascorbic acid		
Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition	Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition
10	0.9813±0.0014	39.6	10	0.8240±0.0015	49.3
20	0.9030±0.0004	44.4	20	0.7620±0.0022	53.2
30	0.8316±0.0014	48.9	30	0.6830±0.0002	57.9
40	0.6810±0.0002	58.1	40	0.5540±0.005	65.8
50	0.5410±0.0014	66.8	50	0.4810±0.0002	70.2

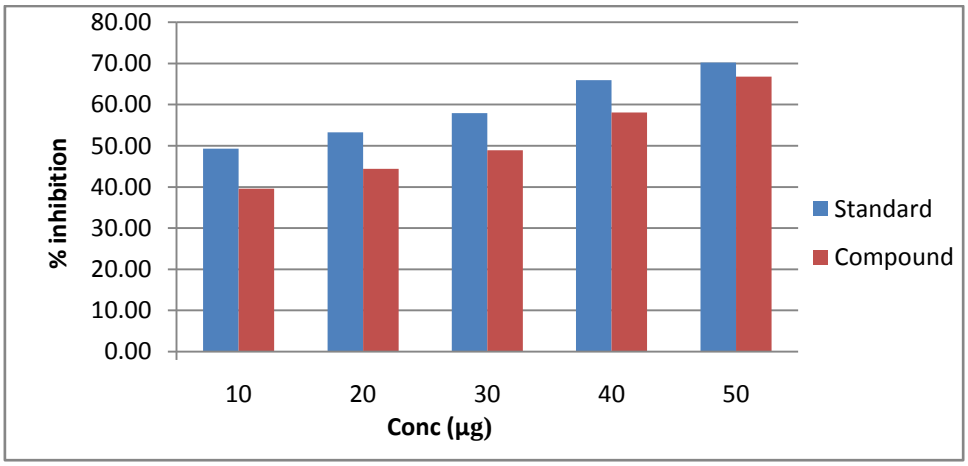


Figure 3: DPPH Radical scavenging assay of Compd ISS-3 and Ascorbic acid

TABLE 4: DPPH Radical scavenging assay of Compd ISS-4 and Ascorbic acid

Compd ISS-4			Ascorbic acid		
Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition	Conc. µg/ml	Mean Abs ± S.E.M	% Inhibition
10	1.0116±0.0014	37.80	10	0.8240±0.0015	49.3
20	0.9230±0.0005	43.20	20	0.7620±0.0022	53.2
30	0.8336±0.0014	48.80	30	0.6830±0.0002	57.9
40	0.7813±0.0014	51.80	40	0.5540±0.005	65.8
50	0.6516±0.0014	59.90	50	0.4810±0.0002	70.2

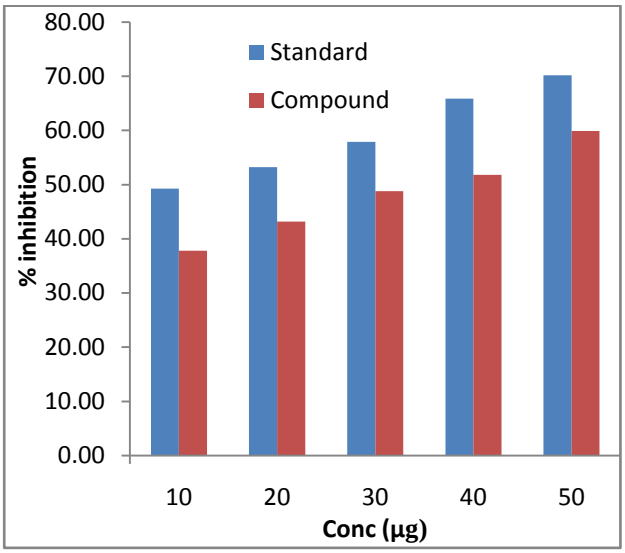


Fig 4: DPPH Radical scavenging assay of Compd ISS-4 and Ascorbic acid

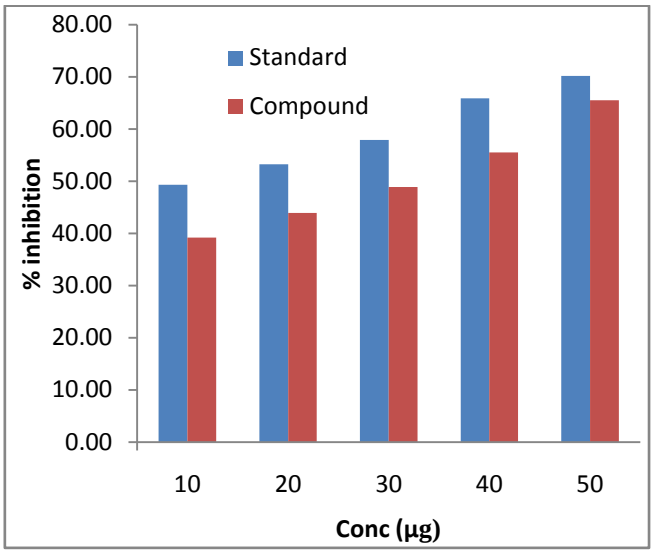


Figure 5: DPPH Radical scavenging assay of Compd ISS-5 and Ascorbic acid

TABLE 5: DPPH Radical scavenging assay of Compd ISS-5 and Ascorbic acid

Compd ISS-5			Ascorbic acid		
Conc. µg/ml	**Mean Abs ± S.E.M**	**% Inhibition**	**Conc. µg/ml**	**Mean Abs ± S.E.M**	**% Inhibition**
10	**0.9870±0.0002**	**39.20**	**10**	**0.8240±0.0015**	**49.3**
20	**0.9116±0.0014**	**43.9**	**20**	**0.7620±0.0022**	**53.2**
30	**0.8336±0.0014**	**48.9**	**30**	**0.6830±0.0002**	**57.9**
40	**0.7230±0.0005**	**55.5**	**40**	**0.5540±0.005**	**65.8**
50	**0.5616±0.0014**	**65.50**	**50**	**0.4810±0.0002**	**70.2**

TABLE 6: Physicochemical data of synthesized compound

S.No.	R_1=R_2	Molecular formula	%age yield	M.P.* (°C)	R_f value
1.		$C_6H_{10}N_2O_2S$	49	120-122	0.8
2.		$C_8H_{12}N_2O_3S$	57	199-201	0.7
3.		$C_9H_{12}N_2O_2S$	60	230-233	0.69
4.		$C_9H_{14}N_2O_2S$	70	155-158	0.79
5.		$C_8H_{14}N_2O_2S$	60	199-201	0.72

REPRESENTATIVE SPECTRAL ANALYSIS:

3-{(dimethylamino) methyl} thiazolidine-2, 4-dione (1SS-1)

Yield: 49%, m.p.: 120-122˚C,^{1}H NMR(400 MHZ, DMSO-d_6, δ, ppm), 4.11(s, 2H, $COCH_2$), 4.55(s, 2H, $NHCH_2$), 2.26(s, 6H, $NH(CH_3)_2$), IR V_{max} (cm^{-1}) (KBr): 1350-1000 (C-N), 3000-2850 (C-H-str,alk), 1450-1375 (-CH_3-bend), 1465 (-CH_2-bend), 1725-1705 (C=O).

3-(Morpholinomethyl)thiazolidine-2,4-dione (1SS-2) Yield:57%, m.p.: 199-201^{0}C,^{1}H NMR(400MHZ, DMSO-d_6, δ, ppm): 4.11(s,

2H,$COCH_2$),4.55(s,2H,$NHCH_2$),2.50(t, 4H, CH_3-morpholine ring),3.65(t,4H,CH_2-morpholine ring), IR V_{max}(cm^{-1})(KBr), 1465 (C=O-str), 3150-3050 (-CH_2-str),

3(pyrrolidin-1-ylmethyl) thiazolidine-2, 4-dine (1SS-3)

CH_2-pyrrolidine ring), IR V_{max} (cm^{-1}) (KBr): 1725-1705(C=O-str), 1350-1000(CN-str), 3150-3050(CH_2-str-aro), 3000-2850(CH-str).

3(piperdin-1-ylmethyl) thiazolidine-2, 4-dione (1SS-4)

Yield: 70%, m.p.: 155-158˚C,^{1}H NMR(400MHZ, DMSO-d_6, δ, ppm): 4.11(s, 2H, $COCH_2$), 4.55(s, 2H, $NHCH_2$), 2.45(t, 4H, CH_2-piperidine ring), 1.53(t, 4H, CH_2- piper dine ring), IR V_{max} (cm^{-1})(KBr): 1350-1000(CN str), 1725-1705(C=O-str), 3150-3050(CH_2-str-aro).

3((diethyl amino) methyl) thiazloidine-2, 4-dione (1SS-5)

Yield: 60%, m.p.: 199-201˚C, ^{1}H NMR(400MHZ, DMSO-d_6, δ, ppm): 4.11(s, 2H, $COCH_2$), 4.55(s, 2H, $NHCH_2$), 2.64(q, 4H, $NH(CH_2)_2$), 3.65(t, 6H, $NH(CH_3)_2$), IR V_{max} (cm^{-1})(KBr): 1465(CH_2-bend), 1450-1375(CH_3-bend), 1725-1705(C=O-str), 1350-1000(CN-str)

RESULTS AND DISCUSSION:

The synthesized compounds of 2, 4-thiazloidinedione (Iss-1 to Iss-5) showed diversified antioxidant activity. A series of mannish base of derivatives of 2, 4-thiazolidinedione were synthesized by mannish reaction with five different secondary amines and formaldehyde. The antioxidant activity was evaluated by the free radical scavenging activity by using DPPH assay method. In the series of mannish base derivatives, compounds Iss-3 and Iss-5 were showed significant antioxidant activity. DPPH (1, 1-diphenyl-2-picryl-hydrazil) is stable free radical. Methanol solution of DPPH is used to evaluate the antioxidant activity of several synthetic compounds. Antioxidant on interaction with DPPH, both transfer electron on hydrogen atom to DPPH, thus neutralizing its free radical character and convert it to 1, 1-diphenyl-2-picryl hydrazine. The degree of discoloration indicates the scavenging activity of the drug. The compound number Iss-1 to Iss-5 show promising antioxidant activity at concentrations (10, 20, 30, 40, and 50µg/ml). But two (Iss-3 and Iss-5) out of five synthesized compounds which were tested by free radical scavenging activity by DPPH assay method showed most significant antioxidant activity at concentration 50µg /ml.

CONCLUSION:

The five compounds were synthesized with the standard chemicals and procedure. The compounds were characterized through their respective IR, ^{1}H NMR, UV and TLC. The compound number Iss-3 and Iss-5 show promising antioxidant activity.

ACKNOWLEDGEMENT

The author wishes to acknowledge the principal Shivalik college of Pharmacy, Nangal for providing the chemicals and Labs and also Registrar JJT University, Jhunjhunu Rajasthan as well as SAIF, Punjab University Chandigarh for Providing IR and NMR data's.

CONFLICT OF INTEREST: The author does not have any conflict of interest.

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