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Antioxidant evaluation of 2,4-thiazolidinedione and rhodanine derivatives

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ABSTRACT

*A series of derivatives of thiazolidinedione and rhodanine **(TZP, RHP, RHB, MB01-MB09)** were synthesized using Scheme **1** and **2**. The spectral analysis of the derivatives was carried out using IR and ^{1}HNMR techniques. The synthesized compounds were screened for their in vitro antioxidant potential. **TZP, RHP and MB01** showed significant antioxidant activity which illustrates the requirement of free NH for antioxidant activity and proved unsubstituted derivatives to be more potent. **MB07** and **MB08** also exhibited moderate antioxidant activity and highlighted the presence of heterocyclic ring to be good for its radical scavenging activity. N-3 unsubstituted and aromatic amines substituted mannich bases were revealed to show better activity.*

Keywords: Thiazolidinedione, antioxidant, rhodanine, mannich bases, DPPH, IC_{50}

INTRODUCTION

Antioxidants are the substances, which when present at low concentrations compared to those of an oxidisable substrate, significantly delay or prevent oxidation of that substrate. The key role of antioxidants is to intercept and react with free radicals so that cascade effect of ROS propagation is prevented by readily donating its proton to the ROS (Reactive Oxygen Species). The antioxidant property of a compound is attributed to its ability of

a) Oxygen radical scavenging
b) Inhibiting cellular Microsomal P-450 linked Mixed Function oxidation (MFO) reaction
c) Suppressing the formation of ROS [1]

In vitro antioxidant activity of the synthesized compounds was quantitatively measured by **DPPH radical scavenging assay**. DPPH (2, 2-diphenyl 1-picrylhydrazyl) is a stable free radical at room temperature and accepts an electron or hydrogen radical to become stable diamagnetic molecule. DPPH radical is scavenged by antioxidants through the donation of protons forming the reduced DPPH. Antioxidant molecule can quench DPPH free radicals and convert them to a colorless / bleached product ultimately resulting in a decrease in absorbance [2].The medicinal properties of thiazolidinediones initiated us to synthesize N-substituted-5-benzylidene-2, 4- TZD and rhodanine derivatives and evaluate their antioxidant activity. The synthesized 2, 4-thiazolidinedione and rhodanine derivatives were substituted at N-3 with secondary amines to obtain their Mannich bases.

MATERIALS AND METHODS

Chemistry

Melting points were determined on an ELICO melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 400 MHz on a Brucker Advance II 400 spectrometer using TMS as an internal standard. Infrared spectra were recorded using KBr pellets on a Perkin-Elmer FTIR spectrophotometer. All compounds exhibited ^{1}H NMR and IR spectral data consistent with the proposed structures. The progress of the reaction was monitored by TLC using silica gel G as adsorbent. The plates were developed by exposing to the iodine vapours.

General procedure for thiazolidine-2, 4-dione (1) [3]

The solution containing chloroacetic acid (56.4 g, 0.6mol) in 60 ml of water and thiourea (45.6 g, 0.6mol) dissolved in 60 ml of water. The mixture was stirred for 15 min. to form a white precipitate, accompanied by considerable cooling. To the contents of the flask, added slowly 60 ml of concentrated hydrochloric acid from a dropping funnel, the flask was then connected with a reflux condenser and gentle heat applied to effect complete solution, after which the reaction mixture was stirred and refluxed for 8-10 hour at 100-110°C. On cooling, the contents of the flask solidified to a cluster of white needles. The product was filtered and washed with water to remove traces of hydrochloric acid and dried. It was purified by recrystallization from ethyl alcohol. Yield: 85%; m.p.:123-125°C.

General procedure for 4- Hydroxy-5-benzylidene-2, 4-thiazolidinedione (2) [3]

4-Hydroxy benzaldehyde (20 g, 0.188mol) and 2, 4-thiazolidinedione (22 g, 0.188mol) were together suspended in ethanol. To this, a catalytic amount of piperidine (1 ml) was added. The mixture was stirred and refluxed. After the complete removal of water and when the temperature reached above 110°C the reaction mixture was stirred for a further1 hour. On cooling, the product precipitated out from ethanol. The compound was filtered and washed with cold dry toluene and dry ethanol. Yield: 93%; m.p.:240-242°C.

General procedure for N-(Benzothiazol-2-yl)-2-chloroacetamide (3) [4]

2-Amino benzothiazole (15 gm, 0.1mol) in chloroform (10 ml) was stirred in a conical flask and to this, chloroacetyl chloride (12.01ml, 0.15mol) was added drop wise under cold condition. Reaction mixture was stirred till completion of reaction, which was monitored by TLC.

General procedure for N-(Benzothiazol-2-yl)-2-(4-((2, 4-dioxothiazolidin-5 ylidene)methyl)phenoxyacetamide (4) [4]

5-Benzylidene-2, 4-thiazolidinedione (22.12gm, 0.1mol) and anhydrous potassium carbonate (20.72gm, 0.15mol) in dimethyl formamide (DMF) was stirred in a flask and to this reaction mixture, N-(Benzothiazol-2-yl)-2-chloroacetamide (34.0gm, 0.15mol) in DMF was added. Reaction mixture was stirred at room temperature till the completion of reaction, which was monitored by TLC. After completion of reaction, water was added to get the solid final product.

General procedure for N-mannich bases (5) (MB01-MB09) [5]

To a solution of N-(Benzothiazol-2-yl)-2-(4-((2, 4-dioxothiazolidin-5-ylidene) methyl) phenoxy)acetamide (5.09gm, 0.01 mol) in DMF, formaldehyde (0.6ml, 0.02 mol) was added under stirring. The reaction-mixture was stirred at RT for 0.5 hr to complete the reaction of formaldehyde and yield methylol derivative of compound. To this, the solution of secondary amine in DMF was added drop wise and refluxed for 2 hr. The reaction was poured into ice cold water and filtered off and wash with hot water. Finally it was dried and purified by recrystallization from chloroform to get the desired compound.

Spectral data

(E)-5-(4-hydroxybenzylidene) thiazolidine-2, 4-dione: IR (KBr, cm^{-1}): 3405 (NH str.), 3125 (C-H str., aromatic), 1720 and 1678 (C=O str., cyclic imide), 1510 (C=C bend, aromatic), 1279 (C-O str.), 1156 (C-N str.), 614 (C-S str.); ^{1}H NMR (DMSOd_6) δ (ppm): 6.92 (d, 2H, aromatic), 7.46 (d, 2H, aromatic), 7.695 (s, 1H, benzylidene proton)

N-(Benzothiazol-2-yl)-2-chloroacetamide: IR (KBr, cm^{-1}): 3368 (NH str.), 1695 (C=O str., amide), 1450 (C=C bend, aromatic), 1268 (C-O str.), 1177 (C-N str.), 677 (C-Cl str.)

N-(Benzothiazol-2-yl)-2-(4-((2, 4-dioxothiazolidin-5 ylidene)methyl)phenoxyacetamide **(TZP):** IR (KBr, cm^{-1}): 3197 (NH str.), 3065 (H-C=C str.), 1733 and 1677 (C=O str., cyclic imide), 1267 (C-O str.), 1176 (C-N str.), 611

(C-S str.); ^{1}H NMR (DMSOd_6) δ (ppm): 8.5 (2H, s, Ar-H), 8.3 (1H, s, NH), 7.56 (2H, s, Ar-H), 7.14 (2H, m, Ar-H), 4.68 (2H, s, CH_2)

***(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide* (RHP):** IR (KBr, cm^{-1}): 3190 (NH str.), 3070 (H-C=C str.), 1643 (C=O, aliphatic) and 1664 (C=O str., imide), 1271 (C-O str.), 1170 (C-N str.), 621 and 632 (C-S str.); ^{1}H NMR (DMSOd_6) δ (ppm): 7.96 (1H, s, NH), 7.75 (2H, s, Ar-H), 7.32 (3H, m, Ar-H), 6.68 (2H, s, Ar-H), 3.7 (2H, s, CH_2)

***(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((2, 4-dioxo-3-(piperazin-1-ylmethyl) thiazolidin-5-ylidene) methyl)phenoxy) acetamide* (MB01):** IR (KBr, cm^{-1}): 3402 (NH str.), 2935 (H-C=C str.), 1664 (C=O str., imide), 1459 (CH_2 str.), 1278 (C-O str.), 1169 (C-N str.), 620 (C-S str.); ^{1}H NMR (DMSOd_6) δ (ppm): 8.25 (2H, s, Ar-H), 7.95 (1H, s, NH), 7.10 (3H, m, Ar-H), 6.8 (3H, m, Ar-H), 3.34 (2H, s, CH_2), 2.5 (4H, m, CH_2)

***(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((3-((diphenylamino)methyl)-2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy) acetamide* (MB02):** IR (KBr, cm^{-1}): 3385 (NH str.), 3045 (H-C=C str.), 1680 and 1656 (C=O str., cyclic imide), 1494 (C=C bend, aromatic), 1477 (CH_2, bend), 1234 (C-O str.), 1172 (C-N str.), 615 (C-S str.); ^{1}H NMR (DMSOd_6) δ (ppm): 8.14 (1H, s, NH), 7.2 (4H, m, Ar-H), 7.07 (6H, d, Ar-H), 6.8 (1H, s, H-C=C)

***(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((3-((dimethylamino)methyl)-2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy) acetamide* (MB03):** IR (KBr, cm^{-1}): 3381 (NH str.), 3061 (H-C=C str.), 1664 (C=O str., imide), 1593 (C=C bend, aromatic), 1471 (CH_2, bend), 1273 (C-O str.), 1176 (C-N str.), 619 (C-S str.); ^{1}H NMR (DMSOd_6) δ (ppm): 7.9 (1H, s, NH), 7.72 (4H, m, Ar-H), 7.33 (4H, m, Ar-H), 6.84 (1H, s, H-C=C), 3.4 (4H, s, CH_2), 2.5 (6H, s, CH_3)

***(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((3-((4-methylpiperazin-1-yl)methyl)-2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy)acetamide* (MB04):** IR (KBr, cm^{-1}): 3288 (NH str.), 3062 (H-C=C str.), 1681 and 1598 (C=O str., cyclic imide), 1494 (C=C bend, aromatic), 1377 (CH_3, bend), 1273 (C-O str.), 1188 (C-N str.), 617 (C-S str.); ^{1}H NMR (DMSOd_6) δ (ppm): 8.1 (2H, s, Ar-H), 7.96 (1H, s, NH), 7.17 (4H, m, Ar-H), 6.98 (1H, s, H-C=C), 3.42 (4H, s, CH_2), 2.5 (8H, s, CH_2), 2.2 (3H, s, CH_3)

***(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((3-((diethylamino)methyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy) acetamide* (MB05):** IR (KBr, cm^{-1}): 3413 (NH str.), 2921 (CH_3 str., aliphatic), 1666 (C=O str., imide), 1494 (C=C bend, aromatic), 1377 (CH_3, bend), 1273 (C-O str.), 1188 (C-N str.), 617 (C-S str.)

***(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((3-((dibenzylamino)methyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy) acetamide* (MB06):** IR (KBr, cm^{-1}): 3406 (NH str.), 1664 (C=O str., imide), 1506 (C=C bend, aromatic), 1467(-CH_2-), 1250 (C-O str.), 1176 (C-N str.), 622(C-S str.); ^{1}H NMR (DMSOd_6) δ (ppm): 7.95 (1H, s, NH), 7.68 (4H, m, Ar-H), 7.09 (4H, m, Ar-H), 3.34 (4H, s, CH_2), 2.5 (4H, s, CH_2), 1.2 (6H, s, CH_3)

***(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((4-oxo-3-(piperazin-1-ylmethyl)-2-thioxothiazolidin-5-ylidene)methyl) phenoxy) acetamide* (MB07):** IR (KBr, cm^{-1}): 3240 and 3304 (NH str.), 3078 (H-C=C str.), 1666 (C=O str., amide), 1460 (CH_2, bend), 1278 (C-O str.), 1159 (C-N str.), 646 (C-S str.)

***(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((3-((4-methylpiperazin-1-yl)methyl)-4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenoxy)acetamide* (MB08):** IR (KBr, cm^{-1}): 3213 (NH str.), 3061 (H-C=C str.), 1664 (C=O str., amide), 1481 (C=C bend, aromatic), 1465 (CH_2 bend), 1365 (CH_3, bend), 1278 (C-O str.), 1151 (C-N str.), 624 (C-S str.); ^{1}H NMR (DMSOd_6) δ (ppm): 8.23 (2H, s, Ar-H), 7.89 (1H, s, NH), 7.23 (4H, m, Ar-H), 6.91 (1H, s, H-C=C), 3.38 (4H, s, CH_2), 2.43 (8H, s, CH_2), 2.32 (3H, s, CH_3)

***(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((3-((dimethylamino)methyl)-4-oxo-2-thioxothiazolidin-5-ylidene)methyl) phenoxy) acetamide* (MB09):** IR (KBr, cm^{-1}): 3381 (NH str.), 3061 (H-C=C str.), 1664 (C=O str., amide), 1593 (C=C bend, aromatic), 1471 (CH_2, bend), 1273 (C-O str.), 1176 (C-N str.); ^{1}H NMR (DMSOd_6) δ (ppm): 7.85 (1H, s, NH), 7.69 (4H, m, Ar-H), 7.39 (4H, m, Ar-H), 6.78 (1H, s, H-C=C), 3.5 (4H, s, CH_2), 2.8 (6H, s, CH_3)

***(E)-5-(4-hydroxybenzylidene)-2-thioxothiazolidin-4-one* (RHB):** IR (KBr, cm^{-1}): 3383 (NH str.), 3101 (C-H str., aromatic), 1685 (C=O str., amide), 1506 (C=C bend, aromatic), 1282 (C-O str.), 1174 (C-N str.), 624 (C-S str.); ^{1}H

NMR ($DMSOd_6$) δ (ppm): 10.4 (1H, s, NH), 7.13 (3H, m, Ar-H), 6.68 (2H, m, Ar-H)

Antioxidant Assay

The solutions of synthesized mannich bases of 2, 4-thiazolidinedione and rhodanine derivatives were prepared in DMSO in concentrations ranging from 10-500 µg/ml. A DPPH blank was prepared and methanol was used for the baseline correction. The well-known antioxidant ascorbic acid was used as a positive control. 2 ml of each compound solution having different concentration (10-500µg/ml) were taken in different test tubes and 2 ml of 0.1mM methanolic solution of DPPH was added and shaken vigorously. The tubes were then incubated at 37°C for 30 min. Changes in absorbance were measured at 517nm using a UV/visible spectrophotometer. Measurement was performed in triplicate and the DPPH radical scavenging activity was expressed as % inhibition of DPPH and was calculated using the equation:

Percent Inhibition of DPPH (%) = $(A_0 - A_1/A_0) \times 100$
Where, A_0 is the absorbance of control (blank, without compound)

A_1 is the absorbance of the test compound

The DPPH Scavenging Activity of ascorbic acid at various concentrations was also measured and compared with those of the newly synthesized compounds [6].

Table I: Physicochemical Characterization of the Synthesized Compounds

S.No.	Product code	Molecular formula	Molecular weight	Melting Point	R_f	% yield
1.	TZP	$C_{19}H_{13}O_4N_3S_2$	411.454	168-180	0.61	58.4
2.	RHP	$C_{19}H_{13}O_3N_3S_3$	427.52	170-185	0.86	60.3
3.	MB01	$C_{24}H_{23}O_4N_5S_2$	509.601	62-64	0.73	67.89
4.	MB02	$C_{32}H_{24}O_4N_4S_2$	592.687	56-60	0.72	48.56
5.	MB03	$C_{22}H_{20}O_4N_4S_2$	468.549	54-58	0.65	56.7
6.	MB04	$C_{25}H_{25}O_4N_5S_2$	523.627	60-64	0.70	44.9
7.	MB05	$C_{34}H_{28}O_4N_4S_2$	620.741	63-65	0.82	78.25
8.	MB06	$C_{24}H_{24}O_4N_4S_2$	496.602	55-57	0.76	62.8
9.	MB07	$C_{24}H_{23}O_3N_5S_3$	525.666	58-62	0.60	53.65
10.	MB08	$C_{25}H_{25}O_3N_5S_3$	539.693	60-65	0.79	70.48
11.	MB09	$C_{22}H_{20}O_3N_4S_3$	484.614	55-59	0.57	64.45
12.	RHB	$C_{10}H_7O_2NS_2$	237.298	150-155	0.67	78.42

TLC Mobile Phase: Benzene:Methanol ; 8.5:1.5 (v/v)

RESULTS AND DISCUSSION

Chemistry

The mannich bases of thiazolidinedione and rhodanine derivatives were synthesized using scheme 1 and scheme 2 (Fig 2) respectively. Thiazolidinedione and rhodanine was condensed with benzaldehyde by Knoevenagel condensation to form 5-benzylidene thiazolidinedione and 5-benzylidene rhodanine which was condensed with acetamide derivative of 2-aminobenzothiazole (**2)** to form compound (**3)**. This compound was further reacted with formaldehyde by stirring and then refluxed with desired secondary amines to get the mannich bases. The completion of reaction was confirmed by single spot TLC. The synthesized derivatives were characterized by their physical parameters such as R_f, melting point and % yield.The results are summarized in Table I.

A Knoevenagel condensation is a **nucleophilic addition of an active hydrogen compound** to a carbonyl group followed by a dehydration reaction in which a molecule of water is eliminated (hence condensation). The product is often an alpha, beta conjugated enone. Knoevenagel reaction of aldehyde with the cyclic dione in the presence of a catalytic amount of base such as piperidine and an acid such as benzoic acid can provide the phenoxy cyclic dione. The Knoevenagel reaction is typically performed in an aprotic solvent such as toluene at a temperature preferably between 100-200°C [9].

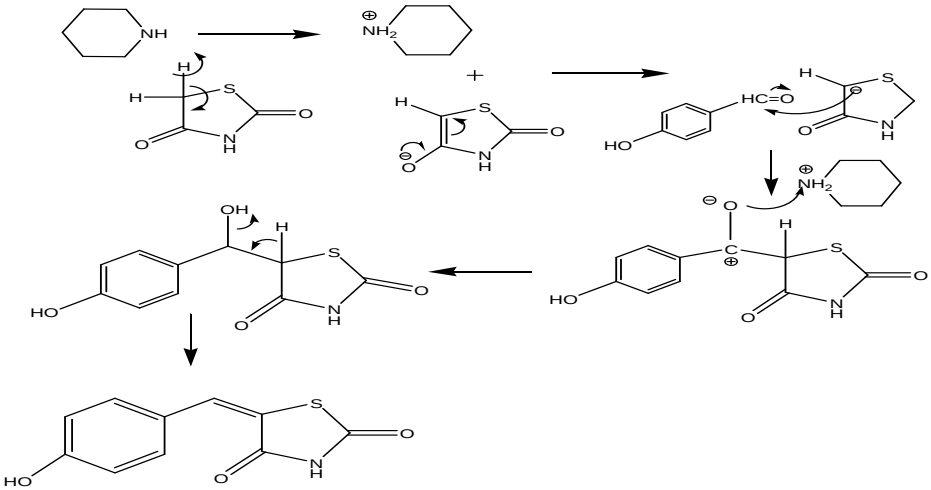


Fig 1. Mechanism of Knoevenagel condensation

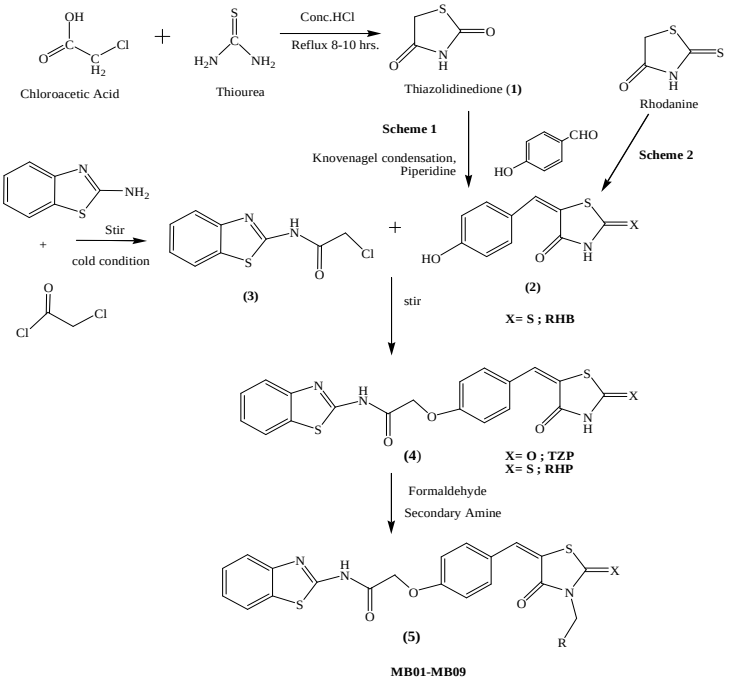
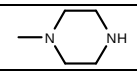
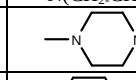
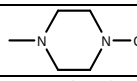
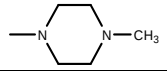


Fig 2. The general synthetic scheme for synthesis of mannich bases of thiazolidinedione and rhodanine derivatives

S. No.	Product code	R	X	S.No.	Product code	R	X
1.	TZP	-	O	**7.**	MB05	$-N(CH_2.C_6H_5)_2$	O
2.	RHP	-	S	**8.**	MB06	$-N(CH_2.CH_3)_2$	O
3.	MB01		O	**9.**	MB07		S
4.	MB02	$-N(C_6H_5)_2$	O	**10.**	MB08		S
5.	MB03	$-N(CH_3)_2$	O	**11.**	MB09	$-N(CH_3)_2$	S
6.	MB04		O	**12.**	RHB	-	S

The structure of the synthesized compounds was confirmed by IR, ^{1}HNMR. The IR Spectrum of benzylidene 2, 4-thiazolidinedione illustrates the presence of H-C=C bond at 3065cm^{-1} which confirmed the unsaturation at C-5 in all

the synthesized derivatives. The absence of O-H peak at 3600 cm^{-1} in TZP confirmed the formation of ether linkage in the pharmacophore.

The IR spectra of synthesized derivatives (MB01-MB09) exhibited the absorption bands for aromatic ring vibrations in the region of 3150-3050 cm^{-1}. The carbonyl stretching band was observed at 1680 and 1664 cm^{-1}.The $–CH_2$- bend was observed at 1465cm^{-1} which confirmed the presence of methylene group in mannich bases. The synthesized compounds also showed C-O str. in the range of 1270-1278cm^{-1}, C-N str. in the range of 1100-1200cm^{-1} and C-S str. in the range of 620-630cm^{-1} which indicated the synthesis of respective compounds.

The ^{1}HNMR spectrum of benzylidene derivatives of thiazolidinedione and rhodanine displayed the characteristic peak at δ 7.1 for unsaturation at C-5 of the ring and NH peak at δ 10.0. The pharmacophore showed the peak at δ 4.7 for $–CH_2$ which confirmed the condensation of 2-amino benzothiazole ring with benzylidene thiazolidinedione and rhodanine, with the peaks for aromatic hydrogen at δ 7.2 and δ 7.7. The NH peak at 8.0 for amide linkage at benzothiazole and NH peak at δ 10.0 for unsubstituted N-3 can be easily distinguished in the spectrum of TZP and RHP. The mannich bases of thiazolidinedione gave characteristic peak for $–CH_2$ at δ 2.2-3.5 which confirmed the methylene group between secondary amines and N-3. The absence of NH peak at δ 10.0 further confirms the substitution at N-3 of thiazolidinedione and rhodanine instead of at amide linkage of benzothiazole which was also one of the probable sites for substitution of secondary amine. The peaks of respective amines containing hydrogens were also noted. The spectral data thus confirmed the synthesis of mannich bases of thiazolidinedione and rhodanine derivatives with respective amines at N-3 of TZP and RHP.

Antioxidant Activity

Antioxidant activity of the synthesized compounds compared to ascorbic acid is shown in Table 2. The IC_{50} values were noted by measuring the concentration showing 50% inhibition of DPPH and the results are shown in Table 3. The results indicated that the synthesized compounds showed moderate to good antioxidant activity as compared to ascorbic acid. It may be due to thiazolidinedione and rhodanine moiety to initiate the free radical scavenging activity due to its –NH and –C=O groups. The compounds having more NH and C=O moieties are expected to show good results. The SAR findings can be summarized as follows

- Benzylidene compound (RHB) showed less antioxidant activity than RHP, as after the fusion of benzothiazole ring with benzylidene ring the number of radical scavenging moieties like NH and C=O groups increased which further contributed towards the antioxidant activity.
- TZP and RHP showed significant activity with optimal IC_{50} values 75 and 85 µg/ml respectively, due to NH and C=O group which increased in number after condensing benzylidene and benzothiazole moiety in the synthesis of TZP and RHP.
- Mannich bases of thiazolidinedione and rhodanine were found to be moderate to less active than other synthesized derivatives which may be due to absence of free NH group at N-3 in thiazolidinedione and rhodanine moiety.
- Mannich bases with alicyclic amines *viz*. piperazine having free NH group (**MBO1**and **MB07**) showed better activity with IC_{50} values 80 and 92µg/ml respectively.
- Aliphatic and aromatic amine substituted mannich bases showed moderate activity with IC_{50} in the 110-130µg/ml.

Table 2. Percent inhibition of DPPH by synthesized compounds at different concentrations

S. No.	Product Code	10µg/ml	50µg/ml	100µg/ml	250µg/ml	500µg/ml
1.	Ascorbic Acid	35.84	41.35	58.83	69.28	84.67
2.	TZP	8.73	35.39	46.59	57.34	62.65
3.	RHP	10.96	40.68	65.794	71.047	78.29
4.	MB01	10.56	45.76	59.58	65.45	71.98
5.	MB02	22.61	38.69	45.45	71.69	72.56
6.	MB03	22.79	29.77	58.59	68.06	72.10
7.	MB04	21.96	32.58	46.50	64.24	71.23
8.	MB05	19.30	40.67	49.44	55.69	66.86
9.	MB06	22.38	39.75	51.51	59.14	72.68
10.	MB07	21.96	36.03	56.27	61.33	71.00
11.	MB08	18.84	24.49	51.74	68.93	76.47
12.	MB09	15.94	25.87	45.22	65.24	71.04
13.	RHB	22.74	35.01	46.00	58.40	74.54

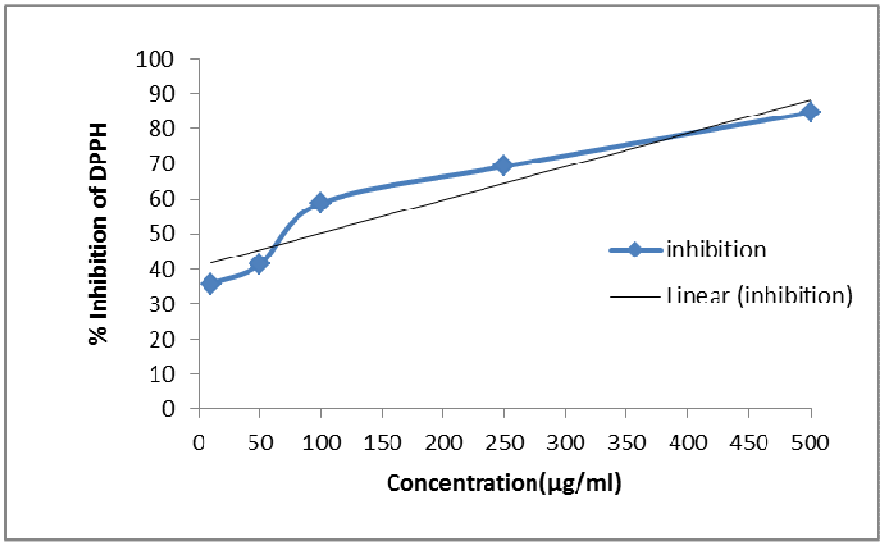


Fig. II Standard curve of percent inhibition of DPPH Vs Ascorbic acid

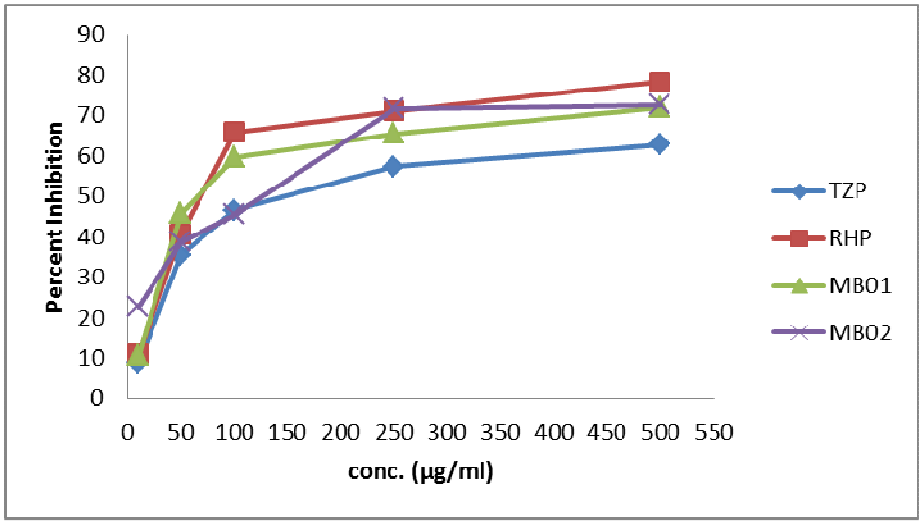


Fig.III Percent inhibition of DPPH by test compounds (TZP, RHP, MB01 and MB02)

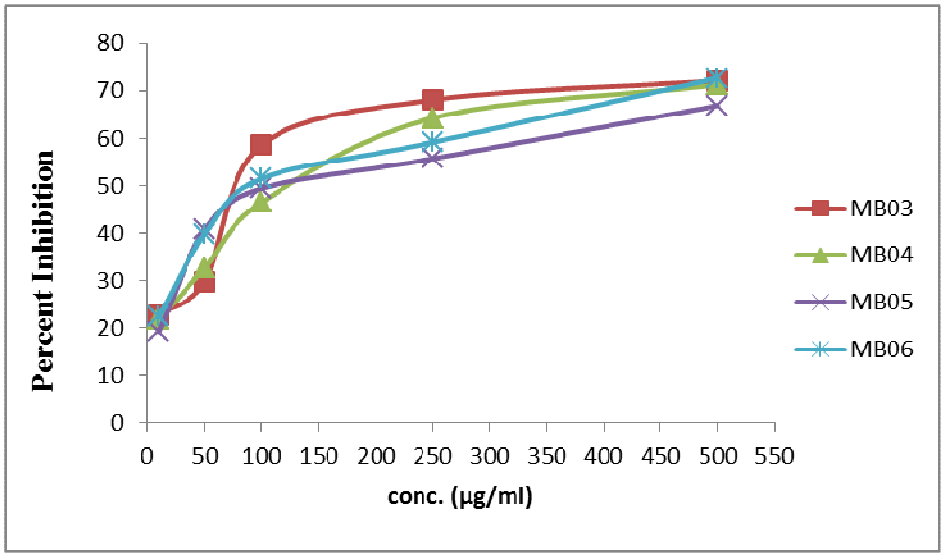


Fig. IV: Percent inhibition of DPPH by test compounds (MB03, MB04, MB05 and MB06)

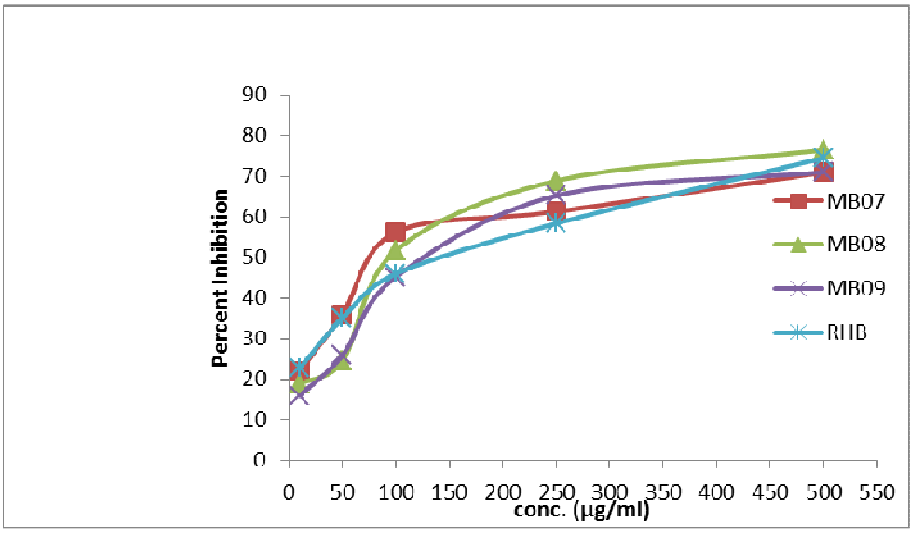


Fig.V: Percent inhibition of DPPH by test compounds (MB07, MB08, MB09 and RHB)

Table 3. IC_{50} values of synthesized derivatives

S. No.	Product Code	IC_{50} (µg/ml)
1.	Ascorbic acid	26
2.	TZP	**75**
3.	RHP	**85**
4.	MBO1	**80**
5.	MB02	122
6.	MB03	120
7.	MB04	125
8.	MB05	110
9.	MB06	130
10.	MB07	**92**
11.	MB08	**95**
12.	MB09	130
13.	RHB	120

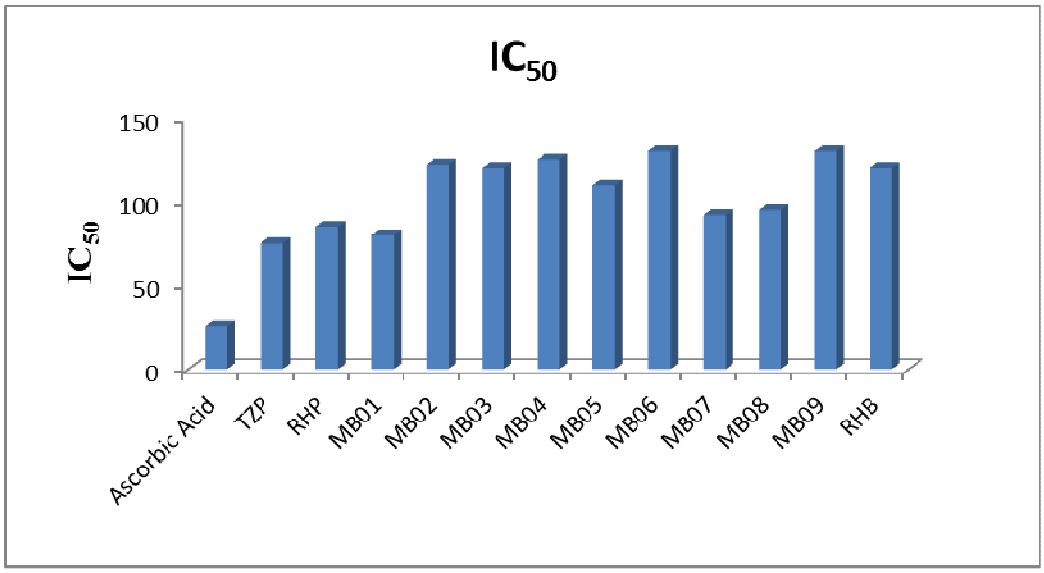


Fig.VI: IC_{50} values of test compounds in comparison with ascorbic acid as standard

CONCLUSION

Mannich bases of novel derivatives of thiazolidinedione and rhodanine were synthesized and characterized by spectral techniques. The derivatives screened for their antioxidant capability showed radical scavenging activity which illustrated the requirement of free NH for antioxidant activity and proved unsubstituted derivatives to be more potent. Further, N-3 substituted derivatives are displayed significant activity than N-unsubstituted compounds and aromatic substitution at N-3 is showed better activity than alicyclic and aliphatic substitution.

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