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Synthesis of novel thiazolidin-4-one derivatives their antimicrobial and molecular docking studies

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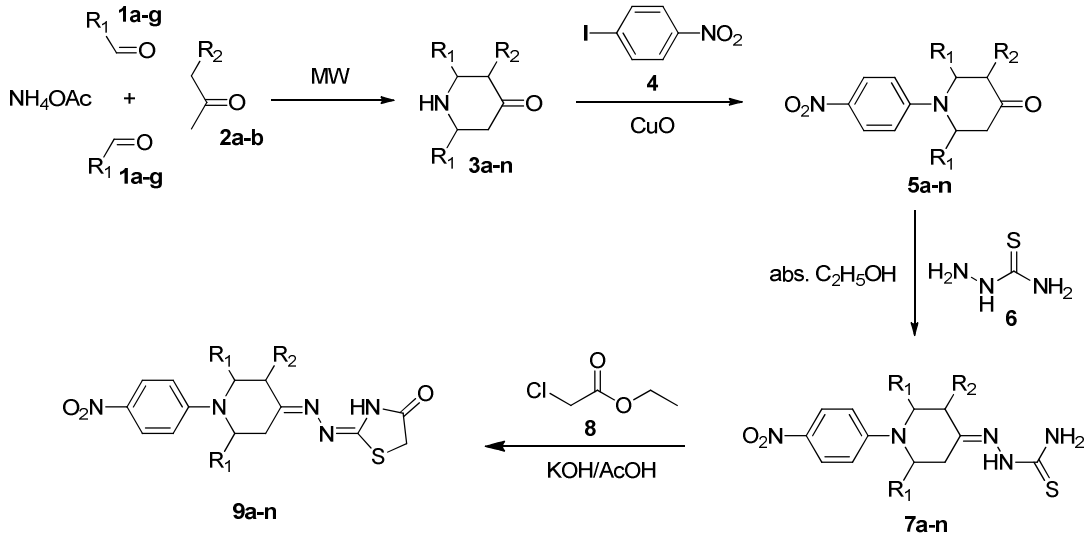
ABSTRACT

Novel thiazolidin-4-one derivatives have been synthesized via Ullmann reaction followed by condensation of 1-nitrophenyl-4-piperidones with thiosemicarbazides and then cyclisation with ethylchloroacetate. Chemical structureof the synthesised compounds was elucidated by FT-IR, ^{1}H NMR, ^{13}C NMR, Mass and elemental analysis. The title compounds were tested for their antimicrobial activity and found to exhibit a variable degree of activity. Molecular docking studies done to investigate plausible mechanism of action towards the DNA topoisomerase IV receptor.

Key words: Thiazolidin-4-one; Ullmann reaction; piperidone; antimicrobial activity.

INTRODUCTION

The main objective of organic and medicinal chemists is design, synthesis and production of molecules having significance as human therapeutic agents. Heterocyclic compounds, which contain nitrogen, oxygen or sulphur, are important class of compounds receiving special attention as they belong to a class of compounds with proven utility in medicinal chemistry. In particular, numerous natural and synthetic compounds which contain thiazolidin-4-one scaffold as the core moiety have been identified as the efficient drugs for various diseases [1-3].Thiazolidin-4-one compounds have found uses as antibacterial [4-5], antifungal [6], anti-inflammatory[7-8], anti-HIV [9],anticancer [10-11], immunostimulating agent [12], antimycobacterial[13], nematicidal agents [14], anticonvulsant [15-16], antidiabetic [17], muscarinic receptor 1 agonist [18], FSH receptor agonist [19], trypanocidal[20] and antiarrhythmic activity [21].In continuation of our earlier work[22-25], we herein report the synthesis of thiosemicarbazones and then thiazolidin-4-one derivatives. The required starting material, 4-piperidinone derivatives (**3a-n**) have been prepared by Mannich-enamine substitution reaction using aldehyde (**1a-g**), ketone (**2a-b**) and ammonium acetate. Then1-(4-nitrophenyl)-4-piperidone derivatives (**5a-n**) have been prepared by Ullmann reaction between 4-piperidone derivatives (**3a-n**) and 4-nitroiodobenzene (**4**) using cuprous salt as a catalyst[26]. The thiosemicarbazones(**7a-n**) have been prepared by condensation of appropriate 1-(4-nitrophenyl)-4-piperidone (**5a-n**) compounds with thiosemicarbazide (**6**) in abs.ethanol[27]. Finally, thiosemicarbazone derivatives (**7a-n**) reacted with ethylchloroacetate (**8**) and KOH in acetic acid underwent intermolecular cyclization[28] to yield five-membered heterocyclic compounds, thiazolidine-4-one derivatives(**9a-n**) (**Scheme 1**).



Scheme 1. Synthesis of thiazolidin-4-one compounds (9a-n)

MATERIALS AND METHODS

2.1. *Measurements*

Melting points of synthesized compounds were recorded in ºC by applying open capillary method and are uncorrected. Purity of the compounds was routinely checked on silica gel-G TLC plates. FT-IR spectra were recorded on Perkin-Elmer 237 spectrophotometer using potassium bromide (KBr) powder andvalues are given in cm^{-1}. The ^{1}H NMR spectra were recorded on Brucker F 300 MHz spectrophotometer and ^{13}C NMR on Brucker F 75 MHz spectrophotometer, in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard (chemical shift in δ, ppm) and coupling constant (J) values are given in Hertz (Hz). Mass spectra were recorded on a ThermoFinnigan LCQ Advantage MAX 6000 ESI spectrometer and elemental analysis data were recorded using ThermoFinnigan FLASH EA 1112 CHN analyser.

2.2. *General method for synthesis of 4-piperidones (**3a-n**)*

A green synthetic approach was reported for the facile synthesis of various 4-piperidones (**3a-n**), presumably is a Mannich-enamine substitution reaction using Montmorillonite K-10 catalyst[29]. The present developed method for the synthesis of 4-piperidones (**3a-n**) offers many advantages including excellent yields of products, operational simplicity, involvement of cost effective and non-toxic reagents which make the process environmentally benign, and possibility of recycling the solid clay. Dry ammonium acetate (0.1 mol) was mixed with Montmorillonite K-10 (200mg) in a dry condition. Then, freshly distilled appropriate aldehyde (**1a-g**) (0.2mol) and ketone (**2a-b**) (0.1mol) were added to the mixture and kept in the microwave oven for 3 min at 130 watts. The reaction mixture was than dissolved in dry ether (50 mL) followed by conc. HCl (30mL). The hydrochloride was suspended into acetone and basified with liq.NH_3. The crude solid was recrystallised from ethanol to afford pure 4-piperidone compounds (**3a-n**).

2.3. *General procedure for synthesis of 1-(4-nitrophenyl)-4-piperidones (**5a-n**)*

A mixture of 4-piperidones (**3a-n**) (0.01mol), 4-nitroiodobenzene (**4**) (0.01 mol), anhydrous potassium carbonate (1.5g) and cupric oxide (0.5 g) in pyridine (20mL) was heated under reflux for 24 h. The cooled mixture was filtered and the residue washed thoroughly with hot pyridine. The filtrate was poured into ice cold dil.HCl. The separated solid was recrystallised from ethanol to afford pure 1-(4-nitrophenyl)-4-piperidones **(5a-n)**.

The structure of synthesised 1-(4-nitrophenyl)-4-piperidone derivatives by Ullmann reaction was elucidated with the help of FT-IR, ^{1}H NMR, ^{13}C NMR and mass data as illustrated for compound **5a**. In the FT-IR spectrum, the sharp band appeared at 1713 cm^{-1} corresponds to the C=O stretching and the peak at 1519 cm^{-1} corresponds to NO_2stretching of the product **5a**. In the ^{1}H NMR, peaks in the range of δ: 7.03-8.22 ppm confirms 14 aromatic protons. The signal at δ: 1.11 ppm for three protons confirmed the presence of $-CH_3$ group. In the ^{13}C NMR, the peaks at δ: 12.1 correspond to $-CH_3$ carbon and the peaks at δ: 52.2, 49.2, 67.6 and 67.9 ppm show the presence of four other aliphatic carbons. The peak around δ: 112.7 to 151.8 ppm confirmed the aromatic carbons and the peak at δ: 211.0 ppm confirmed the presence of carbonyl group. A distinguishing peak observed at m/z: 387 in the mass spectrum for $[M+H]^+$ ion further conforms the product **5a**.

FT-IR (KBr) (cm^{-1}): 1713 (C=O), 1519 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ: 8.12 (d, 2H, *J* = 7.2 Hz, Ar-H); 7.22-7.40 (10H, m, Ar-H); 7.03 (d, 2H, *J* = 7.1 Hz, Ar-H); 4.30 (m, 2H, 2CH); 2.56 (dd, 1H, *J* = 7.4, 10.2 Hz, CH_2); 2.49 (dd, 1H, *J* =7.8, 10.1 Hz, CH_2); 2.34 (m, 1H, CH); 1.14 (d, 3H, *J* = 7.4 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ: 211.0, 151.8,142.3,141.6,137.4,129.2,128.4,128.1, 127.9,127.5,126.0,125.1,112.7,67.9,67.6,52.2,49.2,12.1;MS *m/z*: 386 $[M]^+$, 387$[M+H]^+$; Anal. Calcd. For $C_{24}H_{22}N_2O_3$. C74.59, H5.74, N7.25. Found C74.55, H5.70, N7.26.

2.4.*General procedure for synthesis of thiosemicarbazone derivatives (**7a-n**)*

Thiosemicarbazide(**6**) (0.01mol) was added to 1-(4-nitrophenyl)-4-piperidones **(5a-n)** (0.01mol) dissolved in abs.ethanol (50 mL), the reaction mixture was refluxed for 10 h and the completion of the reaction was monitored by TLC. Then the reaction mixture was cooled to room temperature and poured into ice water, the solid obtained was filtered, washed with water and recrystallised from acetic acid to afford pure thiosemicarbazones **(7a-n)**.

The structure of synthesised thiosemicarbazones **(7a-n)** derivatives was elucidated with the help of FT-IR, ^{1}H NMR, ^{13}C NMR and mass data as illustrated for compound **7a**. In the FT-IR spectrum, the band appeared at 3431 cm^{-1} corresponds to the NH_2 stretching and the band at 1612 cm^{-1} corresponds to NH_2 bending and also the band appeared at 1524 cm^{-1} corresponds to the NO_2stretching and the band at 1172 cm^{-1} corresponds to C=Sstretching of the product **7a**. In the ^{1}H NMR, peaks in the range of δ: 7.02-8.20 ppm confirms 14 aromatic protons. The signal at δ: 8.70 ppm for two protons and 7.15 ppm for one proton correspond to $–NH_2$ and –NH protons. The signal at δ: 1.02 ppm for three protons confirmed the presence of $–CH_3$ group. In the ^{13}C NMR, the peaks at δ: 16.4 correspond to $–CH_3$ carbon and the peaks at δ: 25.2, 37.1, 68.6 and 68.8 ppm show the presence of four other aliphatic carbons. The peak around δ: 112.7 to 151.6 ppm confirmed the aromatic carbons and the peak at δ: 158.3 and 181.5 ppm confirmed the presence of C=N and C=S group respectively. A distinguishing peak observed at *m/z*: 460 in the mass spectrum for $[M+H]^+$ ion further conforms the product **7a**.

FT-IR (KBr) (cm^{-1}): 3431 (NH_2), 1612 (NH), 1524 (NO_2), 1172 (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ: 8.70 (s, 2H, NH_2); 8.20 (d, 2H, *J* = 7.0 Hz, Ar-H); 7.24-7.44 (m, 10H, Ar-H); 7.15 (s, 1H, NH); 7.02 (d, 2H, *J* = 7.2 Hz, Ar-H); 4.02 (m, 2H, 2CH); 2.50 (dd, 1H, *J* = 7.3, 10.0 Hz, CH_2); 2.44 (dd, 1H, *J* =7.9, 10.0 Hz, CH_2); 2.32 (m, 1H, CH); 1.08 (d, 3H, *J* = 7.2 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ: 181.5, 158.3, 151.6,142.2, 141.8, 137.6,129.2, 128.5,128.2, 127.9,127.4, 126.0,125.2, 112.7, 68.8,68.6, 37.1,25.2, 16.4.; MS: *m/z* 460 $[M+H]^+$.MS *m/z*: 459 $[M]^+$, 460$[M+H]^+$; Anal. Calcd. For $C_{25}H_{25}N_5O_2S$. C65.34, H5.48, N15.24. Found C65.37, H5.43, N15.28.

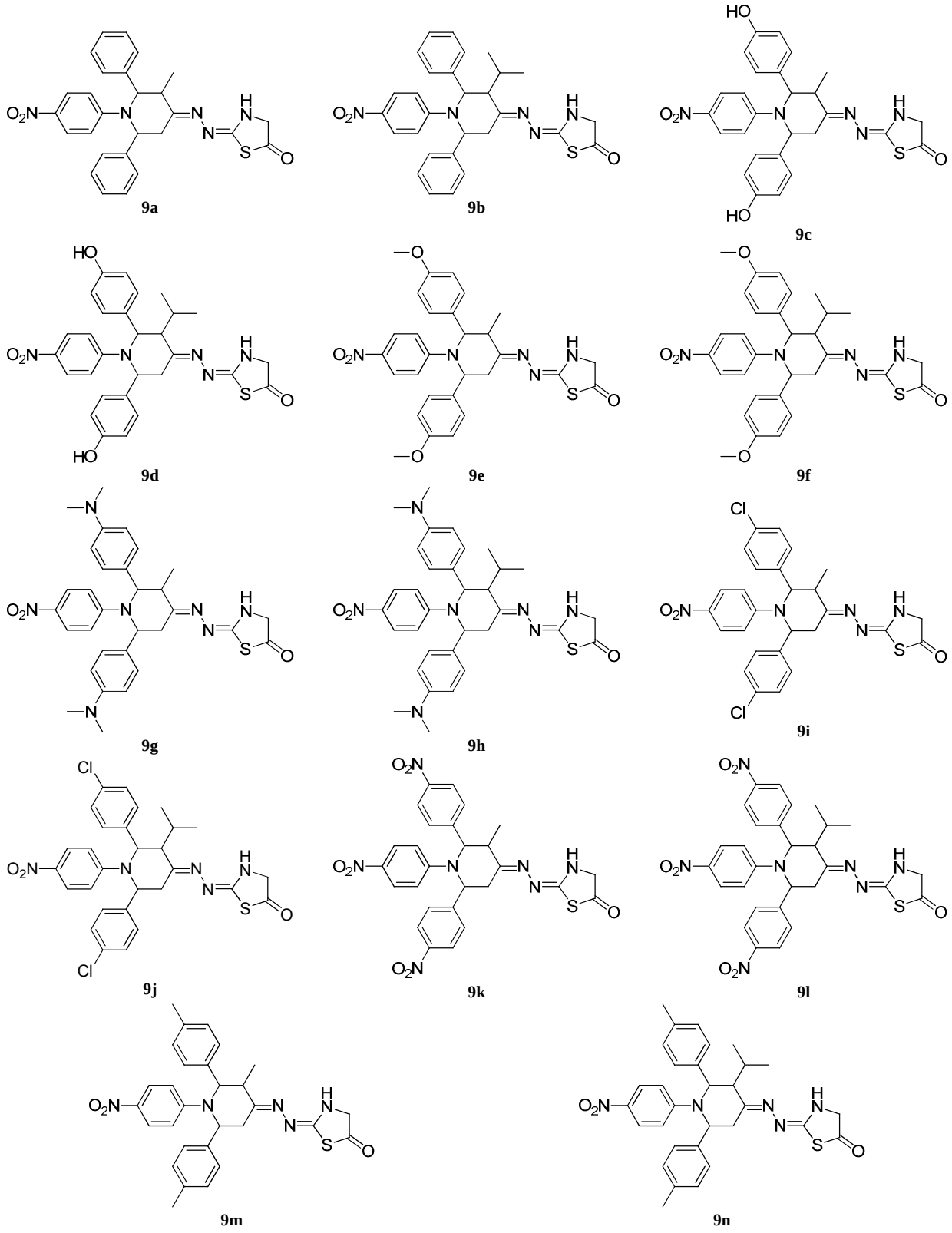
2.5.*General procedure for synthesis of thiazolidin-4-one derivatives (**9a-n**)*

To aequimolar mixture of thiosemicarbazone derivatives **(7a-n)**, ethylchloroacetate (**8**) and KOH were added to the solvent acetic acid (50mL). The reaction mixture was refluxed for 10 h. The precipitate obtained was filtered and recrystallized from ethanol to afford pure thiozolidin-4-one derivatives **(9a-n)** (**Table 1**) (**Figure 1**).

Table 1. Synthesis of thiazolidin-4-one compounds (9a-n)

S. No.	Thiazolidin-4-one	R_1	R_2	Yield (%)
1	9a	$C_6H_5–$	$CH_3–$	78
2	9b	$C_6H_5–$	$(CH_3)_2CH–$	74
3	9c	$4–OH–C_6H_4–$	$CH_3–$	72
4	9d	$4–OH–C_6H_4–$	$(CH_3)_2CH–$	70
5	9e	$4–CH_3–O–C_6H_4–$	$CH_3–$	72
6	9f	$4–CH_3–O–C_6H_4–$	$(CH_3)_2CH–$	76
7	9g	$4–(CH_3)_2N–C_6H_4–$	$CH_3–$	72
8	9h	$4–(CH_3)_2N–C_6H_4–$	$(CH_3)_2CH–$	77
9	9i	$4–Cl–C_6H_4–$	$CH_3–$	82
10	9j	$4–Cl–C_6H_4–$	$(CH_3)_2CH–$	78
11	9k	$4–NO_2–C_6H_4–$	$CH_3–$	68
12	9l	$4–NO_2–C_6H_4–$	$(CH_3)_2CH–$	70
13	9m	$4–CH_3–C_6H_4–$	$CH_3–$	76
14	9n	$4–CH_3–C_6H_4–$	$(CH_3)_2CH–$	74

Figure 1. List of synthesised thiazolidin-4-one compounds (9a-n)



The structure of synthesised thiazolidin-4-onederivatives **(9a-n)**was elucidated with the help of FT-IR, ^{1}H NMR, ^{13}C NMR and mass data as illustrated for compound **9a**. In the FT-IR spectrum, the sharp band appeared at 1680 cm^{-1} corresponds to the C=O stretching vibration and the band appeared at 3215 cm^{-1} corresponds to the NHstretching vibration. Also the band appeared at 1517 cm^{-1} corresponds to the NO_2stretching of the product **9a**. In the ^{1}H NMR, the signal at δ: 9.20 ppm for one proton confirmed the presence of NH group and the peaks in the range of δ: 7.02-8.12 ppm confirms 14 aromatic protons. The signal at δ: 1.12 ppm for three protons confirmed the presence of –CH_3

group. In the ^{13}C NMR, the peaks at δ: 173.6 and 33.4 confirmed the presence of C=O and CH_2 group of thiazole-4-one ring respectively. The peak at δ: 16.3 correspond to $-CH_3$ group. A distinguishing peak observed at *m/z*: 500 in the mass spectrum for $[M+H]^+$ ion further conforms the product **9a**.

2.6. *Characterization of synthesized compounds*

2.6.1. 2-((3-Methyl-1-(4-nitrophenyl)-2,6-diphenylpiperidin-4-ylidene)hydrazono)thiazolidin-4-one (**9a**)

Yield 78%; m.p.102-104 ˚C; FT-IR(KBr)(cm^{-1}): 3215 (N–H), 3045 (Ar.C–H), 2930 (C–H), 1680 (C=O), 1619 (C=N), 1532 (C=C), 1517 (–NO_2), 1075 (N–N); ^1H NMR (300 MHz, $CDCl_3$): δ: 9.20 (s, 1H, NH), 8.12 (d, 2H, *J* = 7.2 Hz, Ar-H), 7.24-7.35 (m, 10H, Ar-H), 7.02 (d, 2H, *J* = 7.0 Hz, Ar-H), 4.05 (m, 2H, 2CH), 3.62 (s, 2H, CH_2), 2.52 (dd, 1H, *J* = 7.0, 10.2 Hz, CH_2), 2.46 (dd, 1H, *J* =7.8, 10.2 Hz, CH_2), 2.35 (m, 1H, CH), 1.12 (d, 3H, *J* = 7.2 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCI_3$): δ: 173.6, 162.6, 158.3, 151.2, 142.5, 141.3, 137.7, 128.8, 128.5, 128.2, 127.5, 127.2, 126.0, 125.8, 112.3, 68.7, 68.0, 37.2, 33.4, 24.9, 16.3; MS *m/z*: 499 $[M]^+$, 500 $[M+H]^+$; Anal. Calcd. For $C_{27}H_{25}N_5O_3S$. C64.91, H5.04, N14.02. Found C64.88, H5.06, N14.16.

2.6.2. 2-((3-Isopropyl-1-(4-nitrophenyl)-2,6-diphenylpiperidin-4-ylidene)hydrazono)thiazoli-din-4-one (**9b**)

Yield 74%; m.p.127-129 ˚C; FT-IR(KBr)(cm^{-1}): 3218 (N–H), 3040 (Ar.C–H), 2928 (C–H), 1678 (C=O), 1621 (C=N), 1536 (C=C), 1516 (–NO_2), 1072 (N–N); ^1H NMR (300 MHz, $CDCl_3$): δ: 9.22 (s, 1H, NH), 8.14 (d, 2H, *J* = 7.1 Hz, Ar-H), 7.25-7.43 (m, 10H, Ar-H), 7.04 (d, 2H, *J* = 7.2 Hz, Ar-H), 4.02 (m, 2H, 2CH), 3.60 (s, 2H, CH_2), 2.54 (dd, 1H, *J* = 7.2, 10.1 Hz, CH_2), 2.48 (dd, 1H, *J* =7.9, 10.1 Hz, CH_2), 1.85 (m, 1H, CH), 1.72 (s, 1H, CH),1.01 (d, 6H, $2CH_3$);^{13}C NMR (75 MHz, $CDCI_3$): δ: 173.6, 162.5, 158.5, 151.3, 142.4, 141.4, 137.9, 128.8, 128.4, 128.1, 127.4, 127.1, 126.4, 125.6, 112.7, 68.9, 68.1, 33.4, 27.3, 25.1, 24.3, 21.6; MS *m/z*: 527 $[M]^+$, 528 $[M+H]^+$; Anal. Calcd. For $C_{29}H_{29}N_5O_3S$. C66.01, H5.54, N13.28. Found C65.97, H5.58, N13.23.

2.6.3.2-((2,6-Bis(4-hydroxyphenyl)-3-methyl-1-(4-nitrophenyl)piperidin-4-ylidene)hydrazo-no)thiazolidin-4-one (**9c**)

Yield 72%; m.p.114-116 ˚C; FT-IR(KBr)(cm^{-1}): 3220 (N–H), 3041 (Ar.C–H), 2935 (C–H), 1677 (C=O), 1622 (C=N), 1536 (C=C), 1512 (–NO_2), 1070 (N–N); ^1H NMR (300 MHz, $CDCl_3$): δ: 9.22 (s, 1H, NH), 8.14 (d, 2H, *J* = 7.0 Hz, Ar-H), 7.16 (d, 4H, *J* = 7.6 Hz, Ar-H), 7.04 (d, 2H, *J* = 7.2 Hz, Ar-H), 6.74 (d, 4H, *J* = 7.8 Hz, Ar-H), 5.38 (s, 2H, OH), 4.11 (m, 2H, 2CH), 3.66 (s, 2H, CH_2), 2.54 (dd, 1H, *J* = 7.1, 10.0 Hz, CH_2), 2.48 (dd, 1H, *J* =7.8, 10.1 Hz, CH_2), 2.34 (m, 1H, CH), 1.18 (d, 3H, *J* = 7.4 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCI_3$): δ: 173.5, 162.4, 158.5, 156.9, 155.8, 151.2, 137.5,134.5, 134.1, 128.3, 128.0, 125.9,116.1, 115.7, 112.3, 68.8, 68.1, 37.3, 33.5, 24.7, 16.1; MS *m/z*: 531 $[M]^+$, 532 $[M+H]^+$; Anal. Calcd. For $C_{27}H_{25}N_5O_5S$. C61.00, H4.74, N13.17. Found C60.97, H4.68, N13.19.

2.6.4.2-((2,6-Bis(4-hydroxyphenyl)-3-isopropyl-1-(4-nitrophenyl)piperidin-4-ylidene)hydra-zono)thiazolidin-4-one (**9d**)

Yield 70%; m.p.153-155 ˚C; FT-IR(KBr)(cm^{-1}): 3225 (N–H), 3055 (Ar.C–H), 2926 (C–H), 1682 (C=O), 1622 (C=N), 1537 (C=C), 1515 (–NO_2), 1055 (N–N); ^1H NMR (300 MHz, $CDCl_3$): δ: 9.24 (s, 1H, NH), 8.08 (d, 2H, *J* = 7.0 Hz, Ar-H), 7.18 (d, 4H, *J* = 7.4 Hz, Ar-H), 7.02 (d, 2H, *J* = 7.4 Hz, Ar-H), 6.75 (d, 4H, *J* = 7.5 Hz, Ar-H), 5.35 (s, 2H, OH), 4.06 (m, 2H, 2CH), 3.62 (s, 2H, CH_2), 2.55 (dd, 1H, *J* = 7.2, 10.3 Hz, CH_2), 2.42 (dd, 1H, *J* =7.8, 10.2 Hz, CH_2), 1.84 (m, 1H, CH), 1.75 (s, 1H, CH),1.02 (d, 6H, $2CH_3$); ^{13}C NMR (75 MHz, $CDCI_3$): δ: 173.5, 162.4, 158.5, 156.7, 155.8, 151.2, 137.5,134.8, 134.2, 128.4, 128.0, 125.8,116.2, 115.7, 112.3, 68.9, 68.1, 33.4, 27.3, 25.1, 24.3, 21.6;MS *m/z*: 559 $[M]^+$, 560 $[M+H]^+$; Anal. Calcd. For $C_{29}H_{29}N_5O_5S$. C62.24, H5.22, N12.51. Found C62.29, H5.24, N12.50.

*2.6.5.2-((2,6-Bis(4-methoxyphenyl)-3-methyl-1-(4-nitrophenyl)piperidin-4-ylidene)hydrazo-no)thiazolidin-4-one(***9e**)

Yield 72%; m.p.132-133 ˚C; FT-IR(KBr)(cm^{-1}): 3223 (N–H), 3039 (Ar.C–H), 2935 (C–H), 1676 (C=O), 1624 (C=N), 1533 (C=C), 1512 (–NO_2), 1256 (C–O), 1065 (N–N); ^1H NMR (300 MHz, $CDCl_3$): δ: 9.20 (s, 1H, NH), 8.16 (d, 2H, *J* = 7.2 Hz, Ar-H), 7.18 (d, 4H, *J* = 7.4 Hz, Ar-H), 7.02 (d, 2H, *J* = 7.8 Hz, Ar-H), 6.96 (d, 4H, *J* = 7.6 Hz, Ar-H), 4.15 (m, 2H, 2CH), 3.68 (s, 2H, CH_2), 3.34 (s, 6H, OCH_3), 2.52 (dd, 1H, *J* = 7.1, 10.0 Hz, CH_2), 2.48 (dd, 1H, *J* =7.9, 10.0 Hz, CH_2), 2.34 (m, 1H, CH), 1.18 (d, 3H, *J* = 7.3 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCI_3$): δ: 173.6, 162.7, 158.3, 156.6, 155.9, 151.4, 137.7,134.6, 134.1, 128.4, 128.1, 126.0,116.2, 115.6, 112.4, 68.5, 68.0, 56.6, 37.4, 33.7, 24.3, 16.4; MS *m/z*: 559 $[M]^+$, 560 $[M+H]^+$; Anal. Calcd. For $C_{29}H_{29}N_5O_5S$. C62.24, H5.22, N12.51. Found C62.21, H5.26, N12.55.

2.6.6.2-((3-Isopropyl-2,6-bis(4-methoxyphenyl)-1-(4-nitrophenyl)piperidin-4-ylidene)hydr-azono)thiazolidin-4-one (**9f**)

Yield 76%; m.p.126-128 ˚C; FT-IR(KBr)(cm^{-1}): 3225 (N–H), 3044 (Ar.C–H), 1682 (C=O), 2929 (C–H), 1628 (C=N), 1535 (C=C), 1515 (–NO_2), 1260 (C–O), 1072 (N–N); ^1H NMR (300 MHz, $CDCl_3$): δ: 9.22 (s, 1H, NH), 8.14 (d, 2H, *J* = 7.4 Hz, Ar-H), 7.16 (d, 4H, *J* = 7.2 Hz, Ar-H), 7.04 (d, 2H, *J* = 7.6 Hz, Ar-H), 6.92 (d, 4H, *J* = 7.5 Hz, Ar-H), 4.14 (m, 2H, 2CH), 3.65 (s, 2H, CH_2), 3.36 (s, 6H, OCH_3), 2.56 (dd, 1H, *J* = 7.0, 10.2 Hz, CH_2), 2.40 (dd,

1H, *J* =7.7, 10.2 Hz, CH_2), 1.86 (m, 1H, CH), 1.74 (s, 1H, CH), 1.03 (d, 6H, $2CH_3$);^{13}C NMR (75 MHz, $CDCl_3$): *δ*: 173.7, 162.4, 158.2, 156.7, 155.5, 151.5, 137.8,134.3, 134.0, 128.5, 128.1, 125.7,116.0, 115.9, 112.6, 68.6, 68.0, 56.6, 33.7, 27.5, 25.1, 24.2, 21.9;MS *m/z*: 587 $[M]^+$, 588 $[M+H]^+$; Anal. Calcd. For $C_{31}H_{33}N_5O_5S$. C63.36, H5.66, N11.92. Found C63.32, H5.64, N11.98.

2.6.7. 2-((2,6-Bis(4-(dimethylamino)phenyl)-3-methyl-1-(4-nitrophenyl)piperidin-4-ylidene)hydrazono)thiazolidin-4-one (**9g**)
Yield 72%; m.p.135-136 ˚C; FT-IR(KBr)(cm^{-1}): 3220 (N–H), 3033 (Ar.C–H), 2925 (C–H), 1686 (C=O), 1622 (C=N), 1532 (C=C), 1517 (–NO_2), 1075 (N–N); ^{1}H NMR (300 MHz, $CDCl_3$): *δ*: 9.22 (s, 1H, NH), 8.14 (d, 2H, *J* = 7.1 Hz, Ar-H), 7.10 (d, 4H, *J* = 7.0 Hz, Ar-H), 7.04 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.70 (d, 4H, *J* = 7.5 Hz, Ar-H), 4.16 (m, 2H, 2CH), 3.68 (s, 2H, CH_2), 3.02 (s, 12H, NCH_3), 2.52 (dd, 1H, *J* = 7.2, 10.0 Hz, CH_2), 2.43 (dd, 1H, *J* =7.8, 10.1 Hz, CH_2), 2.35 (m, 1H, CH), 1.16 (d, 3H, *J* = 7.4 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): *δ*: 173.5, 162.4, 158.5, 151.2, 149.5, 148.0, 137.5,131.7, 131.1, 130.4, 130.1, 125.8,113.0, 112.7, 112.3, 68.9, 68.1, 42.4, 37.3, 33.4, 24.7, 16.2; MS *m/z*: 585 $[M]^+$, 586 $[M+H]^+$; Anal. Calcd. For $C_{31}H_{35}N_7O_3S$. C63.57, H6.02, N16.74. Found C63.52, H6.00, N16.79.

2.6.8.2-((2,6-Bis(4-(dimethylamino)phenyl)-3-isopropyl-1-(4-nitrophenyl)piperidin-4-ylide-ne)hydrazono) thiazolidin-4-one (**9h**)
Yield 77%; m.p.148-149 ˚C; FT-IR(KBr)(cm^{-1}): 3237 (N–H), 3052 (Ar.C–H), 2921 (C–H), 1668 (C=O), 1609 (C=N), 1533 (C=C), 1515 (–NO_2), 1081 (N–N); ^{1}H NMR (300 MHz, $CDCl_3$): *δ*: 9.20 (s, 1H, NH), 8.18 (d, 2H, *J* = 7.2 Hz, Ar-H), 7.14 (d, 4H, *J* = 7.4 Hz, Ar-H), 7.06 (d, 2H, *J* = 7.6 Hz, Ar-H), 6.72 (d, 4H, *J* = 7.6 Hz, Ar-H), 4.18 (m, 2H, 2CH), 3.63 (s, 2H, CH_2), 3.04 (s, 12H, NCH_3), 2.55 (dd, 1H, *J* = 7.3, 10.2 Hz, CH_2), 2.42 (dd, 1H, *J* =7.8, 10.2 Hz, CH_2), 1.84 (m, 1H, CH), 1.78 (s, 1H, CH), 1.01 (d, 6H, $2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): *δ*: 173.3, 162.6, 158.2, 151.0, 149.4, 148.1, 137.5,131.6, 131.0, 130.4, 130.0, 125.8,113.1, 112.7, 112.1, 68.8, 68.0, 42.1, 33.5, 27.7, 25.2, 24.7, 21.5;MS *m/z*: 613 $[M]^+$, 614 $[M+H]^+$; Anal. Calcd. For $C_{33}H_{39}N_7O_3S$. C64.58, H6.40, N15.97. Found C64.62, H6.45, N15.92.

2.6.9. 2-((2,6-Bis(4-chlorophenyl)-3-methyl-1-(4-nitrophenyl)piperidin-4-ylidene)hydrazono)thiazolidin-4-one (**9i**)
Yield 82%; m.p.107-109 ˚C; FT-IR(KBr)(cm^{-1}): 3233 (N–H), 3055 (Ar.C–H), 2922 (C–H), 1681 (C=O), 1632 (C=N), 1534 (C=C), 1509 (–NO_2), 1071 (N–N); ^{1}H NMR (300 MHz, $CDCl_3$): *δ*: 9.20 (s, 1H, NH), 8.16 (d, 2H, *J* = 7.2 Hz, Ar-H), 7.46 (d, 4H, *J* = 7.1 Hz, Ar-H), 7.42 (d, 4H, *J* = 7.4 Hz, Ar-H), 7.06 (d, 2H, *J* = 7.5 Hz, Ar-H), 4.18 (m, 2H, 2CH), 3.64 (s, 2H, CH_2), 2.54 (dd, 1H, *J* = 7.2, 10.1 Hz, CH_2), 2.44 (dd, 1H, *J* =7.8, 10.1 Hz, CH_2), 2.34 (m, 1H, CH), 1.18 (d, 3H, *J* = 7.2 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): *δ*: 173.3, 162.4, 158.3, 151.6, 140.3, 139.7, 137.4,132.0, 131.2, 128.9, 128.4, 128.2, 127.9,125.8,112.4, 68.5, 68.0, 37.3, 33.6, 24.5, 16.3; MS *m/z*: 567 $[M]^+$, 569 $[M+2]^+$; Anal. Calcd. For $C_{27}H_{23}Cl_2N_5O_3S$. C57.05, H4.08, N12.32. Found C57.09, H4.01, N12.35.

2.6.10.2-((2,6-Bis(4-chlorophenyl)-3-isopropyl-1-(4-nitrophenyl)piperidin-4-ylidene)hydra-zono)thiazolidin-4-one **9j**)
Yield 78%; m.p.98-99 ˚C; FT-IR(KBr)(cm^{-1}): 3245 (N–H), 3039 (Ar.C–H), 2941 (C–H), 1687 (C=O), 1623 (C=N), 1541 (C=C), 1511 (–NO_2), 1079 (N–N); ^{1}H NMR (300 MHz, $CDCl_3$): *δ*: 9.18 (s, 1H, NH), 8.21 (d, 2H, *J* = 7.2 Hz, Ar-H), 7.45 (d, 4H, *J* = 7.1 Hz, Ar-H), 7.40 (d, 4H, *J* = 7.2 Hz, Ar-H), 7.08 (d, 2H, *J* = 7.4 Hz, Ar-H), 4.16 (m, 2H, 2CH), 3.65 (s, 2H, CH_2), 2.54 (dd, 1H, *J* = 7.0, 10.2 Hz, CH_2), 2.44 (dd, 1H, *J* =7.8, 10.2 Hz, CH_2), 1.85 (m, 1H, CH), 1.78 (s, 1H, CH), 1.02 (d, 6H, $2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): *δ*: 173.5, 162.6, 158.7, 151.2, 140.0, 139.7, 137.7,132.6, 131.5, 128.9, 128.5, 128.1, 127.5,125.8,112.4, 68.8, 68.1, 33.5, 27.3, 25.2, 24.3, 21.7;MS *m/z*: 595 $[M]^+$, 597 $[M+2]^+$; Anal. Calcd. For $C_{29}H_{27}Cl_2N_5O_3S$. C58.39, H4.56, N11.74. Found C58.30, H4.52, N11.79.

2.6.11. 2-((3-Methyl-1,2,6-tris(4-nitrophenyl)piperidin-4-ylidene)hydrazono)thiazolidin-4-one (**9k**)
Yield 68%; m.p.144-146 ˚C; FT-IR(KBr)(cm^{-1}): 3217 (N–H), 3045 (Ar.C–H), 2933 (C–H), 1680 (C=O), 1616 (C=N), 1537 (C=C), 1512 (–NO_2), 1069 (N–N); ^{1}H NMR (300 MHz, $CDCl_3$): *δ*: 9.26 (s, 1H, NH), 8.28 (d, 4H, *J* = 7.0 Hz, Ar-H), 8.15 (d, 2H, *J* = 7.2 Hz, Ar-H), 7.52 (d, 4H, *J* = 7.4 Hz, Ar-H), 7.06 (d, 2H, *J* = 7.6 Hz, Ar-H), 4.15 (m, 2H, 2CH), 3.62 (s, 2H, CH_2), 2.55 (dd, 1H, *J* = 7.0, 10.2 Hz, CH_2), 2.45 (dd, 1H, *J* =7.8, 10.1 Hz, CH_2), 2.35 (m, 1H, CH), 1.16 (d, 3H, *J* = 7.4 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): *δ*: 173.2, 162.4, 158.5, 151.1, 148.1, 147.6, 146.2, 145.7, 137.5,125.2,124.7, 124.4, 124.0, 123.7, 112.3, 68.6, 68.1, 37.5, 33.5, 24.7, 16.0; MS *m/z*: 589 $[M]^+$, 590 $[M+H]^+$; Anal. Calcd. For $C_{27}H_{23}N_7O_7S$. C55.00, H3.93, N16.63. Found C54.97, H3.91, N16.69.

2.6.12. 2-((3-Isopropyl-1,2,6-tris(4-nitrophenyl)piperidin-4-ylidene)hydrazono)thiazolidin-4-one (**9l**)
Yield 70%; m.p.131-132 ˚C; FT-IR(KBr)(cm^{-1}): 3219 (N–H), 3043 (Ar.C–H), 2931 (C–H), 1681 (C=O), 1613 (C=N), 1528 (C=C), 1517 (–NO_2), 1072 (N–N); ^{1}H NMR (300 MHz, $CDCl_3$): *δ*: 9.24 (s, 1H, NH), 8.24 (d, 4H, *J* = 7.1 Hz, Ar-H), 8.14 (d, 2H, *J* = 7.0 Hz, Ar-H), 7.54 (d, 4H, *J* = 7.4 Hz, Ar-H), 7.03 (d, 2H, *J* = 7.4 Hz, Ar-H), 4.14 (m, 2H, 2CH), 3.64 (s, 2H, CH_2), 2.55 (dd, 1H, *J* = 7.5, 10.1 Hz, CH_2), 2.44 (dd, 1H, *J* =7.9, 10.1 Hz, CH_2), 1.86

(m, 1H, CH), 1.75 (s, 1H, CH), 1.01 (d, 6H, $2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): *δ*: 173.4, 162.2, 158.7, 151.4, 148.0, 147.7, 146.1, 145.1, 137.3,125.9,124.6, 124.3, 124.0, 123.6, 112.2, 68.9, 68.2, 33.4, 27.4, 25.2, 24.1, 21.3;MS *m/z*: 617 $[M]^+$, 618 $[M+H]^+$; Anal. Calcd. For $C_{29}H_{27}N_7O_7S$. C56.39, H4.41, N15.87. Found C56.44, H4.43, N15.83.

2.6.13. 2-((3-Methyl-1-(4-nitrophenyl)-2,6-di-p-tolylpiperidin-4-ylidene)hydrazono)thiazolidin-4-one (**9m**)
Yield 76%; m.p.123-124 ˚C; FT-IR(KBr)(cm^{-1}): 3223 (N–H), 3053 (Ar.C–H), 2932 (C–H), 1678 (C=O), 1616 (C=N), 1533 (C=C), 1511 (–NO_2), 1076 (N–N); ^1H NMR (300 MHz, $CDCl_3$): *δ*: 9.22 (s, 1H, NH), 8.15 (d, 2H, *J* = 7.1 Hz, Ar-H), 7.22 (d, 4H, *J* = 7.5 Hz, Ar-H), 7.14 (d, 4H, *J* = 7.5 Hz, Ar-H), 7.04 (d, 2H, *J* = 7.6 Hz, Ar-H), 4.14 (m, 2H, 2CH), 3.66 (s, 2H, CH_2), 3.32 (s, 6H, OCH_3), 2.54 (dd, 1H, *J* = 7.2, 10.4 Hz, CH_2), 2.46 (dd, 1H, *J* =7.8, 10.3 Hz, CH_2), 2.35 (m, 1H, CH), 2.17 (s, 6H, CH_3), 1.15 (d, 3H, *J* = 7.3 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): *δ*: 173.5, 162.4, 158.5, 151.2, 139.0, 138.6, 137.5,136.7, 135.7, 129.1, 128.8, 126.5, 126.3, 125.8,112.3, 68.8, 68.1, 37.3, 33.5, 24.7, 22.2, 16.1; MS *m/z*: 527 $[M]^+$, 528 $[M+H]^+$; Anal. Calcd. For $C_{29}H_{29}N_5O_3S$. C66.01, H5.54, N13.27. Found C66.07, H5.56, N13.23.

2.6.14. 2-((3-Isopropyl-1-(4-nitrophenyl)-2,6-di-p-tolylpiperidin-4-ylidene)hydrazono)thiazolidin-4-one (**9n**)
Yield 74%; m.p.116-118 ˚C; FT-IR(KBr)(cm^{-1}): 3223 (N–H), 3043 (Ar.C–H), 2924 (C–H), 1687 (C=O), 1622 (C=N), 1534 (C=C), 1513 (–NO_2), 1078 (N–N); ^1H NMR (300 MHz, $CDCl_3$): *δ*: 9.25 (s, 1H, NH), 8.16 (d, 2H, *J* = 7.3 Hz, Ar-H), 7.24 (d, 4H, *J* = 7.4 Hz, Ar-H),7.15 (d, 4H, *J* = 7.4 Hz, Ar-H), 7.05 (d, 2H, *J* = 7.3 Hz, Ar-H), 4.15 (m, 2H, 2CH), 3.68 (s, 2H, CH_2), 3.34 (s, 6H, OCH_3), 2.56 (dd, 1H, *J* = 7.0, 10.2 Hz, CH_2), 2.46 (dd, 1H, *J* =7.8, 10.2 Hz, CH_2), 2.18 (s, 6H, CH_3), 1.88 (m, 1H, CH), 1.76 (s, 1H, CH), 1.02 (d, 6H, $2CH_3$);*δ*: 173.4, 162.2, 158.5, 151.1, 139.0, 138.4, 137.5,136.8, 135.7, 129.2, 128.8, 126.6, 126.0, 125.8,112.2, 68.8, 68.1, 33.5, 27.3, 25.2, 24.3, 22.5, 21.7;MS *m/z*: 555 $[M]^+$, 556 $[M+H]^+$; Anal. Calcd. For $C_{31}H_{33}N_5O_3S$. C67.00, H5.99, N12.60. Found C67.06, H6.01, N12.69.

2.7. *Determination of antimicrobial activity*
The following bacteria were used for the experiments; *Salmonella typhimurium, Klebsiella pneumoniae, Proteus vulgaris, Shigella flexneri, Micrococcus luteus, Enterobacter aerogenes, Staphylococcus aureus* and *Staphylococcus aureus (MRSA- methicillin resistant),* and two fungi namely *Candida albicans* and *Malassesia pachydermatis.* All cultures were obtained from the Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India. Streptomycin (Sigma) was used as a positive control against bacteria and ketoconazole (Himedia, Mumbai) was used as a positive control against fungi. Bacterial inoculums were prepared by growing cells in Mueller Hinton Broth (MHA) (Himedia) for 24 h at 37 ˚C. These cell suspensions were diluted with sterile MHB to provide initial cell counts of about 104 CFU/mL. The filamentous fungi were grown on sabouraud dextrose agar (SDA) slants at 28 ˚C for 10 days and the spores were collected using sterile doubled distilled water and homogenized. Yeast was grown on sabouraud dextrose broth (SDB)at 28 ˚C for 48 h.

Minimum inhibitory concentration studies of isolated compounds were performed according to the standard reference method for bacteria, for filamentous fungi and yeasts. The required concentrations (1000 µg/mL, 500 µg/mL, 250 µg/mL,125 µg/mL, 62.5 µg/mL, 31.25 µg/mLand 15.62 µg/mL) of the compound was dissolved in DMSO (2%), and diluted to give serial two-fold dilutions that were added to each medium in 96 well plates. An inoculum of 100 mL from each well was inoculated. The anti-fungal agents ketoconazole for fungi and streptomycin for bacteria were included in the assays as positive controls. For fungi, the plates were incubated for 48-72 hat 28 ˚C and for bacteria the plates were incubated for 24 h at 37 ˚C.The MIC for fungi was defined as the lowest extract concentration, showing no visible fungal growth after incubation time. 5 mL of tested broth was placed on the sterile MHA plates for bacteria and incubated at respective temperature. The MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate.

2.8.*Molecular docking studies*
Molecular docking studies have been done using the AutoDock Tools (ADT) version 1.5.6 and AutoDock version 4.2.5.1 docking program. The DNA topoisomerase IV structure was obtained from the Protein Data Bank (PDB ID: 4EMV). The co-crystallized ligand in the DNA topoisomerase IV structure was removed. Then, the polar hydrogen atoms were added, lower occupancy residue structures were deleted, and any incomplete side chains were replaced using the ADT. Further, ADT was used to remove crystal water, Gasteiger charges were added to each atom, and merged the non-polar hydrogen atoms to the protein structure. The distance between donor and acceptor atoms that form a hydrogen bond was defined as 1.9 Å with a tolerance of 0.5 Å, and the acceptor– hydrogen–donor angle was not less than 120˚. The structures were then saved in PDBQT file format, for further studies in ADT. Ligand 2D structures were drawn using ChemDraw Ultra 7.0 (ChemOffice 2002). Chem3D Ultra 7.0 was used to convert 2D structure into 3D and the energy minimized using semi-empirical AM1 method. Minimize energy to minimum RMS gradient of 0.100 was set in each iteration. All structures were saved as .pdb file format for input to ADT. All the

ligand structures were then saved in PDBQT file format, to carry out docking in ADT.A grid box with dimension of 40 × 40 × 40 $Å^3$ with 0.375 Å spacing and centred on 14.860, 29.555, 6.941 was created around the binding site of the lignad on DNA topoisomerase IV using ADT. The centre of the box was set at ligand centre and grid energy calculations were carried out. For the AutoDock docking calculation, default parameters were used and 10 docked conformations were generated for each compound. The energy calculations were done using genetic algorithms. The outputs were exported to PyMOL for visual inspection of the binding modes and interactions of the compounds with amino acid residues in the active sites.

RESULTS AND DISCUSSION

3.1. *Chemistry*

Novel thiazolidin-4-one derivatives have been prepared by four steps. First, 4-piperidinone derivatives (**3a-n**) have been prepared by Mannich-enamineand then1-(4-nitrophenyl)-4-piperidone derivatives (**5a-n**) have been prepared by copper catalyzed Ullmann reaction between 4-piperidone derivatives (**3a-n**) and 4-nitroiodobenzene (**4**). The thiosemicarbazones (**7a-n**) have been prepared by condensation of appropriate 1-(4-nitrophenyl)-4-piperidone (**5a-n**) compounds with thiosemicarbazide (**6**). Finally, thiosemicarbazone derivatives (**7a-n**) reacted with ethylchloroacetate (**8**) and KOHin acetic acid to yield thiazolidin-4-one derivatives (**9a-n**). These thiazoldin-4-one compounds have been analysed by FT-IR, ^{1}H NMR, ^{13}C NMR, Mass and elemental analysis.

3.2.*Antimicrobial activity*

All the synthesised thiazolidin-4-one compounds were assessed for their *in vitro* antimicrobial activity against eight bacteria namely *Salmonella typhimurium, Klebsiella pneumoniae, Proteus vulgaris, Shigella flexneri, Micrococcus luteus, Enterobacter aerogenes, Staphylococcus aureus* and *Staphylococcus aureus (MRSA- methicillin resistant)*, and two fungi namely *Candida albicans* and *Malassesia pachydermatis*. The antimicrobial potency of the thiazolidin-4-one compounds was compared with standard drugs streptomycin and ketoconazole for antibacterial and antifungal studies respectively. The minimum inhibitory concentration (MIC) values were calculated as summarized in **Table 2**.

Table 2: Minimum inhibitory concentration (µg/mL) of synthesized compounds against tested microbes

S	Gram negative bacteria				Gram positive bacteria				Fungi	
	S. typhimurium	*K. pneumoniae*	*P. vulgaris*	*S. flexneri*	*M. luteus*	*E. aerogenes*	*S. aureus*	*(MRSA)S. aureus*	*C. albicans*	*M. pachydermatis*
9a	62.5	15.6	125	31.25	62.5	125	31.25	62.5	125	125
9b	250	250	250	62.5	125	250	125	250	500	125
9c	31.25	31.25	62.5	15.6	15.6	125	31.25	250	125	125
9d	125	62.5	250	62.5	125	62.5	62.5	125	125	250
9e	500	250	500	125	250	125	500	250	500	250
9f	125	250	250	62.5	125	250	125	250	125	125
9g	-	250	250	500	500	250	500	500	500	125
9h	500	250	500	500	250	500	500	500	250	250
9i	125	62.5	62.5	31.25	125	31.25	62.5	250	125	250
9j	125	125	250	125	500	125	125	125	250	500
9k	500	500	-	500	250	500	500	-	500	250
9l	250	125	250	500	-	250	125	500	-	500
9m	125	125	125	250	250	250	250	250	500	500
9n	250	250	125	250	125	250	62.5	125	250	125
C	30	6.25	-	6.25	6.25	25	6.25	6.25	25	-

The results obtained from the antimicrobial study of synthesised thiazolidin-4-one compounds revealed that all the tested compounds exhibited significant antimicrobial activity against tested microbes. Some of the thiazolidn-4-one compounds showed excellent activity against all tested bacterial and fungal strains. Compound **9d** and **9i** exhibited good to excellent activity against tested bacteria, notably compound **9i** shows MIC value 31.25 µg/mL against *Shigella flexneri* and *Enterobacter aerogens.* Compound **9b**, **9f** and **9n** showed good to moderate activity against some of the tested bacteria. Among the entire compound tested, compounds **9a** and **9c** showed very excellent activity against bacterial strains. Compound **9a** shows MIC value 15.6 µg/mL against *Klebsiella pneumoniae* and 31.25 µg/mL against *Shigella flexneri* and *Staphylococcus aureus*. Interestingly, compound **9c** exhibits MIC value 15.6 µg/mL against *Shigella flexneri* and *Micrococcus luteus* and also 31.25 µg/mL against *Salmonella typhimurium, Klebsiella pneumoniae* and *Staphylococcus aureus*. Compound **9c** showed better activity against *Candida albicans* with MIC value 62.5 µg/mL.

3.3.*Molecular docking studies*

All the synthesised compounds were subjected to molecular docking studies using the AutoDock Tools (ADT) [30] version 1.5.6 and Auto Dock version 4.2.5.1 docking program to investigate the potential binding mode of

inhibitors. Molecular docking sturdies of the synthesized compounds was done using DNA topoisomerase IV as the receptor site. DNA topoisomerase IV receptor is involved in the transcription and replication process of the bacteria, which is required for maintaining the proper DNA topology [31].

In order to verify the reproducibility of the docking calculations, the bound ligand was extracted from the complexes and submitted for one-ligand run calculation. This reproduced top scoring conformation falling within root-mean-square deviation (rmsd) value of 1.82 Å from bound X-ray conformation for DNA topoisomerase IV receptor, suggesting this method is valid enough to be used for docking studies of other compounds **(Figure 2)**.

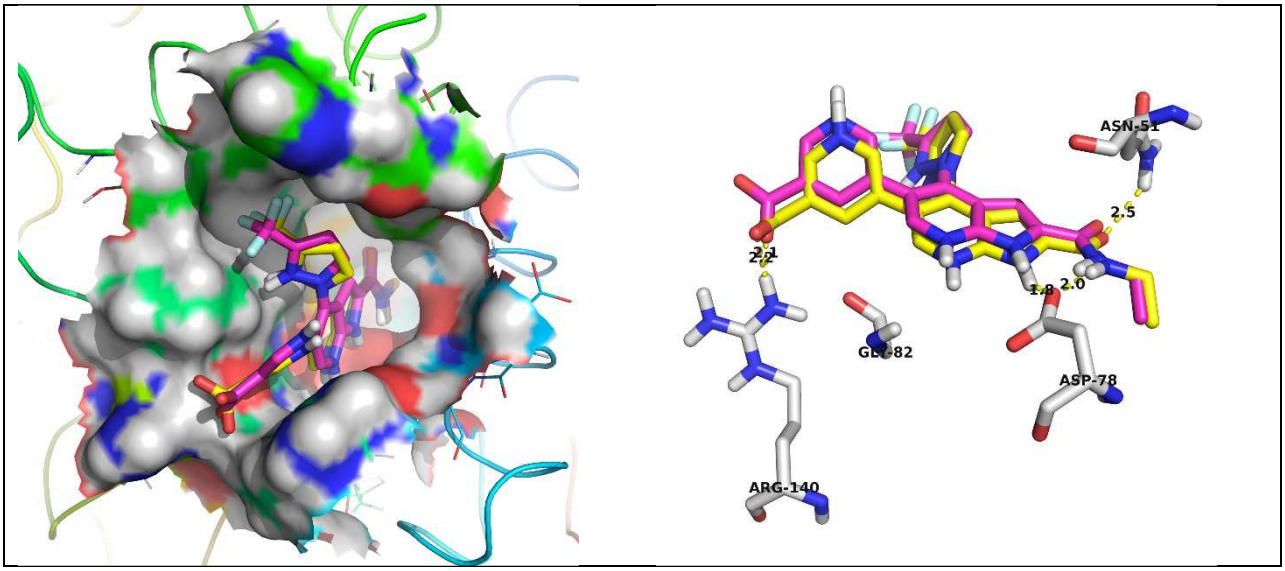


Figure 2. Method validation using crystallised and docked ligand with DNA topoisomerase IV receptor

Docking of different ligands to protein was performed using AutoDock, following the same protocol used in as that of validation study. Receptor and ligand docked conformations were analysed in terms of energy, hydrogen bonding, and hydrophobic interactions. Detailed analyses of the ligand-receptor interactions were carried out, and final coordinates of the ligand and receptor were saved. PyMol software was used for the visualization of the receptor with the ligand binding site [32]. The free energy of binding (FEB) of all the compounds were calculated from the docking score of best docked confirmation of ligand with the receptor active site (**Table 3**).

Table 3: The free energy of binding (FEB) of all compounds

Compound	Binding energy (kcal/mol)[a]
	DNA Topoisomerase IV
9a	-10.13
9b	-9.50
9c	-10.40
9d	-9.59
9e	-8.18
9f	-8.60
9g	-7.21
9h	-6.65
9i	-9.65
9j	-9.51
9k	-6.61
9l	-7.52
9m	-8.28
9n	-9.24
[a]*Calculated by Autodock*	

The molecular docking studies of the synthesized thiazolidin-4-one compounds with receptor DNA topoisomerase IV exhibited well established bonds with one or more amino acids in the receptor active pocket. Molecular docking studies revealed that all the synthesized molecules showed good to excellent binding energy toward the target receptor DNA topoisomerase IV, ranging from -6.61 to -10.40 kcal /mol. Interestingly, hydroxyl group substituted compounds exhibit higher binding energy values compare with other groups. Notably, compound **9c** shows the highest binding energy value -10.40 kcal /mol compare with all the docked compounds with the DNA topoisomerase

IV receptor. And also, NO_2 group substitution at C_4 position of phenyl ring decreased the binding energy. For example, 4-NO_2 phenyl substituted compound **9k** shows very low binding energy value -6.61 kcal /mol.

Among all the compounds docked, compound **9c**, which fits appropriately in the active site and forms five hydrogen bonds with three amino acid of the 4EMV protein. NO_2 group oxygen of **9c**interact with ARG140 and forms two hydrogen bonds with bond length of 1.9 and 2.6 Å. Also, phenolic OH of **9c** interact with GLY82 and ASP78 amino acids, and forms three hydrogen bonds with bond length of 1.8, 2.2 and 2.4 Å. Hence, compound **9c**exhibits very high binding energy value -10.40 kcal /mol. Synthesized compound **9c**also exhibits very good binding towards DNA topoisomerase IV receptor. NO_2 group oxygen of **9a** interacts with ARG140 and forms one hydrogen bond with bond length of 1.8 Å. Also, NH and C=O of thiazolidine ring interact with ASP78 amino acid, and forms two hydrogen bonds with bond length of 2.1 and 2.9 Å. Hence, compound **9a** exhibits very good binding energy value -10.13 kcal /mol. (**Figure 3**)

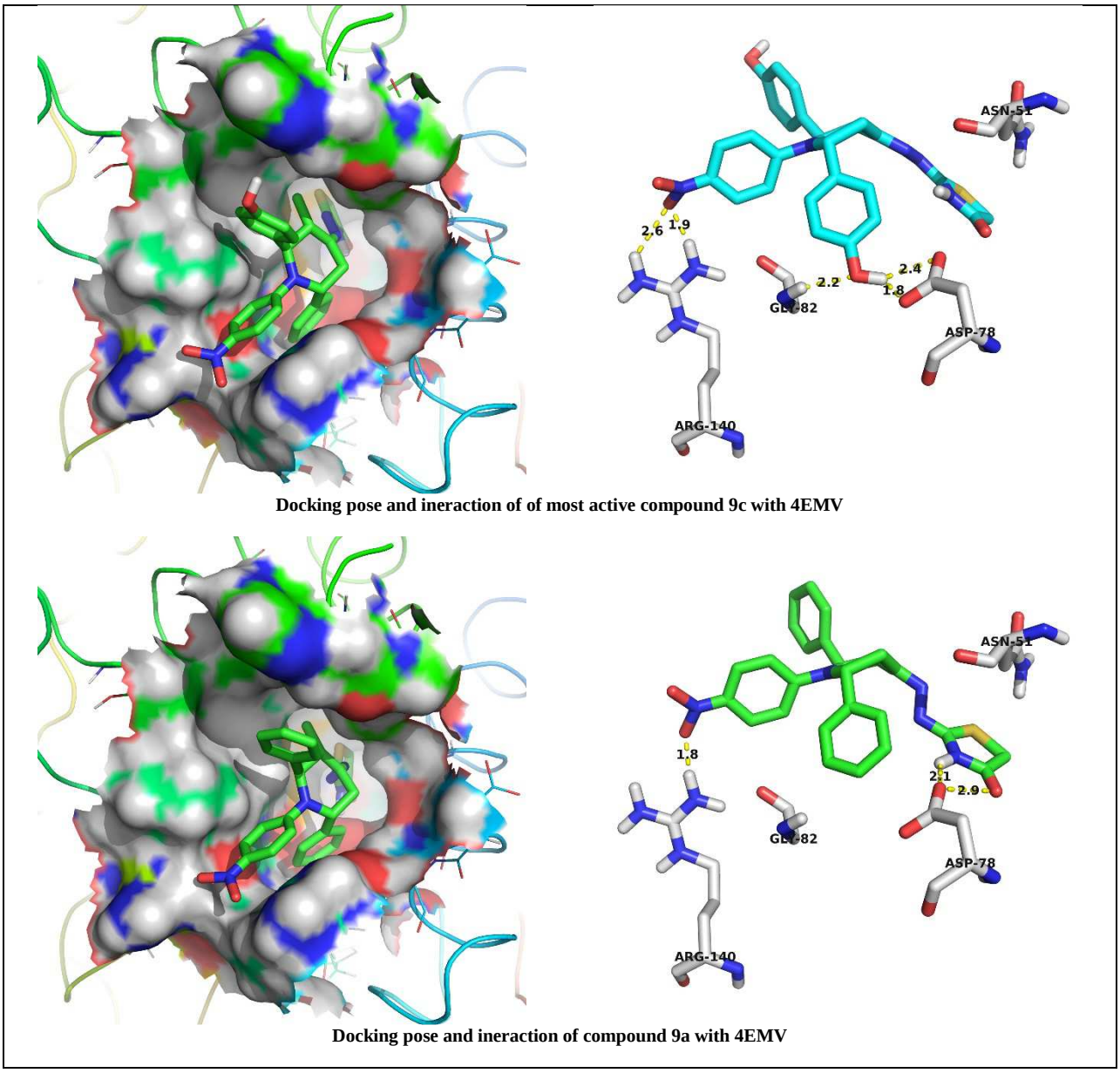


Figure 3. Docking pose of 9c and 9a with 4EMV receptor

CONCLUSION

Novel thiazolidin-4-one derivatives have been synthesized from Ullmann reaction followed by condensation of 1-nitrophenyl-4-piperidones with thiosemicarbazides and then cyclisation with ethylchloroacetate. Chemical structures were elucidated using by FT-IR, ^{1}H NMR, ^{13}C NMR, Mass and elemental analysis. The antimicrobial activity of

these novel thiazolidn-4-one compounds, have been done using eight bacteria and two fungi. Some of the compound shown significant activity against tested bacteria and fungi compare with standard drugs. Among all the compounds tested, **9c** exhibits very high antimicrobial activity with MIC value 15.6 µg/mL against *Shigella flexneri* and *Micrococcus luteus* and also 62.5 µg/mL against *Candida albicans*. Molecular docking studies has been done with DNA topoisomerase IV receptor to find out the possible binding interaction with active site amino acids.

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