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ORIGINAL ARTICLE

Synthesis and pharmacological studies of 1-(2-amino-1-(4-methoxyphenyl) ethyl) cyclohex anol analogs as potential microbial agents

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Abstract A novel series of Schiff bases **4a–n** was prepared from 2-hydrazinyl-*N*-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl)acetamide. Thiazolidinone **5a–n** derivatives were prepared from the reaction of Schiff base and thioglycolic acid. The structures of the synthesized compounds were assigned on the basis of elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data. All the compounds were screened against different strains of bacteria and fungi. These active compounds impelled us to study their antitubercular activity. Compounds **4b**, **5a**, **5b**, **5d**, **5e**, **5f**, **5k**, **5l** and **5n** emerged as promising antimicrobials. It was also observed that the promising antimicrobials have proved to be better antituberculars. Compound **5k** showed better antitubercular activity compared to Rifampicin.

1. Introduction

Tuberculosis (TB), a disease long considered substantially eradicated in the developed countries, has resurged dramatically in the last decades, establishing itself as one of the infectious diseases resulting in the highest number of human deaths worldwide (World Health Organization, 2006; Ballell et al., 2005; Janin, 2007). Likely, the first underlying reason for such escalation in number of infections with the TB pathogen, *Mycobacterium tuberculosis* (MT), is the deadly synergy with human immunodeficiency virus (HIV) indeed, an impressive number of HIV-infected individuals succumb to MT aggression (Morris et al., 1995). The second, important cause is the emergence of multi-drug resistant strains (MDR) of MT (Ballell et al., 2005; Janin, 2007; Morris et al., 1995; Telzak et al., 1995; Basso and Blanchard, 1998; Bastian and Colebuuders, 1999), together with the spread of severe opportunistic disseminated infections produced by Mycobacterium other than tuberculosis (MOTT), particularly *Mycobacterium avium* (Inderlied et al., 1993).

The standard therapy (Janin, 2007) for TB includes isoniazid, targeting both the NADH-dependent enoyl reductase

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(InhA) and the 3-oxoacyl ACP synthase (KasA) (Mdluli et al., 1998; Rozwarski et al., 1998) and Rifampicin, a well characterized inhibitor of the DNA-dependent RNA-polymerase (Cole, 1994). There are two basic approaches to develop a new drug for TB: (i) synthesis of analogs, modifications or derivatives of existing compounds for shortening and improving TB treatment and, (ii) searching novel structures, that the TB organism has never been presented with before, for the treatment of multi-drug resistant TB (Crabb, 2002).

To pursue this goal, our research efforts are directed to find new chemical classes of antitubercular active agents with different modes of action. Thiazolidin-4-ones are an important group of heterocyclic compounds, having valuable biological activities in the areas of medicine. Recently, antimicrobial and antimycobacterial activities (de Aquino et al., 2008; Verma and Saraf, 2008; Küçükgüzel et al., 2006) of this framework containing compounds were explored well whereas, their 2,3-disubstituted analogs have proved to be predominantly effective non-nucleoside HIV reverse transcriptase inhibitors (Barreca et al., 2001).

Thiazolidinone and its derivative are known to possess a variety of physiological properties; viz. analgesic, local and spiral anesthetics, antibacterial, (Mistry and Desai, 2004; Sayyed et al., 2006) anti-inflammatory, (Yadav et al., 2005) antitubercular, (Patel et al., 2006) anticancer, anti HIV (Bhatt et al., 1994) and fungicidal (Hui-Ling et al., 2000) activities. After an extensive literature search, it was observed that, till date enough effort has not been made to combine these two moieties as a single molecular scaffold and to identify new candidates that may be of value in designing new, potent, selective and less toxic antitubercular and antimicrobial agents. In view of this data, we reported the synthesis of new thiazolidinone and Schiff base which possessed a wide variety of biological activities encouraging antitubercular activity against *M. tuberculosis* H_{37}Rv and antimicrobial activity.

The present work deals with the synthesis of the title compounds starting from 1-(2-amino-1-(4-methoxyphenyl) ethyl) cyclohexanol, followed by their antimicrobial, antifungal and antitubercular screening.

2. Chemistry

1-(2-Amino-1-(4-methoxyphenyl) ethyl) cyclohexanol (**1**), on condensation with chloroacetyl chloride yielded 2-chloro-*N*-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**2**), which on amination with hydrazine hydrate yielded in turn 2-hydrazinyl-*N*-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**3**). Compound **3**, on condensation with various aromatic aldehydes afforded a series of 2-(2-benzylidenehydrazinyl)-*N*-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)ethyl) acetamides **4a–n**, which upon reaction in the presence of Thioglycolic acid and dimethyl formamide yielded 2-(4-oxo-2-substituted phenylthiazolidin-3-ylamino)-*N*-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide **5a–n** (Scheme 1).

3. Biology

The MICs of synthesized compounds were carried out by broth micro dilution method as described by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) (Husbands et al., 1984). Antibacterial activity was screened against one gram positive bacterium (*Staphylococcus aureus* ATCC 6538P) and two gram negative bacteria (*Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 9027). Ampicillin and Penicillin-G were used as a standard antibacterial agent. Antifungal activity was screened against three fungal species *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16404 and *A. clavatus* ATCC 9600. Greseofulvin was used as a standard antifungal agent.

All ATCC cultures were collected from the National Chemical Laboratory, Pune and tested against the above mentioned known drugs. Mueller Hinton broth was used as a nutrient medium to grow and dilute the drug suspension for the test. Inoculums' size for the test strain was adjusted to 10^8 CFU (Colony Forming Unit) per milliliter by comparing the turbidity. DMF was used as diluents to get the desired concentration of drugs to test upon standard bacterial strains. MIC of compounds was determined against *M. tuberculosis* H37Rv strain by using Lowenstein–Jensen medium (conventional method) as described by Rattan (EUCAST; Rattan, 2000).

4. Result and discussion

4.1. Analytical results

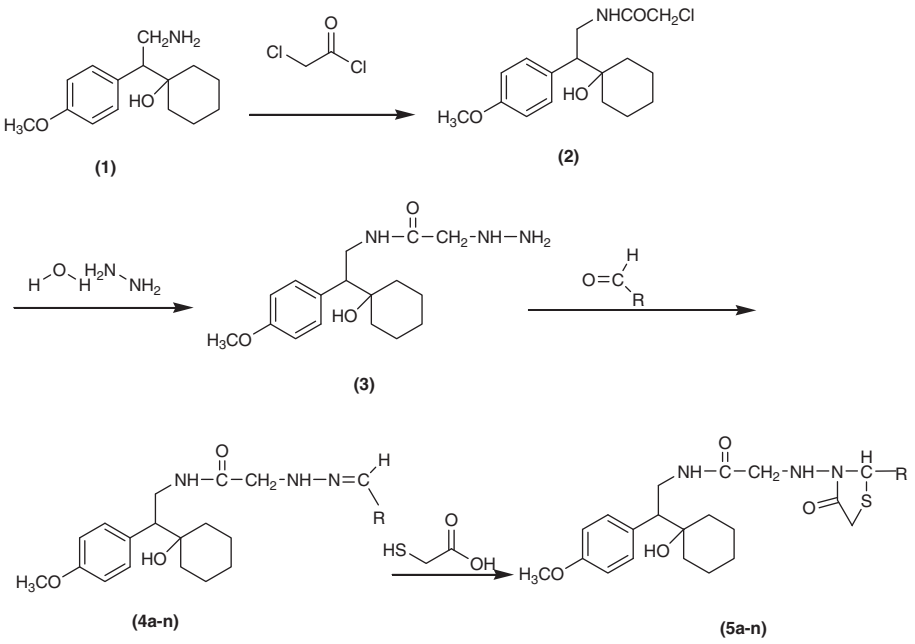
A series of analogs has been synthesized in good yields by using the synthetic route as outlined in Scheme 1. IR, ^{1}H NMR, ^{13}C NMR and mass spectral data are in well agreement with the proposed structures of all newly synthesized compounds.

The IR spectra of Compound **3** showed a broad stretching band around 3425 and 3200 cm^{-1} for NH and NH_2 with ^{1}H NMR a singlet at δ 2.1 and δ 8.1 accounted for NH_2 and NH accordingly. Mass spectrum of compound **3** displayed a molecular ion peak at m/z 321, confirmed its molecular weight. The synthesized compounds **4a–n** and **5a–n** were confirmed by IR, NMR and mass spectra. A typical sharp characteristic absorption band for –C═O was observed at ν_{max} 1717. The bands of –C═N of Schiff base derivatives clearly appeared at 1570 cm^{-1}. In ^{1}H NMR, a singlet at δ 8.2 attributed to the N═CH– protons while in the ^{13}C NMR spectra, the high δ value at 144.1 ppm attributed to the N═CH– group present in Schiff base. In ^{1}H NMR doublet at 3.55 value and in ^{13}C NMR spectra 54.52 ppm indicate the presence of –CO═CH_2–NH– group.

In ^{1}H NMR, a singlet at δ 5.90 and in ^{13}C NMR spectra value of 57.10 attributed to the presence of –CH–S of thiazolidinone ring. Mass spectrum of **4b** displayed a molecular ion peak at m/z 514, confirmed its molecular weight.

4.2. Biological results

The antibacterial screening results are summarized in Table 1.The results revealed that substituted Schiff base showed moderate activity against all the bacterial strains except compound **4b** having 4-methoxy substituent showed good activity against *S. aureus* and *E. coli*, while thiazolidinone exhibited good activity against *S. aureus* and *P. aeruginosa*. Most of thiazolidinone showed good activity (256 μg/ml) while compounds **5e** and **5f** containing 2,3,4-trimethoxy and 2-chloro substituents possessed pronounced activity (128 μg/



Substituents of compounds **4a-n** and **5a-n**

R = a. 4-OHC_6H_4,	f. 2-ClC_6H_4	k. 4-OH, 3-$OCH_3C_6H_4$
b. 4-$OCH_3C_6H_4$,	g. 4-$NO_2C_6H_4$	l. 3-Br,4-$OCH_3C_6H_4$
c. C_6H_5,	h. 3,4,5-$OCH_3C_6H_4$	m. 2-$NO_2C_6H_4$
d. 2, 3-ClC_6H_4	i. 3-phenoxy C_6H_4	n. 3-ClC_6H_4
e. 2,3,4-$OCH_3C_6H_4$	j. N, N -$CH_3C_6H_4$	

Scheme 1 Synthetic protocol for 2-(4-oxo-2-substituted phenylthiazolidin-3-ylamino)-*N*-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide **5a–n**.

ml) against *S. aureus*. Compounds **5a**, **5b** and **5d** having 4-hydroxy, 4-methoxy and 2,3-dichloro substituents showed good activity (128 μg/ml) then other compounds against *Streptococcus pyogenes*. Compound **5k** containing 4-hydroxy 3-methoxy substituent has higher activity (16.0 μg/ml) against *E. coli* and (32 μg/ml) against *P. aeruginosa*. Good activity was observed with compounds **5a**, **5e**, **5g**, **5l** and **5m** containing 4-hydroxy, 2,3,4-trimethoxy, 4-nitro, 3-bromo and 2-nitro substituents while others displayed moderate activity against *E. coli*. Compounds **5e**, **5f** and **5j** having 2,3,4-tri methoxy, 2-dichloro and *N*,*N*-dimethyl substituents showed good activity (128 μg/ml) whereas others displayed moderate activity against *P. aeruginosa*. Compounds **5a**, **5b**, **5d**, **5k**, **5l** and **5n** exhibited very good activity against gram positive bacteria whereas **5b**, **5e**, **5f** and **5k** showed very good activity toward gram negative bacteria.

The results of antifungal activity are summarized in Table 2. The results showed that Schiff base **4a–n** possessed good activity (256–512 μg/ml) against *C. albicans*. Compounds **4a–n** displayed moderate to weak activity (256–512 μg/ml) against *A. niger*. Compounds **5f**, **5h** and **5n** having 2-chloro, 3,4,5-trimethoxy and 3-chloro substituent exhibited better activity (128 μg/ml) whereas other compounds showed good activity (256 μg/ml) except **5g**, **5j** and **5l** against *C. albicans*. All the compounds showed weak activity against *A. niger* and *Aspergillus clavatus*.

The encouraging results from the antibacterial studies that impelled us to go for preliminary screening of synthesized compounds against *M. tuberculosis* are summarized in Table 3. Compound **4b** containing 4-methoxy substituent showed better activity (64 μg/ml) against *M. tuberculosis* and compounds **5b**, **5f**, **5l** and **5n** showed good activity (32–64 μg/ml) which is attributed due to 4-methoxy, 2-chloro, 3-bromo and 3-chloro substituents whereas compound **5k** which is having 4-hydroxy 3-methoxy substituent on thiazolidinone ring showed better activity (16 μg/ml). Due to the better activity against tested microorganisms and mycobacteria, compound **5k** has been selected for further development and studies to acquire more information about structure–activity relationships that are in progress in our laboratories.

5. Conclusion

A series of newer analogs of thiazolidinone were synthesized by introduction of Schiff base to thiazolidinone using aromatic

Table 1 Minimum inhibitory concentrations (MICs, μg/ml).

Compounds	Minimal bactericidal concentration μg/ml			
	Gram positive		Gram negative	
	S. aureus ATCC 6538P	*S. pyogenes* ATCC 8668	*E. coli* ATCC 8739	*P. aeruginosa* ATCC 9027
4a	512	512	128	512
4b	256	256	64	128
4c	512	256	512	512
4d	256	512	128	256
4e	512	256	256	512
4f	512	256	512	512
4g	512	512	512	512
4h	512	128	512	512
4i	512	512	256	256
4j	512	512	256	512
4k	128	256	128	128
4l	512	512	512	512
4m	512	512	256	512
4n	512	256	512	256
5a	128	128	256	256
5b	128	128	64	64
5c	512	128	256	512
5d	128	128	128	256
5e	256	256	256	128
5f	256	256	512	128
5g	512	512	256	512
5h	128	128	64	512
5i	512	256	128	256
5j	512	256	128	128
5k	64	256	16	32
5l	128	128	256	256
5m	256	256	256	512
5n	128	128	512	256
Ampicillin	**250**	**100**	**100**	**100**

aldehyde in the presence of acetic acid and assessed for their antimicrobial and antituberculosis activity. The antibacterial data indicated that the analogs with halogen, methoxy and nitro substituents emerged as promising antimicrobials showed moderate to better activity while analogs bearing chloro substituent showed better antifungal activity. It was also observed that the promising antimicrobials had proved to be better antituberculars. Specifically, compound **5k**, due to its better activity against $H_{37}Rv$ strain, would be the best choice for the preparation of new derivatives in order to improve antitubercular activity in the future.

6. Experimental

6.1. Chemistry

All chemicals were of analytical grade and used directly. Melting points were determined in PMP–DM scientific melting point apparatus and are uncorrected. The purity of compounds was checked by TLC using Merck silica gel $^{60}F_{254}$ and visualized by exposure to iodine vapors or UV light. IR spectra were recorded on a Perkin–Elmer RX 1 FTIR spectrophotometer, using potassium bromide pellets, the frequencies are expressed in cm^{-1}. The ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker Avance II 400 NMR spectrometer, using tetramethylsilane as the internal reference, with chloroform ($CDCl_3$) as solvent. The chemical shifts are reported in parts per million (d ppm). Elemental analyses were performed on a Heraeus Carlo Erba 1180 CHN analyzer. The mass spectra were recorded on micromass Q–T of micro (TOF MS ESþ). All spectral data were consistent with the proposed structure and micro analysis within ±0.4% of theoretical values.

*6.1.1. Procedure for the synthesis of 2-chloro-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)ethyl)acetamide (**2**)*

A mixture of 1-(2-amino-1-(4-methoxyphenyl) ethyl) cyclohexanol (**1**) (Husbands et al., 1984) (0.1 mol) and chloroacetyl chloride (0.1 mol) in Toluene (30 ml) was stirred at 30–35 °C in the presence of sodium carbonate (0.1 mol) for about 6.0 h. Completion of reaction was monitored by T.L.C (toluene/acetone, 5:5). The excess solvent was distilled off and then the remaining residue was poured into ice cold water. The separated solid was filtered, washed and recrystallized from ethanol to have a white color solid product. Yield 90%; m.p. 90 °C.

*6.1.2. Procedure for the synthesis of 2-hydrazinyl-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)ethyl)acetamide (**3**)*

A mixture of 2-chloro-*N*-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (2, 0.1 mol) and hydrazine hydrate (0.1 mol) in methanol (30 ml) was refluxed for about 6 h. Completion of reaction was monitored by T.L.C (toluene/acetone, 5:5). The mixture was then cooled down and

Table 2 Minimum inhibitory concentrations (MICs, μg/ml).

Compounds	Minimal fungicidal concentration μg/ml		
	C. albicans ATCC 10231	*A. niger* ATCC 16404	*A. clavatus* ATCC 9600
4a	512	256	512
4b	512	512	256
4c	512	256	256
4d	512	256	512
4e	512	512	512
4f	256	512	512
4g	512	512	256
4h	256	256	256
4i	512	256	256
4j	512	512	512
4k	512	512	512
4l	512	512	256
4m	256	256	256
4n	512	256	256
5a	256	512	256
5b	256	256	128
5c	256	256	128
5d	256	128	256
5e	256	512	256
5f	128	256	512
5g	512	512	512
5h	128	256	256
5i	256	256	256
5j	512	512	512
5k	256	512	256
5l	512	512	512
5m	256	256	256
5n	128	256	256
Griseofulvin	**500**	**100**	**100**

pH was adjusted to 4.0 by addition of 10% Acetic acid. Product was extracted in methylene dichloride by adjusting pH 9.0 by the addition of 10% sodium carbonate. Methylene dichloride was distilled out under vacuum to get colorless oil. Yield 70%.

*6.1.3. General preparation of the compounds (**4a–n**)*

A mixture of 2-hydrazinyl-*N*-(2-(1-hydroxycyclohexyl)-2-(4-ethoxyphenyl) ethyl) acetamide (3, 0.01 mol), aromatic aldehyde (0.01 mol) and 2–3 drops of glacial acetic acid in methanol (30 ml) was refluxed for 5 h. The completion of reaction was monitored by TLC (Eluent: toluene/acetone 5:5). The excess solvent was distilled off and the remaining residue was then poured into ice cold water. The separated solid was filtered, washed and recrystallized from ethanol to give compounds (**4a–n**).

*6.1.3.1. 2-(2-(4-Hydroxybenzylidene) hydrazinyl)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**4a**)*. Yield 77%; m.p. 180–182 °C; light yellow color powder, IR (KBr, cm^{-1}) 3470 (NH), 1719 (C═O), 1654 (CONH), 1542 (N═CH), ^{1}H NMR: (400 MHz, $CDCl_3$) δ: 3.72 (s, 3H, Ar–OCH_3), 2.0 (d, 1H, –OH) (cyclohexanol), 1.4–1.68 (m, 10H, –CH_2 (cyclohexanol), 3.45 (dd, 1H, –CH), 3.61 (dd, –2H, –CH_2), 8.01 (d, 1H, –N*H*–C═O), 3.55 (d, 2H, –C═O, –CH_2), 2.01 (d, 1H, N*H*-N), 8.2 (s, 1H, N═CH), 7.41–6.82 (s, 1H, Ar-OH), 6–8 (m, Ar–H, 1–8 H), ^{13}C NMR: (50 MHz; $CDCl_3$) δ: 55.8 (*C*–OCH_3), 19.8–71.5 (Cyclohexane), 50.79 (CH), 36.4 (–NH–*C*H_2), 54.52 (O═C–*C*H_2), 144.1 (CH═N), 116.4–1601.0 (Aromatic), Anal. calcd for $C_{24}H_{31}N_3O_4$: C 67.74, H 7.34, N 9.87, O; found C 67.70, H 7.31, N 9.82.

*6.1.3.2. 2-(2-(4-Methoxybenzylidene) hydrazinyl)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**4b**)*. Yield 72%; m.p. 210 °C, off white color powder, IR (KBr, cm^{-1}) 3465 (NH), 1715 (C═O), 1648 (CONH), 1545 (N═CH), ^{1}H NMR: 3.75 (s, 3H, Ar–OCH_3), 2.02 (d, 1H, –OH) (cyclohexanol), 1.44–1.70 (m, 10H, –CH_2 (cyclohexanol), 3.48 (dd, 1H, –CH), 3.65 (dd, –2H, –CH_2), 8.05 (d, 1H, –N*H*–C═O), 3.54 (d, 2H, –C═O, –CH_2), 2.01 (d, 1H, N*H*–N), 8.11 (s, 1H, N═CH), 6.7–8.0 (m, Ar–H, 1–8 H). ^{13}C NMR (50 MHz; $CDCl_3$) δ: 55.6 (*C*–OCH_3), 19.9–71.9 (Cyclohexane), 50.75 (CH), 36.4 (–NH–*C*H_2), 54.57 (O═C–*C*H_2), 143.8 (CH═N), 114.4–163.0 (Aromatic), Anal. calcd for $C_{25}H_{33}N_3O_4$: C 68.31, H 7.57, N 9.56; found C 68.29, H 7.52, N 9.50.

*6.1.3.3. 2-(2-Benzylidenehydrazinyl)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**4c**)*. Yield 68%; m.p.92–95 °C, Yellow color powder, IR (KBr, cm^{-1}) 3460 (NH), 1700 (C═O), 1664 (CONH), 541 (N═CH), ^{1}H NMR: 3.73 (s, 3H, Ar–OCH_3), 2.04 (d, 1H, –OH) (cyclohexanol), 1.45–1.69 (m, 10H, –CH_2) (cyclohexanol), 3.48 (dd, 1H, –CH), 3.64 (dd, –2H, –CH_2), 8.01 (d, 1H, –N*H*–C═O), 3.59 (d, 2H, –C═O, –CH_2), 2.01 (d, 1H, N*H*–N), 8.15 (s, 1H, N═CH), 7.0–7.9 (m, Ar–H, 1–8 H).^{13}C NMR (50 MHz; $CDCl_3$) δ: 55.9 (*C*–OCH_3), 19.95–71.1 (Cyclohexane), 50.81

Table 3 Minimum inhibitory concentrations (MICs, μg/ml).

Compounds	MIC values (μg/ml) of *M. tuberculosis* $H_{37}Rv$	Inhibition (%)
4a	512	99
4b	64	98
4c	512	98
4d	256	99
4e	256	99
4f	128	98
4g	512	98
4h	256	99
4i	512	99
4j	512	98
4k	64	98
4l	256	99
4m	512	99
4n	256	98
5a	256	98
5b	32	99
5c	256	99
5d	256	98
5e	128	98
5f	64	99
5g	256	99
5h	128	98
5i	256	98
5j	256	99
5k	16	99
5l	64	98
5m	128	98
5n	64	99
Rifampicin	**40**	**98**

(CH), 36.4 (–NH–*C*H_2), 54.50 (O═C–*C*H_2), 143.05 (CH═N), 128.9.4–134.0 (Aromatic), Anal. calcd for $C_{24}H_{31}N_3O_3$: C 70.39, H 7.63, N 10.26; found C 70.34, H 7.60, N 10.21.

*6.1.3.4. 2-(2-(2, 3-Dichlorobenzylidene) hydrazinyl)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**4d**)*. Yield 64%; m.p. 210–215 °C, Off white color powder, IR (KBr, cm^{-1}) 3448 (NH), 1705 (C═O), 1654 (CONH), 1534 (N═CH), ^{1}H NMR: 3.75 (s, 3H, Ar–OCH_3), 2.06 (d, 1H, –OH) (cyclohexanol), 1.40–1.68 (m, 10H, –CH_2 (cyclohexanol), 3.47 (dd, 1H, –CH), 3.66 (dd, –2H, –C*H*$_2$), 8.01 (d, 1H, –N*H*–C═O), 3.54 (d, 2H, –C═O, –C*H*$_2$), 2.01 (d, 1H, N*H*–N), 8.12 (s, 1H, N═CH), 6.6–6.90 (m, 8H, Ar–H).^{13}C NMR (50 MHz; $CDCl_3$) *δ*: 55.9 (*C*–OCH_3), 19.92–71.65 (Cyclohexane), 50.78 (CH), 36.4 (–NH–*C*H_2) 54.57 (O═C–*C*H_2), 143.8 (CH═N), 114.4–163.0 (Aromatic), Anal. calcd for $C_{24}H_{29}Cl_2N_3O_3$: C 60.25, H 6.11, N, 8.78; found C 60.20, H 6.06, N, 8.72.

*6.1.3.5. 2-(2-(2, 3, 4-Trimethoxybenzylidene) hydrazinyl)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**4e**)*. Yield 61%; m.p. 180–183 °C, Off white color powder, IR (KBr, cm^{-1}) 3450 (NH), 1710 (C═O), 1654 (CONH), 1560 (N═CH), ^{1}H NMR: 3.76 (s, 3H, Ar–OCH_3), 2.07 (d, 1H, –OH) (cyclohexanol), 1.40–1.69 (m, 10H, –CH_2) (cyclohexanol), 3.47 (dd, 1H, –CH), 3.65 (dd, –2H, –C*H*$_2$), 8.05 (d, 1H, –N*H*–C═O), 3.58 (d, 2H, –C═O, –C*H*$_2$), 2.04 (d, 1H, N*H*–N), 8.10 (s, 1H, N═CH), 6.6–6.90 (m, 4H, Ar–H).^{13}C NMR *δ*: 56.02 (*C*–OCH_3), 19.95–71.35 (Cyclohexane), 50.84 (CH), 36.9 (–NH–*C*H_2) 54.59 (O═C–*C*H_2), 143.05 (CH═N), 110.4–155.0 (Aromatic), Anal. calcd for $C_{27}H_{37}N_3O_6$: C 64.91, H 7.46, N 8.41; found C 64.86, H 7.41, N 8.37.

*6.1.3.6. 2-(2-(2-Chlorobenzylidene) hydrazinyl)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**4f**)*. Yield 69%; m.p. 140–145 °C, Light yellow color powder, IR (KBr, cm^{-1}) 3448 (NH), 1717 (C═O), 1647 (CONH), 1542 (N═CH), ^{1}H NMR: 3.78 (s, 3H, Ar–OCH_3), 2.03 (d, 1H, –OH) (cyclohexanol), 1.42–1.70 (m, 10H, –CH_2) (cyclohexanol), 3.45 (dd, 1H, –CH), 3.63 (dd, –2H, –C*H*$_2$), 8.01 (d, 1H, –N*H*–C═O), 3.58 (d, 2H, –C═O, –C*H*$_2$), 2.05 (d, 1H, N*H*–N), 8.13 (s, 1H, N═CH), 6.6–6.90 (m, 8H, Ar–H).^{13}C NMR *δ*: 55.8 (*C*–OCH_3), 19.90–71.40 (Cyclohexane), 50.85 (CH), 36.45 (–NH–*C*H_2) 54.59 (O═C–*C*H_2), 143.0 (CH═N), 127.0.–135.0 (Aromatic), Anal. calcd for $C_{24}H_{30}ClN_3O_3$: C 64.93, H 6.81, N 9.46; found C 64.90, H 6.76, N 9.41.

*6.1.3.7. 2-(2-(4-Nitrobenzylidene) hydrazinyl)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**4g**)*. Yield 76%; m.p. 275–278 °C, Yellow color powder, IR (KBr, cm^{-1}) 3422 (NH), 1708 (C═O), 1654 (CONH), 1560 (N═CH), ^{1}H NMR: 3.75 (s, 3H, Ar–OCH_3), 2.04 (d, 1H, –OH) (cyclohexanol), 1.42–1.68 (m, 10H, –CH_2) (cyclohexanol), 3.47 (dd, 1H, –CH), 3.66 (dd, –2H, –C*H*$_2$), 8.05 (d, 1H, –N*H*–C═O), 3.57 (d, 2H, –C═O, –C*H*$_2$), 2.05 (d, 1H, N*H*–N), 8.13 (s, 1H, N═CH), 6.6–6.90 (m, 8H, Ar–H).^{13}C NMR *δ*: 55.80 (*C*–OCH_3), 19.90–71.39 (Cyclohexane), 50.85 (CH), 36.30 (–NH–*C*H_2) 54.55 (O═C–*C*H_2), 143.50 (CH═N), 121.0–140.0 (Aromatic), Anal. calcd for $C_{24}H_{30}N_4O_5$: C 63.42, H 6.65, N 12.33; found C 63.37, H 6.60, N 12.30.

*6.1.3.8. 2-(2-(3, 4, 5-Trimethoxybenzylidene) hydrazinyl)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**4h**)*. Yield 73%; m.p. 190–197 °C, off white color powder, IR (KBr, cm^{-1}) 3448 (NH), 1743 (C═O), 1647 (CONH), 1577 (N═CH), ^{1}H NMR: 3.79 (s, 3H, Ar–OCH_3), 2.04 (d, 1H, –OH) (cyclohexanol), 1.40–1.70 (m, 10H, –CH_2) (cyclohexanol), 3.49 (dd, 1H, –CH), 3.64 (dd, –2H, –C*H*$_2$), 8.01 (d, 1H, –N*H*–C═O), 3.58 (d, 2H, –C═O, –C*H*$_2$), 2.05 (d, 1H, N*H*–N), 8.13 (s, 1H, N═CH), 6.6–6.90 (m, 8H, Ar–H).^{13}C NMR *δ*: 55.80 (*C*–OCH_3), 19.92–71.35 (Cyclohexane), 50.84 (CH), 36.40 (–NH–*C*H_2) 54.57 (O═C–*C*H_2), 143.0 (CH═N), 121.4–139.9 (Aromatic), Anal. calcd for $C_{27}H_{37}N_3O_6$: C 64.91, H 7.46, N 8.41; found C 64.86, H 7.41, N 8.37.

*6.1.3.9. 2-(2-(3-Phenoxybenzylidene) hydrazinyl)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**4i**)*. Yield 70%; m.p. 130–132 °C, light brown color powder, IR (KBr, cm^{-1}) 3422 (NH), 1718 (C═O), 1636 (CONH), 1577 (N═CH), ^{1}H NMR: 3.79 (s, 3H, Ar–OCH_3), 2.05 (d, 1H, –OH) (cyclohexanol), 1.42–1.69 (m, 10H, –CH_2) (cyclohexanol), 3.48 (dd, 1H, –CH), 3.66 (dd, –2H, –C*H*$_2$), 8.01 (d, 1H, –N*H*–C═O), 3.57 (d, 2H, –C═O, –C*H*$_2$), 2.05 (d, 1H, N*H*–N), 8.13 (s, 1H, N═CH), 6.6–6.90 (m, 8H, Ar–H).^{13}C NMR *δ*: 55.85 (*C*–OCH_3), 19.75–71.50 (Cyclohexane), 50.75 (CH), 36.45 (–NH–*C*H_2) 54.55 (O═C–*C*H_2), 143.20 (CH═N), 119.4–160.0 (Aromatic), Anal. calcd for $C_{30}H_{35}N_3O_4$: C 71.83, H 7.03, N 8.38; found C 71.79, H 7.00, N 8.33.

*6.1.3.10. 2-(2-(4-Dimethylaminobenzylidene) hydrazinyl)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**4j**)*. Yield 74%; m.p. 229–232 °C, off white color powder, IR (KBr, cm^{-1}) 3422 (NH), 1734 (C═O), 1637 (CONH), 1578 (N═CH), ^{1}H NMR: 3.75 (s, 3H, Ar–OCH_3), 2.03 (d, 1H, –OH) (cyclohexanol), 1.41–1.75 (m, 10H, –CH_2) (cyclohexanol), 3.49 (dd, 1H, –CH), 3.68 (dd, –2H, –CH_2), 8.09 (d, 1H, –N*H*–C═O), 3.58 (d, 2H, –C═O, –CH_2), 2.05 (d, 1H, N*H*–N), 8.13 (s, 1H, N═CH), 6.6–6.90 (m, 8H, Ar–H).^{13}C NMR δ: 55.84 (*C*–OCH_3), 19.90–71.44 (Cyclohexane), 50.84 (CH), 36.37 (–NH–*C*H_2) 54.58 (O═C–*C*H_2), 143.5 (CH═N), 114.4–163.0 (Aromatic), Anal. calcd for $C_{26}H_{36}N_4O_3$: C 69.00, H 8.02, N 12.38; found C 68.95, H 8.00, N 12.34.

*6.1.3.11. 2-(2-(4-Hydroxy3-methoxybenzylidene) hydrazinyl)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**4k**)*. Yield 75%; m.p. 218–222 °C, orange color powder, IR (KBr, cm^{-1}) 3462 (NH), 1718 (C═O), 1648 (CONH), 1571 (N═CH), ^{1}H NMR: 3.76 (s, 3H, Ar–OCH_3), 2.05 (d, 1H, –OH) (cyclohexanol), 1.41–1.67 (m, 10H, –CH_2) (cyclohexanol), 3.48 (dd, 1H, –CH), 3.65 (dd, –2H, –CH_2), 8.03 (d, 1H, –N*H*–C═O), 3.55 (d, 2H, –C═O, –CH_2), 2.06 (d, 1H, N*H*–N), 8.13 (s, 1H, N═CH), 6.6–6.90 (m, 8H, Ar–H).^{13}C NMR δ: 55.9 (*C*–OCH_3), 19.92–71.65 (Cyclohexane), 50.78 (CH), 36.4 (–NH–*C*H_2) 54.57 (O═C–*C*H_2), 143.8 (CH═N), 114.4–163.0 (Aromatic), Anal. calcd for $C_{25}H_{33}N_3O_5$: C 65.91, H 7.30, N 9.22; found C 65.86, H 7.26, N 9.18.

*6.1.3.12. 2-(2-(3-Bromo-4-methoxybenzylidene) hydrazinyl)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**4l**)*. Yield 70%; m.p. 133–137 °C, light yellow color powder, IR (KBr, cm^{-1}) 3468 (NH), 1735 (C═O), 1647 (CONH), 1570 (N═CH), ^{1}H NMR: 3.76 (s, 3H, Ar–OCH_3), 2.05 (d, 1H, –OH) (cyclohexanol), 1.41–1.69 (m, 10H, –CH_2) (cyclohexanol), 3.48 (dd, 1H, –CH), 3.68 (dd, –2H, –CH_2), 8.04 (d, 1H, –N*H*–C═O), 3.57 (d, 2H, –C═O, –CH_2), 2.07 (d, 1H, N*H*–N), 8.10 (s, 1H, N═CH), 6.6–6.90 (m, 8H, Ar–H).^{13}C NMR δ: 55.5 (*C*–OCH_3), 19.90–71.50 (Cyclohexane), 50.85 (CH), 36.9 (–NH–*C*H_2) 54.55 (O═C–*C*H_2), 143.4 (CH═N), 116.4–135.0 (Aromatic), 159.8 (C–Br), Anal. calcd for $C_{25}H_{32}BrN_3O_4$: C 57.92, H 6.22, N 8.11; found C 57.86, H 6.17, N 8.07.

*6.1.3.13. 2-(2-(2-Nitrobenzylidene) hydrazinyl)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**4m**)*. Yield 77%; m.p. 206–212 °C, yellow color powder IR (KBr, cm^{-1}) 3465 (NH), 1734 (C═O), 1649 (CONH), 1565 (N═CH), ^{1}H NMR: 3.77 (s, 3H, Ar–OCH_3), 2.04 (d, 1H, –OH) (cyclohexanol), 1.41–1.69 (m, 10H, –CH_2) (cyclohexanol), 3.47 (dd, 1H, –CH), 3.65 (dd, –2H, –CH_2), 8.09 (d, 1H, –N*H*–C═O), 3.58 (d, 2H, –C═O, – CH_2), 2.05 (d, 1H, N*H*–N), 8.14 (s, 1H, N═CH), 6.6–6.90 (m, 8H, Ar–H).^{13}C NMR δ: 55.2 (*C*–OCH_3), 19.9–71.3 (Cyclohexane), 50.81 (CH), 36.5 (–NH–*C*H_2) 54.87 (O═C–*C*H_2), 143.1 (CH═N), 121.4–140.0 (Aromatic), Anal. calcd for $C_{24}H_{30}N_4O_5$: C 63.42, H 6.65, N 12.33; found C 63.37, H 6.60, N 12.29.

*6.1.3.14. 2-(2-(3-Chlorobenzylidene) hydrazinyl)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**4n**)*. Yield 74%; m.p. 206–212 °C, light brown color powder, IR (KBr, cm^{-1}) 3461 (NH), 1737 (C═O), 1650 (CONH), 1571 (N═CH), ^{1}H NMR: 3.73 (s, 3H, Ar–OCH_3), 2.05 (d, 1H, –OH) (cyclohexanol), 1.40–1.69 (m, 10H, –CH_2) (cyclohexanol), 3.48 (dd, 1H, –CH), 3.64 (dd, –2H, –CH_2), 8.05 (d, 1H, –N*H*–C═O), 3.58 (d, 2H, –C═O, –CH_2), 2.05 (d, 1H, N*H*–N), 8.12 (s, 1H, N═CH), 6.6–6.90 (m, 8H, Ar–H).^{13}C NMR δ: 55.30 (*C*–OCH_3), 19.95–71.45 (Cyclohexane), 50.80 (CH), 36.45 (–NH–*C*H_2) 54.70 (O═C–*C*H_2), 143.0 (CH═N), 124.4–140.0 (Aromatic), Anal. calcd for $C_{24}H_{30}ClN_3O_3$: C 64.93, H 6.81, N 9.46; found C 64.89, H 6.76, N 9.41.

*6.1.4. General preparation of the compounds (**5a–n**)*

A mixture of compound **4a–n** (0.01 mol) and thioglycolic acid (0.02 mol) was refluxed in the presence of zinc chloride and solvent DMF for 12 h. The completion of reaction was monitored by TLC (toluene: acetone, 5.0:5.0). After completion, reaction mass was dumped in ice cold water. The product formed was isolated washed with water and recrystallized from ethanol to give compound **5a–n.**

*6.1.4.1. 2-(2-(4-Hydroxyphenyl)-4-oxothiazolidin-3-ylamino)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**5a**)*. Yield 58%; m.p. 96–97 °C, off white color powder, IR (KBr, cm^{-1}) 3483 (–OH), 1734 (C═O thiazolidinone), 1654 (CONH), 625 (C–S–C), ^{1}H NMR 3.73 (s, 3H, Ar–OCH_3), 2.01 (s, 1H, –OH) (Cyclohexane), 1.41–1.67 (m, 10H, –CH_2) (Cyclohexane), 3.45 (dd, 1H, –CH), 3.61 (dd, 2H, CH_2), 8.01 (d, 1H, –N*H*–C═O), 3.53 (d, 2H, O═C–CH_2), 2.01 (d, 1H, –N*H*–N), 5.93 (s, 1H, N–C*H* thiazolidinone), 3.37 (s, 2H, –CH_2 (thiazolidinone), ring), 3.73 (s, 1H, Ar–OCH_3), 6.6–6.90 (m, 8H, Ar–H), ^{13}C NMR 55.5 (O–CH_3), 168.55 (C═O), 57.19 (CHS); 115.0–159.0 (Aromatic), MS (*m*/*z*): 501 (M^+), Anal. Calcd for $C_{26}H_{33}N_3O_5S$: C 62.50. H 6.66, N 8.41; found C 62.00, H 6.61, N 8.36.

*6.1.4.2. 2-(2-(4-Methoxyphenyl)-4-oxothiazolidin-3-ylamino)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)ethyl)acetamide (**5b**)*. Yield 50%; m.p. 280 °C, off white color powder, IR (KBr, cm^{-1}) 3484 (–OH), 1735 (C═O thiazolidinone), 1647 (CONH), 626 (C–S–C), ^{1}H NMR 3.74 (s, 3H, Ar–OCH_3), 2.02 (s, 1H, –OH) (Cyclohexane), 1.41–1.67 (m, 10H, –CH_2) (Cyclohexane), 3.47 (dd, 1H, –CH), 3.60 (dd, 2H, CH_2), 8.01 (d, 1H, –N*H*–C═O), 3.53 (d, 2H, O═C–CH_2), 2.01 (d, 1H, –N*H*–N), 5.93 (s, 1H, N–C*H* thiazolidinone), 3.38 (s, 2H, –CH_2 (thiazolidinone ring), 5.90 (s, 1H, Ar–OH), 6.6–6.90 (m, 8H, Ar–H).^{13}C NMR (50 MHz; $CDCl_3$) δ: 169.20 (C═O), 156.10 (C–OH), 57.54 (–OCH_3), 57.14 (CHS); 115.0–160.0 (Aromatic), MS (*m*/*z*): 514.70 (M^+), Anal. Calcd for $C_{26}H_{33}N_3O_5S$: C 63.13, H 6.87, N 8.18; found C 63.09, H 6.61, N 8.14.

*6.1.4.3. 2-(4-Oxo-2-phenylthiazolidin-3-ylamino)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)ethyl)acetamide (**5c**)*. Yield 53%; m.p. 80–85 °C, off white color powder, IR (KBr, cm^{-1}) 3471 (–OH), 1735 (C═O thiazolidinone), 16/54 (CONH), 625 (C–S–C), ^{1}H NMR 3.73 (s, 3H, Ar–OCH_3), 2.01 (s, 1H, –OH) (Cyclohexane), 1.41–1.68 (m, 10H, –CH_2) (Cyclohexane), 3.48 (dd, 1H, –CH), 3.63 (dd, 2H, CH_2), 8.00 (d, 1H, –N*H*–C═O), 3.55 (d, 2H, O═C–*C*H_2), 2.01 (d, 1H, –N*H*–N), 5.93 (s, 1H, N–C*H* thiazolidinone), 3.38 (s, 2H, –CH_2 (thiazolidinone ring), 6.6–6.90 (m, 8H, Ar–H), ^{13}C NMR (50 MHz; $CDCl_3$) δ: 55.9 (O–CH_3), 168.20 (C═O), 57.54 (–OCH_3), 57.14 (CHS); 115.0–160.0 (Aromatic), MS

(*m*/*z*): 484.5 (M^+), Anal. Calcd for $C_{26}H_{33}N_3O_4S$: C 64.57, H 6.88, N 8.69; found C 64.52, H 6.82, N 8.65.

*6.1.4.4. 2-(2-(2,3-Dichlorophenyl)-4-oxothiazolidin-3-ylamino)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)ethyl) acetamide (**5d**)*. Yield 59%; m.p. > 300 °C; Brown color powder, IR (KBr, cm^{-1}) 3481 (–OH), 1737 (C═O thiazolidinone), 1648 (CONH), 620 (C–S–C), ^{1}H NMR 3.75 (s, 3H, Ar–OCH_3), 2.01 (s, 1H, –OH) (Cyclohexane), 1.43–1.69 (m, 10H, –CH_2) (Cyclohexane), 3.46 (dd, 1H, –CH), 3.65 (dd, 2H, CH_2), 8.03 (d, 1H, –N*H*–C═O), 3.56 (d, 2H, O═C–*C*H_2), 2.01 (d, 1H, –N*H*–N), 5.95 (s, 1H, N–C*H* thiazolidinone), 3.38 (s, 2H, –CH_2 (thiazolidinone ring), 6.8–7.03 (m, 8H, Ar–H), ^{13}C NMR 55.8 (O–CH_3), 168.10 (C═O), 48.50 (CHS); 115.0–159.0 (Aromatic), MS (*m*/*z*): 553.5, 555.5 (M + 2), Anal. Calcd for $C_{26}H_{31}Cl_2N_3O_4S$: C 56.52, H 5.66, N 7.61; found C 56.46, H 5.61, N 7.55.

*6.1.4.5. 2-(2-(2,3,4-Trimethoxyphenyl)-4-oxothiazolidin-3-ylamino)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)ethyl) acetamide (**5e**)*. Yield 52%; m.p. 65–70 °C, light yellow color powder, IR (KBr, cm^{-1}) 3471 (–OH), 1730 (C═O thiazolidinone), 1655 (CONH), 623 (C–S–C), ^{1}H NMR 3.74 (s, 3H, Ar–OCH_3), 2.03 (s, 1H, –OH) (Cyclohexane), 1.41–1.67 (m, 10H, –CH_2) (Cyclohexane), 3.45 (dd, 1H, –CH), 3.62 (dd, 2H, CH_2), 8.01 (d, 1H, –N*H*–C═O), 3.53 (d, 2H, O═C–*C*H_2), 2.01 (d, 1H, –N*H*–N), 5.93 (s, 1H, N–C*H* thiazolidinone), 3.38 (s, 2H, –CH_2 (thiazolidinone ring), 3.73 (s, 3H, Ar–OCH_3), 6.6–6.90 (m, 8H, Ar–H), ^{13}C NMR 55.95 (O–CH_3), 168.70 (C═O), 47.55 (CHS); 107.0–155.0 (Aromatic), MS (*m*/*z*): 501 (M^+), Anal. Calcd for $C_{29}H_{39}N_3O_7S$: C 60.71, H 6.85, N 7.32; found C 60.66, H 6.81, N 7.28.

6.1.4.6. 2-(2-(2-chlorophenyl)-4-oxothiazolidin-3-ylamino)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)ethyl)acetamide (5f). Yield 55%; m.p. 80–90 °C; light brown color powder, IR (KBr, cm^{-1}) 3449 (–OH), 1718 (C═O thiazolidinone), 1654 (CONH), 621 (C–S–C), ^{1}H NMR 3.73 (s, 3H, Ar–OCH_3), 2.00 (s, 1H, –OH) (Cyclohexane), 1.40–1.68 (m, 10H, –CH_2) (Cyclohexane), 3.47 (dd, 1H, –CH), 3.65 (dd, 2H, CH_2), 8.04 (d, 1H, –N*H*–C═O), 3.55 (d, 2H, O═C–*C*H_2), 2.01 (d, 1H, –N*H*–N), 5.92 (s, 1H, N–C*H* thiazolidinone), 38 (s, 2H, –CH_2 (thiazolidinone ring), 7.0–7.16 (m, 8H, Ar–H), ^{13}C NMR 55.91 (O–CH_3), 168.70 (C═O), 48.00 (CHS); 135.01 (C–Cl), 125.0–149.0 (Aromatic). MS (*m*/*z*): 519 (M^+), 521 (M + 2), Anal. Calcd for $C_{26}H_{32}ClN_3O_4S$: C 60.28, H 6.23, N, 8.11; found C 60.23, H 6.19, N, 8.06.

*6.1.4.7. 2-(2-(4-nitrophenyl)-4-oxothiazolidin-3-ylamino)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)ethyl)acetamide (**5g**)*. Yield (54%); m.p. 70–75 °C, off white color powder, IR (KBr, cm^{-1}) 3471 (–OH), 1735 (C═O thiazolidinone), 1661 (CONH), 627 (C–S–C), ^{1}H NMR 3.75 (s, 3H, Ar–OCH_3), 2.01 (s, 1H, –OH) (Cyclohexane), 1.42–1.68 (m, 10H, –CH_2) (Cyclohexane), 3.47 (dd, 1H, –CH), 3.65 (dd, 2H, CH_2), 8.01 (d, 1H, –N*H*–C═O), 3.55 (d, 2H, O═C–*C*H_2), 2.01 (d, 1H, –N*H*–N), 5.95 (s, 1H, N–C*H* thiazolidinone), 3.39 (s, 2H, –CH_2 (thiazolidinone ring), 3.73 (s, 3H, Ar–OCH_3), 7.36–8.09 (m, 8H, Ar–H), ^{13}C NMR 55.90 (O–CH_3), 168.80 (C═O), 57.50 (CHS); 120.0–146.0 (Aromatic), MS (*m*/*z*): 530 (M^+), Anal. Calcd for $C_{26}H_{32}N_4O_6S$: C 59.07, H 6.10, N 10.60; found C 59.02, H 6.05, N 10.56.

*6.1.4.8. 2-(2-(3,4,5-trimethoxyphenyl)-4-oxothiazolidin-3-ylamino)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)ethyl)acetamide (**5h**)*. Yield 60%; m.p. 140–145 °C; light brown color powder, IR (KBr, cm^{-1}) 3477 (–OH), 1734 (C═O thiazolidinone), 1636 (CONH), 623 (C–S–C), ^{1}H NMR 3.74 (s, 3H, Ar–OCH_3), 2.03 (s, 1H, –OH) (Cyclohexane), 1.41–1.67 (m, 10H, –CH_2) (Cyclohexane), 3.45 (dd, 1H, –CH), 3.62 (dd, 2H, CH_2), 8.01 (d, 1H, –N*H*–C═O), 3.53 (d, 2H, O═C–*C*H_2), 2.01 (d, 1H, –N*H*–N), 5.93 (s, 1H, N–C*H* thiazolidinone), 3.38 (s, 2H, –CH_2 (thiazolidinone ring), 3.73 (s, 3H, Ar–OCH_3), 6.6–6.90 (m, 8H, Ar–H), ^{13}C NMR 55.82 (O–CH_3), 169.10 (C═O), 58.10 (CHS); 105.0–151.0 (Aromatic), MS (*m*/*z*): 575 (M^+), Anal. Calcd for $C_{29}H_{39}N_3O_7S$: C 60.71, H 6.85, N 7.32; found C 60.66, H 6.81, N 7.28.

*6.1.4.9. 2-(4-oxo-2-(3-phenoxyphenyl) thiazolidin-3-ylamino)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**5i**)*. Yield 60%; m.p. 55 °C; off white color powder, IR (KBr, cm^{-1}) 3477 (–OH), 1734 (C═O thiazolidinone), 1654 (CONH), 620 (C–S–C), ^{1}H NMR 3.74 (s, 3H, Ar–OCH_3), 2.00 (s, 1H, –OH) (Cyclohexane), 1.44–1.69 (m, 10H, –CH_2) (Cyclohexane), 3.47 (dd, 1H, –CH), 3.64 (dd, 2H, CH_2), 8.00 (d, 1H, –N*H*–C═O), 3.55 (d, 2H, O═C–*C*H_2), 2.01 (d, 1H, –N*H*–N), 5.92 (s, 1H, N–C*H* thiazolidinone), 3.40 (s, 2H, –CH_2 (thiazolidinone ring), 3.73 (s, 3H, Ar–OCH_3), 6.72–7.34 (m, 16H, Ar–H), ^{13}C NMR 55.92 (O–CH_3), 168.80 (C═O), 57.50 (CHS); 115.0–158.0 (Aromatic), MS (*m*/*z*): 577 (M^+), Anal. Calcd for $C_{32}H_{37}N_3O_5S$: C 66.76, H 6.48, N, 7.30; found C 66.71, H 6.42, N, 7.25.

*6.1.4.10. 2-(2-(4-(dimethylamino) phenyl)-4-oxothiazolidin-3-ylamino)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**5j**)*. Yield 61%; m.p. 130–135 °C; light brown color powder, IR (KBr, cm^{-1}) 3475 (–OH), 1718 (C═O thiazolidinone), 1654 (CONH), 622 (C–S–C), ^{1}H NMR 3.73 (s, 3H, Ar–OCH_3), 2.02 (s, 1H, –OH) (Cyclohexane), 1.43–1.69 (m, 10H, –CH_2) (Cyclohexane), 3.46 (dd, 1H, –CH), 3.62 (dd, 2H, CH_2), 8.01 (d, 1H, –N*H*–C═O), 3.55 (d, 2H, O═C–*C*H_2), 2.01 (d, 1H, –N*H*–N), 5.93 (s, 1H, N–C*H* thiazolidinone), 3.40 (s, 2H, –CH_2 (thiazolidinone ring), 2.86 (s, 2H, Ar–NH_2), 6.47–6.89 (m, 8H, Ar–H), ^{13}C NMR 56.2 (O–CH_3), 168.00 (C═O), 57.50 (CHS); 115.0–148.0 (Aromatic), MS (*m*/*z*): 528 (M^+), Anal. Calcd for $C_{28}H_{38}N_4O_4S$: C 63.85, H 7.27, N 10.64; found C 63.80, H 7.22, N 10.60.

*6.1.4.11. 2-(2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-ylamino)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl)acetamide (**5k**)*. Yield 59%; m.p. 40–45 °C; off white color powder, IR (KBr, cm^{-1}) 3469 (–OH), 1718 (C═O thiazolidinone), 1648 (CONH), 621 (C–S–C), ^{1}H NMR 3.75 (s, 3H, Ar–OCH_3), 2.05 (s, 1H, –OH) (Cyclohexane), 1.42–1.69 (m, 10H, –CH_2) (Cyclohexane), 3.45 (dd, 1H, –CH), 3.62 (dd, 2H, CH_2), 8.01 (d, 1H, –N*H*–C═O), 3.53 (d, 2H, O═C–*C*H_2), 2.01 (d, 1H, –N*H*–N), 5.91 (s, 1H, N–C*H* thiazolidinone), 3.39 (s, 2H, –CH_2 (thiazolidinone ring), 5.01 (s, 1H, Ar–OH), 6.40–6.55 (m, 6H, Ar–H), ^{13}C NMR 56.8 (O–CH_3), 168.9 (C═O), 57.90 (CHS); 114.0–150.0 (Aromatic), MS (*m*/*z*): 531 (M^+),

Anal. Calcd for $C_{27}H_{35}N_3O_6S$: C 61.23, H 6.66, N 7.93; found C 61.19, H 6.61, N 7.89.

*6.1.4.12. 2-(2-(3-bromo-4-methoxyphenyl)-4-oxothiazolidin-3-ylamino)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl)acetamide (**5l**).* Yield 59%; m.p. 75–80 °C; light brown color powder, IR (KBr, cm^{-1}) 3481 (–OH), 1740 (C═O thiazolidinone), 1649 (CONH), 625 (C–S–C), ^{1}H NMR 3.75 (s, 3H, Ar–OCH_3), 2.02 (s, 1H, –OH) (Cyclohexane), 1.42–1.68 (m, 10H, –CH_2) (Cyclohexane), 3.46 (dd, 1H, –CH), 3.61 (dd, 2H, CH_2), 8.01 (d, 1H, –N*H*–C═O), 3.53 (d, 2H, O═C–*C*H_2), 2.01 (d, 1H, –N*H*–N), 5.92 (s, 1H, N–C*H* thiazolidinone), 3.39 (s, 2H, –CH_2 (thiazolidinone) ring), 3.73 (s, 3H, Ar–OCH_3), 6.87–7.15 (m, 6H, Ar–H), ^{13}C NMR 55.7 (O–CH_3), 168.80 (C═O), 56.50 (CHS), 112.2 (C–Br), 115.0–149.0 (Aromatic), MS (*m*/*z*): 594 (M^+) 596 (M + 2), Anal. Calcd for $C_{27}H_{34}BrN_3O_5S$: C 54.73, H 5.78, N 7.09; found C 54.59, H 5.71, N 7.02.

*6.1.4.13. 2-(2-(2-nitrophenyl)-4-oxothiazolidin-3-ylamino)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)ethyl)acetamide (**5m**).* Yield 59%; m.p. 208–210 °C; pale yellow color powder, IR (KBr, cm^{-1}) 3479 (–OH), 1730 (C═O thiazolidinone), 1647 (CONH), 628 (C–S–C), ^{1}H NMR 3.73 (s, 3H, Ar–OCH_3), 2.00 (s, 1H, –OH) (Cyclohexane), 1.41–1.67 (m, 10H, –CH_2) (Cyclohexane), 3.46 (dd, 1H, –CH), 3.62 (dd, 2H, CH_2), 8.05 (d, 1H, –N*H*–C═O), 3.56 (d, 2H, O═C–*C*H_2), 2.01 (d, 1H, –N*H*–N), 5.95 (s, 1H, N–C*H* thiazolidinone), 3.40 (s, 2H, –CH_2 (thiazolidinone ring), 7.31–8.08 (m, 8H, Ar–H), ^{13}C NMR 55.80 (O–CH_3), 168.80 (C═O), 48.40 (CHS); 125.0–148.90 (Aromatic), MS (*m*/*z*): 530 (M^+), Anal. Calcd for $C26H_{32}N_4O_6S$: C 59.07, H 6.10, N 10.60; found C 59.02, H 6.06, N 10.55.

*6.1.4.14. 2-(2-(3-chlorophenyl)-4-oxothiazolidin-3-ylamino)-N-(2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl) ethyl) acetamide (**5n**).* Yield 59%; m.p. 180–185 °C, pale yellow color powder, IR (KBr, cm^{-1}) 3490 (–OH), 1731 (C═O thiazolidinone), 1654 (CONH), 618 (C–S–C), ^{1}H NMR 3.74 (s, 3H, Ar–OCH_3), 2.05 (s, 1H, –OH) (Cyclohexane), 1.41–1.69 (m, 10H, –CH_2) (Cyclohexane), 3.46 (dd, 1H, –CH), 3.65 (dd, 2H, CH_2), 8.06 (d, 1H, –N*H*–C═O), 3.56 (d, 2H, O═C–*C*H_2), 2.07 (d, 1H, –N*H*–N), 5.97 (s, 1H, N–C*H* thiazolidinone), 3.40 (s, 2H, –CH_2 (thiazolidinone ring), 6.95–7.10 (m, 8H, Ar–H), ^{13}C NMR 55.8 (O–CH_3), 168.01 (C═O), 56.50 (CHS); 135.01 (C–Cl), 125.0–149.0 (Aromatic), MS (*m*/*z*): 519 (M^+) 521 (M + 2), Anal. Calcd for $C_{26}H_{32}ClN_3O_4S$: C 60.28, H 6.23, N 8.11; found C 60.23, H 6.19, N 8.06.

6.2. Biological assay

6.2.1. In vitro evaluation of antimicrobial activity

The MICs of synthesized compounds were carried out by broth micro dilution method as described by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Anargyros et al., 1990; Shah et al., 1985; Desai et al., 1984. Antibacterial activity was screened against two gram positive bacteria (*S. aureus* ATCC 6538P, and Streptococcus pyogenes ATCC 8668) and two gram negative bacteria (*E. coli* ATCC 8739, and *P. aeruginosa* ATCC 9027). Ampicillin was used as a standard antibacterial agent. Antifungal activity was screened against three fungal species *C. albicans* ATCC 10231, *A. niger* ATCC 16404 and *A. clavatus* ATCC 9600. Greseofulvin was used as a standard antifungal agent.

All ATCC cultures were collected from the National Chemical Laboratory, Pune and tested against above mentioned known drugs. The cup well plate method using Hi-Media agar medium was employed to study the antibacterial activity of **4a–n** and **5a–n** against *S. aureus* (ATCC 6538P), P. *aeruginosa* (ATCC 9027) and *E. coli* (ATCC 8739) EUCAST; Rattan, 2000. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. Each test compound (50 mg) was dissolved in dimethyl formamide (50 mL, 1000 μg/mL), which was used as sample solution. Sample size for all the compounds was fixed at 0.1 mL. Using a sterilized cork borer cups were scooped out of agar medium contained in a Petri dish which was previously inoculated with the microorganisms. The test compound solution (0.1 mL) was added in the cups and the Petri dishes were subsequently incubated at 37 °C for 24 h. Ampicillin and Penicillin-G were used as reference drugs and dimethyl formamide as a negative control. Zones of inhibition produced by each compound were measured in mm, and the results are listed in Table 1.

The antifungal activity of compounds **4a–n** and **5a–n** has been assayed in vitro at concentrations of 128, 256 and 512 μg/mL against *C. albicans* (ATCC 10231). Inoculums of std. suspension (0.1 ml) of test organism were added. The plates of sabouraud dextrose agar were incubated at 22 °C for 48 h, which were maintained on sabouraud dextrose agar slants stored at 4 °C.

The compounds were tested by Cup well Method[14] on Muller Hinton Agar for bacteria 4 on sabouraud dextrose agar for yeast or antifungal at concentration of 128, 256 and 512 μg/mL against two Gram negative & a Gram positive bacteria and yeast. The following results were obtained.

6.2.2. Antitubercular activity

Drug susceptibility and determination of MIC of the test compounds against *M. tuberculosis* H_{37}Rv were performed by L.J. agar (MIC) method Anargyros et al., 1990; Shah et al., 1985; Desai et al., 1984 where primary 512, 256 and secondary 128, 64.0, 32.0, 16.0, 8.0, 4.0, 2.0 μg/ml dilutions of each test compound were added liquid L.J. Medium and then media were sterilized by inspissation method. A culture of *M. tuberculosis* H_{37}Rv growing on L.J. medium was harvested in 0.85% saline in bijou bottles. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H_{37}Rv (5 × 104 bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 12 days, 22 days and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H_{37}Rv. The concentration at which no development of colonies occurred or < 20 colonies was taken as MIC concentration of the test compound. The standard strain *M. tuberculosis* H_{37}Rv was tested with known drug Rifampicin.

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