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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis antimicrobial and anticancer activity of N′-arylmethylidene-piperazine-1-carbothiohydrazide

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Abstract Ten newly synthesized thiosemicarbazones of piperazine (**3a–3j**) were evaluated for their antibacterial and antifungal activity against non-pathogenic strains of Escherichia coli (NCIM 2068), *Klebsiella pneumonia* (NCIM 2957), *Staphylococcus aureus* (NCIM 2079), and *Bacillus subtilis* (NCIM 2921); pathogenic strains of *Vibrio cholerae*, *protease*, *Candida albicans* and *Aspergillus niger*. All the 10 compounds (**3a–3j**) were found to be better than Ciprofloxacin against *B. subtilis* and four molecules (**3c**, **3d, 3e,** and **3h)** against *S. aureus*. Compound **3j,** a derivative of benzophenone, has been identified as a potent and promising candidate against *C. albicans*. The compounds were also evaluated for their anticancer activity against HBL-100 and HL60 cell lines. Compound **3a,** a *p*-hydroxy benzaldehyde derivative, has been identified as a potent and promising candidate.

Keywords Thiosemicarbazones · Piperazine · Antibacterial · Antifungal · Anticancer

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Introduction

Many major pathogenic bacteria and parasites have acquired resistance toward chemotherapeutic agents available in the market during the last decade. This led to the adoption of a resolution on antimicrobial resistance in World Health Assembly during 1998 (Surveillance, 1999). Recent reports on emergence of superbugs have raised the fear that infectious diseases may once again become major cause of death worldwide. Now, there is a need to give serious consideration toward development of novel chemotherapeutic agents that are structurally different from the existing molecules and acting on newer targets to efficiently combat Multi-Drug Resistant (MDR) strains and to prevent development of quick resistance by the pathogens. Thiosemicarbazones were very well known for their antimicrobial and anticancer property (Rollas and Küçükgüzel, 2007). Thiosemicarbazones with free primary N_4 amino group were reported for their anticancer property (Liu *et al.,* 1995; Alvero *et al.,* 2006; Finch *et al.,* 1999; Finch *et al.,* 2000). We reported anticancer thiosemicarbazones with secondary N_4 amino group (Chetan *et al.,* 2010; Krishnan *et al.,* 2008;

Kulandaivelu *et al.,* 2011), the present work elucidates the antimicrobial and anticancer property of thiosemicarbazones with tertiary N_4 amino group.

Materials and methods

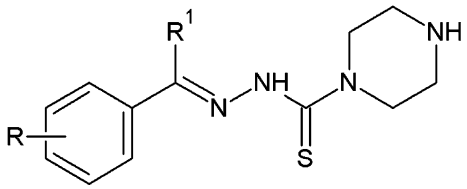
Chemistry

Melting points were determined using Thermonik Melting Point Apparatus (Campbell electronics, India) by capillary method and are uncorrected. Infrared (IR) spectra were taken on a Fourier Transform Infrared Spectrophotometer IR-Prestige 21 (Shimatzu Corporation, Japan) from 4000 to 400 cm^{-1} using KBr disks. ^{1}H-NMR spectra were recorded at 400 MHz in DMSO-d_6 using a Bruker Avance 400 instrument (Bruker Instruments Inc., USA). Chemical shifts were measured at δ units (ppm) relative to Tetramethylsilane (TMS). Fast-atom bombardment (FAB) mass spectra were recorded on a Jeol SX 102/DA-6000 mass spectrometer (Jeol Ltd. Akishima, Tokyo, Japan) using argon/xenon (6 kV, 10 mA) as FAB gas, m-nitrobenzyl alcohol as matrix, and 10 kV as accelerating voltage at room temperature. Elemental analysis was performed on a Vario EL III Elemental Analyser (Elementar, Germany) using sulfanilamide as standard. All chemicals were purchased from Merck, Spectrochem, or CDH, India. Solvents were of reagent grade and were purified and dried by standard procedure. Reactions were monitored by thin-layer chromatography on silica gel plates in either iodine or UV chambers. Intermediates were characterized by IR spectroscopic analysis and Elemental Analysis for CHNS. In the elemental analysis, the observed values were within ±0.4 % of the calculated values. Final compounds were characterized by ^{1}H-NMR and EI-MS. The percentage yields and the physicochemical data of final compounds **3a-3j** are presented in Table 1.

*General procedure for synthesis of methyl hydrazinecarbodithioate **(1)***

To a cooled solution of potassium hydroxide (0.1 M, 6.6 g/7 mL) in 2-propanol (7 mL), hydrazine hydrate (85 % solution, 0.1 M, 6 mL) was added with stirring. Ice-cooled carbondisulfide (0.1 M, 10 mL) was added drop wise to the above stirred solution that was maintained below 10 °C over 1.5 h. The bright yellow mixture obtained was further stirred for 1 h, and then, ice-cooled iodomethane (0.1 M, 7 mL) was added drop wise over a period of 2 h. Stirring was continued for an additional 1.5 h to obtain a white precipitate of **1**. Filtered, washed with ice-cooled water, and recrystallized from dichloromethane. Yield: 43 %; m.p.: 90–92 °C (Klayman *et al.*, 1979).

Table 1 Physico-chemical and spectral data of compounds **3a–3j**



Code	R	R1	MF	MW	MP	% yield	*R_f
3a	*p*-OH	H	$C_{12}H_{16}N_4OS$	264	210–212	76.4	0.67
3b	*p*-OH	CH_3	$C_{13}H_{18}N_4OS$	278	222–224	67.2	0.72
3c	*m*-NO_2	CH_3	$C_{13}H_{17}N_5O_2S$	307	186–188	72.6	0.77
3d	*p*-OCH_3	H	$C_{13}H_{18}N_4OS$	278	216–218	69.6	0.68
3e	*p*-Cl	H	$C_{12}H_{15}ClN_4S$	282	196–198	70.2	0.74
3f	H	CH_3	$C_{13}H_{18}N_4S$	262	212–214	72.6	0.56
3g	H	H	$C_{12}H_{16}N_4S$	248	198–200	73.2	0.65
3h	*p*-Cl	CH_3	$C_{13}H_{17}ClN_4S$	297	220–222	67.6	0.66
3i	*p*-CH_3	H	$C_{13}H_{18}N_4S$	262	198–200	69.3	0.68
3j	H	C_6H_5	$C_{18}H_{20}N_4S$	324	200–202	70.0	0.74

* *n*-Hexane:ethyl acetate; 1:1

*General procedure for synthesis of Schiff bases methylhydrazine carbodithioate **(2a-2j)***

Methyl hydrazinecarbodithioate **1** (0.01 M, 1.22 g) and (un)-substituted aromatic aldehydes/ketone (0.012 M) were dissolved in methanol (10 mL). To this mixture, catalytic amount of concentrated sulfuric acid was added and refluxed for 6–7 h. The reaction mixture turned yellow, as the methylhydrazine carbodithioate dissolved, and the yellow product began to precipitate. The solid obtained was filtered, dried, and recrystallized from suitable solvent. (Klayman *et al.*, 1979).

*General procedure for synthesis of N′-arylmethylidene-piperazine-1-carbothio-hydrazide **(3a–3j)***

Piperazine (0.005 M, 0.685 g) was added to appropriate Schiff's base (**2a–2j,** 0.005 M) in ethanol (25 mL) and refluxed until the evolution of methyl mercaptane almost completely ceased. Solvent present in the reaction mixture was evaporated under vacuum, and the solid was collected and washed with cold ethanol, further purified by recrystallization from suitable solvent (physico-chemical and spectral data in supplementary materials). (Kulandaivelu *et al.*, 2011).

*N′-[(4-hydroxyphenyl)-methylidene]-piperazine-1-carbothio-hydrazide **(3a)*** ^{1}H-NMR (DMSO-d_6, δppm): 1.9 (s, 1H, pip-NH), 2.6 (t, 4H, pip-CH_2), 4.15 (m, 4H, pip-CH_2),

7.4–7.7 (m, 4H, Ar–H), 8.17 (s, 1H, =C–H), 9.7 (s, 1H, Ar–OH), 11.5 (s, 1H, CS–N–H); EI-MS (m/z): 265$[M+1]^+$; Elemental analyses Found (Calcd.): C, 54.48 (54.52); H, 6.14 (6.10); N, 21.32 (21.19); S, 12.25 (12.13).

*N′-[1-(4-hydroxyphenyl)ethylidene]piperazine-1-carbothiohydrazide (**3b**)* ^{1}H-NMR (DMSO-d_6, δppm): 1.7 (s, 3H, $-CH_3$), 1.9 (s, 1H, pip-NH), 2.7 (t, 4H, pip-CH_2), 4.15 (m, 4H, pip-CH_2), 6.8–7.3 (m, 4H, Ar–H), 9.7 (s, 1H, Ar–OH), 11.3 (s, 1H, CS–N–H); EI-MS (m/z): 279$[M+1]^+$; Elemental analyses Found (Calcd.): C, 55.86 (56.09); H, 6.60 (6.52); N, 21.06 (20.13); S, 11.60 (11.52).

*N′-[1-(3-nitrophenyl)ethylidene]piperazine-1-carbothiohydrazide (**3c**)* ^{1}H-NMR (DMSO-d_6, δppm): 1.7 (s, 3H, $-CH_3$), 1.95 (s, 1H, pip-NH), 2.7 (t, 4H, pip-CH_2), 4.1 (m, 4H, pip-CH_2), 7.4–7.8 (m, 4H, Ar–H), 11.5 (s,1H, CS–N–H); EI-MS (m/z): 308$[M+1]^+$; Elemental analyses Found (Calcd.): C, 51.24 (50.80); H, 5.62 (5.57); N, 21.98 (22.78); S, 11.08 (10.43).

*N′-[(4-methoxyphenyl)methylidene]piperazine-1-carbothiohydrazide (**3d**)* ^{1}H-NMR (DMSO-d_6, δppm): 1.98 (s, 1H, pip-NH), 3.75 (t, 4H, pip-CH_2), 2.7 (m, 4H, $-OCH_3$), 4.15 (m, 4H, pip-CH_2), 7.4–7.65 (m, 4H, Ar–H), 8.19 (s, 1H, =C–H), 11.18 (s, 1H, CS–N–H); EI-MS (m/z): 279$[M+1]^+$; Elemental analyses Found (Calcd.): C, 55.98 (56.09); H, 6.78 (6.52); N, 20.88 (20.13); S, 11. 90 (11.52).

*N′-[(4-chlorophenyl)methylidene]piperazine-1-carbothiohydrazide (**3e**)* ^{1}H-NMR (DMSO-d_6, δppm): 2.55 (s, 1H, pip-NH), 3.4 (m, 4H, pip-CH_2), 4.11 (m, 4H, pip-CH_2), 7.39–7.6 (m, 4H, Ar–H), 8.16 (s, 1H, =C–H), 11.15 (s,1H, CS–N–H); EI-MS (m/z): 281$[M-1]^+$; Elemental analyses Found (Calcd.): C, 51.24 (50.97); H, 5.42 (5.35); N, 20.12 (19.81); S, 11.08 (11.34).

*N′-[1-phenylethylidene]piperazine-1-carbothiohydrazide (**3f**)* ^{1}H-NMR (DMSO-d_6, δppm): 2.3 (s, 3H $-CH_3$), 2.7 (s, 1H, pip-NH), 3.4 (m, 4H, pip-CH_2), 4.05 (m, 4H, pip-CH_2), 7.45–7.8 (m, 5H, Ar–H), 9.95 (s,1H, CS–N–H); EI-MS (m/z): 261 $[M-1]^+$; Elemental analyses Found (Calcd.): C, 60.12 (59.51); H, 6.78 (6.91); N, 21.14 (21.35); S, 12.54 (12.22).

*N′-[phenylmethylidene]piperazine-1-carbothiohydrazide (**3g**)* ^{1}H-NMR (DMSO-d_6, δppm): 1.95 (s, 1H, pip-NH), 2.7 (t, 4H, pip-CH_2), 4.1 (m, 4H, pip-CH_2), 7.4–7.7 (m, 5H, Ar–H), 8.15 (s, 1H, =C–H), 11.15 (s, 1H, CS–N–H); EI-MS (m/z): 249$[M+1]^+$; Elemental analyses Found (Calcd.): C, 57.78 (58.04); H, 6.58 (6.49); N, 22.90 (22.56); S, 12.64 (12.91).

*N′-[1-(4-chlorophenyl)ethylidene]piperazine-1-carbothiohydrazide (**3h**)* ^{1}H-NMR (DMSO-d_6, δppm): 1.65 (s, 3H $-CH_3$), 1.95 (s, 1H, pip-NH), 2.85 (t, 4H, pip-CH_2), 4.1 (m, 4H, pip-CH_2), 7.60–7.7 (m, 4H, Ar–H), 11.15 (s, 1H, CS–N–H); EI-MS (m/z): 298$[M+1]^+$; Elemental analyses Found (Calcd.): C, 52.16 (52.60); H, 6.02 (5.77); N, 19.22 (18.88); S, 10.24 (10.80).

*N′-[(4-methylphenyl)methylidene]piperazine-1-carbothiohydrazide (**3i**)* ^{1}H-NMR (DMSO-d_6, δppm): 2.5 (s, 1H, pip-NH), 3.1 (t, 4H, pip-CH_2), 3.7 (s, 3H, CH_3), 4.6 (m, 4H, pip-CH_2), 7.1–7.5 (m, 4H, Ar–H), 8.10 (s, 1H, =C–H), 10.8 (s, 1H, CS–N–H); EI-MS (m/z): 263$[M+1]^+$; Elemental analyses Found (Calcd.): C, 60.04 (59.51); H, 5.88 (6.91); N, 21.68 (21.35); S, 12.04 (12.22).

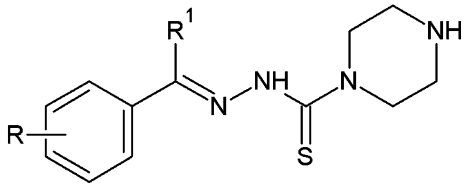
*N′-(diphenylmethylidene)piperazine-1-carbothiohydrazide (**3j**)* ^{1}H-NMR (DMSO-d_6, δppm): 2.4 (s, 1H, pip-NH), 3.15(t, 4H, pip-CH_2), 3.7(m, 4H, pip-CH_2), 7.5–8.1 (m, 10H, Ar–H), 10.5 (s, 1H, N–H); EI-MS (m/z): 325$[M+1]^+$; Elemental analyses Found (Calcd.) C, 66.16 (66.63); H, 5.82 (6.21); N 16.68 (17.27); S, 10.02 (9.88).

Antimicrobial study

Antibacterial studies

The antibacterial activities of the newly synthesized compounds (**3a**–**3j**) were tested using serial double dilution method against non-pathogenic strains of *E. coli* (NCIM 2068), *P. aeuroginosa* (NCIM 2967), *S. aureus* (NCIM 2079), and *B. subtilis* (NCIM 2921) and pathogenic strains of *V. cholera* and *protease* in nutrient agar medium by Cup-plate method. Sterilized media was cooled to 40 °C and 0.5 mL of inoculum for 100 mL of media was added. The flasks were shaken gently to avoid formation of air bubbles. This medium was transferred to Petri dishes of 9-cm diameter in 25 mL portions, so as to obtain 4-5 mm thickness of the media layer. The plates were left at room temperature to allow solidification of the media. In each Petri plate, four cups of suitable diameter were made with a sterile borer. All these procedures were conducted aseptically under laminar air flow workstation. The test compounds and Ciprofloxacin (Symed Lab India Pvt Ltd., Hyderabad, India) were dissolved in DMSO (0.5 %) and solution ranging between 0.1 and 100 μM were prepared. DMSO control was also maintained. Test compounds (40 μL) and standard (40 μL) were added into each cup with the help of a micropipette. Plates were kept undisturbed for at least 2 h at room temperature to allow for proper diffusion. Petri plates were then incubated at 37 ± 1 °C for 24 h. Zone inhibitions (in mm) were measured after incubation, and IC_{50} values are calculated by

Table 2 Antibacterial and antifungal activity of compounds **3a–3j**



Code	R	R1	IC_{50} (μM)*							
			E. coli	*P. aeuroginosa*	*S. aureus*	*B. subtilis*	*V. cholera*	*Protease*	*C. albicans*	*A. niger*
3a	*p*-OH	H	1.64	1.63	1.70	1.62	1.66	1.67	1.94	1.97
3b	*p*-OH	CH_3	3.48	3.51	1.58	3.49	1.59	1.21	2.56	3.55
3c	*m*-NO_2	CH_3	0.57	0.60	0.86	0.32	0.86	0.73	2.00	1.56
3d	*p*-OCH_3	H	1.59	1.57	0.79	0.66	1.53	2.41	2.15	2.09
3e	*p*-Cl	H	1.52	0.60	0.54	0.13	0.60	0.65	1.85	2.14
3f	H	CH_3	1.76	2.11	2.07	1.78	1.17	0.84	3.75	3.76
3g	H	H	3.91	1.97	1.36	0.93	2.23	1.78	1.85	2.21
3h	*p*-Cl	CH_3	0.88	0.71	1.04	0.98	0.78	0.98	3.37	3.32
3i	*p*-CH_3	H	1.74	1.70	1.66	1.70	3.57	1.26	3.72	3.79
3j	H	C_6H_5	3.03	1.44	1.33	1.38	1.38	4.44	1.47	1.62
CIP			0.03	0.05	1.20	4.70	0.20	0.20		
FLU									0.98	>10.00

* Mean value of triplicate

CIP Ciprofloxacin; *FLU* Fluconazole

plotting a graph between log concentrations and percentage inhibition values. All the studies were performed in triplicate and results were presented in Table 2.

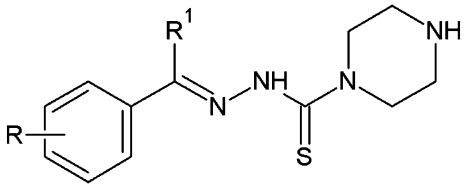
Antifungal studies

The antifungal activities of the test compounds were assayed using serial double dilution method against *C. albicans* and *A. niger* in Sabouraud dextrose agar medium by Cup–plate method. The sterile medium was inoculated using 24 h slant cultures of test organisms and transferred into sterile Petri dishes and allowed to solidify. Four cups of suitable diameter were made on the solidified media. The test compounds and Fluconazole (Symed Lab India Pvt Ltd, Hyderabad, India) were dissolved in DMSO (0.5 %) and solution ranging between 0.1 and 100 μM were prepared. DMSO control was also maintained. Test compounds (40 μL) and standard (40 μL) were added into each cup with the help of a micropipette. Zones of inhibition (in mm) were measured after 24 h of incubation and IC_{50} values are calculated by plotting a graph between log concentrations and percentage inhibition value. All the studies were performed in triplicate and results were presented in Table 2.

Anticancer studies (MTT assay)

The compounds **3a-3j** were evaluated for their anticancer activities on HBL-100 cell lines using MTT assay by serial double dilution method in 96-well plate. Cells seeded in plate at 5000 cells/well. Different dilutions of test and standard (0.1–100 μM) were made with growth medium in such a way that the final DMSO concentration is around 0.5 %. 100 μL of cell suspension and 100 μL of test and standard were transferred aseptically to each well. The plate was then incubated at 37 °C for 72 h in CO_2 incubator. After incubation, 20 μL of MTT was added to each well and plate was wrapped in aluminum foil to prevent the oxidation of the dye. The plate was again incubated for 2 h. 80 μL of lysis buffer was added to each well, and the plate was placed on a shaker overnight. The absorbance was recorded on the ELISA reader at 562-nm wavelength. The absorbance of the test was compared with that of DMSO control to get the percentage inhibition and IC_{50} values are calculated by plotting a graph between log concentrations and percentage inhibition value. All the studies were performed in duplicate and results were presented in Table 3.

Table 3 Anti-cancer activity of compounds **3a–3j**



Code	R	R1	IC_{50} (μM)*	
			HBL-100	HL-60 (48 h)
3a	*p*-OH	H	0.18	50
3b	*p*-OH	CH_3	0.54	60
3c	*m*-NO_2	CH_3	0.99	62
3d	*p*-OCH_3	H	1.62	NT
3e	*p*-Cl	H	1.6	69
3f	H	CH_3	0.29	NT
3g	H	H	2.41	63
3h	*p*-Cl	CH_3	0.26	NT
3i	*p*-CH_3	H	0.8	70
3j	H	C_6H_5	0.34	NT
MTX			0.04	NT

* Mean value of triplicate

MTX methotrexate, *NT* not tested

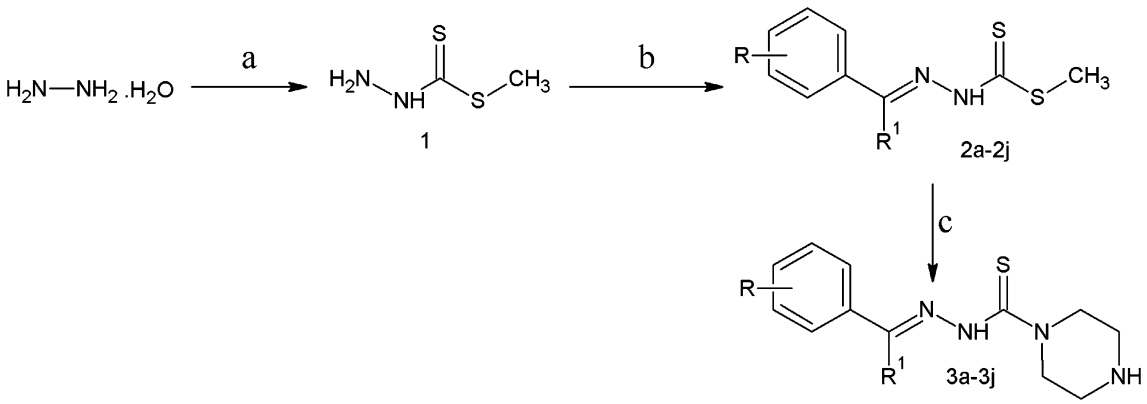
HL-60 cell line assay

The HL-60 human promyelocytic leukemia cell line was purchased from ATCC (American Type Culture Collection, Manassas, VA, USA). Cells were grown in RPMI 1640 medium supplemented with 10 % heat inactivated fetal calf serum (FCS), 1 % L-Glutamine and 1 % Penicillin–streptomycin in a humidified atmosphere containing 5 % CO_2. All media and supplements were obtained from Life Technologies (Paisley, Scotland, UK). Cell counts were determined using a microcell counter CC-108 (SYSMEX, Kobe, Japan). Cells growing in the logarithmic phase of growth were used for all experiments described below. HL-60 cells (0.1×10^6 per mL) were seeded in 25 cm^2 Nunc tissue culture flasks and incubated with increasing concentrations of drugs at 37 °C under cell culture conditions. Cell counts and IC_{50} values were determined after 24, 48, and 72 h using the microcell counter CC-108. Viability of cells was determined by trypan blue exclusion. Results were calculated as number of viable cells. All the studies were performed in triplicate and results were presented in Table 3.

Results and discussion

Chemistry

The final compounds **3a**–**3j** were synthesized following the synthetic route outlined in Scheme 1. Methyl hydrazine carbodithioate (**1**) was prepared by the reaction of hydrazine hydrate (85 %) with carbon disulfide in the presence of potassium hydroxide (Chetan *et al.*, 2010; Klayman *et al.*, 1979). Condensation of **1** with aromatic aldehydes/ketones in the presence of catalytic amount of sulfuric acid in methanol provided **2a**–**2j** (Klayman *et al.*, 1979; Chetan *et al.*, 2010). The final compounds **3a**–**3j** was synthesized by the reaction of piperazine with **2a**–**2j** in ethanol. The reaction comes to completion when evaluation of methyl mercaptan ceases (Chetan *et al.*, 2010; Klayman *et al.*, 1979). Intermediates were characterized by their IR-spectral and elemental analysis data. CHNS microanalysis revealed that variation in experimental values compared with calculated values is within ±0.4 %. Final compounds **3a**–**3j** were characterized by their ^{1}H-NMR and ES-MS spectral data. All the thiosemicarbazone derivatives (**3a–3j**) showed a characteristic peak for the aldehydic proton (=C–H) between δ 8.10–8.19 ppm as a singlet, ketonic methyl proton (–CH_3) between δ 1.65–2.3 ppm as a singlet and piperazine NH proton between δ 1.90–2.70 ppm as singlet.



Scheme 1 Reagents and conditions: *a* KOH/i-PrOH, CS, stirring <10 °C, 2.5 h; CH_3I, stirring, <10 °C, 3.5 h; *b* R-C_6H_4–CO-R/MeOH, H_2SO_4 [cat], reflux, 6–7 h; *c* Piperazine/EtOH, reflux

Eight protons of piperazine displayed a triplet or multiplet between δ 2.60 and 3.75 ppm and a multiplet between δ 3.70 and 4.15 ppm. The EI-MS spectra of all the compounds displayed $(M+1)^+$ peak. The structure, physicochemical characterization of compounds **3a–3j** is presented in Table 1.

Antimicrobial and anticancer studies

All ten thiosemicarbazone derivatives (**3a–3j**) were evaluated for their antibacterial/antifungal activity in serial double dilution method (Barry, 1986) against non-pathogenic strains of *Escherichia coli* (NCIM 2068), *Klebsiella pneumonia* (NCIM 2957), *Staphylococcus aureus* (NCIM 2079), and *Bacillus subtilis* (NCIM 2921) and pathogenic strains of *Vibrio cholerae* and *protease*. Similarly, they were also evaluated for their antifungal activity against *Candida albicans* and *Aspergillus niger*. The results are presented in Table 2. Compound **3c**, **3d**, **3e**, and **3h** were found to be more potent than the standard (Ciprofloxacin) used in the study against *S. aureus*. Thiosemicarbazones derived from benzaldehydes (**3g** and **3e**) were found to be better than those derived from acetophenones (**3f** and **3h**) except hydroxyl derivative (**3b**). Compound with chloro substitution at para position (**3e**) was found to be the most potent in this series. All the ten compounds (**3a–3j**) were found to be more effective than standard against *B. subtilis*. Surprisingly, in this case, also the thiosemicarbazones derived from benzaldehydes (**3g**, **3e** and **3a**) were more effective than those derived from acetophenones (**3f**, **3h** and **3b**). Compound **3e** with chloro substitution at para position was found to be potent within this series. In both the cases, antimicrobial activities were in the following order: *p*-Cl (**3e**) > *p*-OCH_3 (**3d**) > unsub. (**3g**) > *p*-CH_3 (**3i**). All the compounds were found to inhibit *C. albicans* at concentration between 1.47 and 3.75 μM. Thiosemicarbazone derived from benzophenone (**3j**) was found to be the potent within this series and almost equipotent to that of the standard (Fluconazole) used in this study. Against *A. niger*, all the ten compounds were found to be active at concentrations between 1.56 and 3.79 μM and better than the standard.

The compounds were also evaluated for their possible anticancer activity by MTT assay against HBL-100 cell lines (Grever *et al.*, 1992; Boyd and Paull, 1995; Monks *et al.*, 1991). The reports are presented in Table 3. All the compounds exhibited cytotoxic activity against HBL-100 cell lines at concentration between 0.18 and 2.21 μM. Compound **3a** was found to be potent within this series and was 4.5-fold less potent than standard (Methotrexate) used in the study. Thiosemicarbazones derived from acetophenones were found to be better than their benzaldehyde counterpart (except **3a**). The anticancer activity was in the following order: *p*-Cl (**3h**) ≥ unsub. (**3f**) > *p*-OH (**3b**).

Conclusion

The present investigation provided ten molecules (**3a–3j**) better than Ciprofloxacin against *B. subtilis* and four molecules (**3c**, **3d**, **3e**, and **3h**) better than Ciprofloxacin against *S. aureus*. Compounds **3j** was identified as lead molecules for the development of novel antifungal agent and thiosemicarbazones of substituted benzophenone derivatives may provide a promising candidate. Compound **3a** exhibited a potent anticancer activity against HBL-100 cell lines and none of the compounds exhibited an appreciable activity against HL-60 cell lines.

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