Full Paper

Synthesis, Antimicrobial and Anticancer Activity of New Thiosemicarbazone Derivatives

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Thiosemicarbazones of *p*-aminobenzoic acid (PABA) were synthesized and tested for their antimicrobial and anticancer activity. Hydroxamate derivatives **4a–4l** were found to have better antimicrobial and anticancer activity than their acid counterpart. Compound **4d** was found to have good antimicrobial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Vibrio cholerae*, and *Bacillus subtilis* with IC_{50} value of about 1 μM. Compound **4f** showed potent antifungal activity against *Candida albicans* ($IC_{50} = 1.29$ μM) and compound **4h** showed potent anticancer activity ($IC_{50} = 0.07$ μM).

Keywords: Anticancer / Antifungal / Antimicrobial / Hydroxamates / PABA derivatives / Thiosemicarbazones

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Introduction

It has been observed that during the last 10 years many major pathogenic bacteria and parasites have acquired resistance towards chemotherapeutic agents in market. Concern has been echoed in World Health Assembly (1998) with the adoption of a resolution on antimicrobial resistance [1]. This has raised fears that infectious diseases may once again become major cause of death in developing/developed countries. Now, there is a need to give serious consideration towards development of novel chemotherapeutic agents to combat Multi-Drug Resistant (MDR) strains. Sulphonamides, *p*-aminobenzoic acid (PABA) mimetics, launched in 1936 dominated the antibacterial market during the following 50 years [2]. However, derivatives of PABA did not yield a compound better than sulphonamides [3, 4]. PAS (4-amino salicylic acid), a hydroxy analogue of PABA is still on the market for the treatment of tuberculosis. Derivatives of PAS also failed to result in a molecule more active than PAS [5, 6].

Recently, many PABA derivatives were reported for their potential inhibitory property against novel antibacterial targets (MDR-associated proteins [7], NO-carrier [8], DNA binding ligand [9], antifungal [10], antiviral targets (neuraminidase [11], helicase [12]), and anticancer target (proteasome [13]). With this background we designed our molecule incorporating thiosemicarbazones from amino terminal and hydroxamate from carboxylic acid terminal of PABA. Thiosemicarbazones were considered for their antimicrobial & anticancer property [14] and hydroxamates were considered based on their inhibitory activity against bacterial peptide deformylase [15, 16].

Results and Discussion

Chemistry

The acid intermediates **3a–3l** and the hydroxamate final compounds **4a–4l** were synthesized following the synthetic route outlined in Scheme 1. Methyl hydrazine carbodithioate (**1**) was

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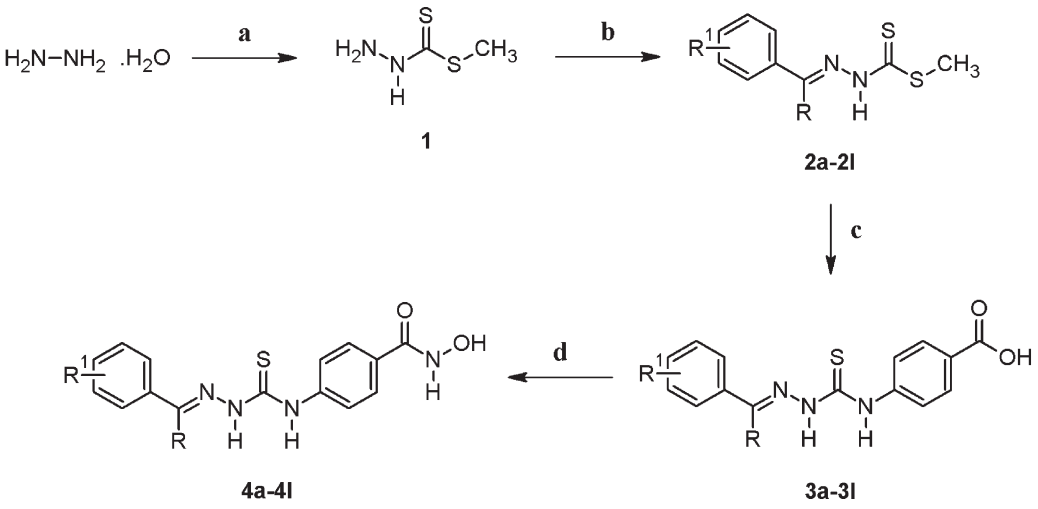


Scheme 1. Reagents and conditions: (a) KOH/*i*-PrOH, CS_2, stirring <10°C, 2.5 h; CH_3I, stirring, <10°C, 3.5 h; (b) R^1-C_6H_4-CO-R/MeOH, H_2SO_4 [cat], reflux, 6-7h; (c) PABA/EtOH, reflux; (d) C_6H_5OCOCl, $(Et)_3NH$, THF, stirring, rt, 4 h.

prepared by the reaction of hydrazine hydrate (85%) with carbon disulfide in the presence of potassium hydroxide [17, 18]. Condensation of **1** with aromatic aldehydes/ketones in the presence of catalytic amount of sulphuric acid in methanol provided **2a–2l** [17, 18]. Intermediate acids **3a–3l** were synthesized by the reaction of PABA with **2a–2l** in ethanol. The reaction comes to completion when evaluation of methyl mercaptan ceases [17, 18]. The final hydroxamate derivatives **4a-4l** were obtained by the reaction of **3a–3l** with phenyl chloroformate followed by hydroxylamine in THF at room temperature [19]. An equimolar amount of triethylamine was added to neutralize the hydrochloric acid liberated during the course of reaction. Intermediate acids **3a–3l** 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%. All acid derivatives (**3a–3l**) displayed characteristic O-H stretch (between 2921–3092 cm^{-1}), N-H stretch (between 3155–3298 cm^{-1}), and C=O stretch (between 1670–1735 cm^{-1}). The final hydroxamate derivatives **4a–4l** were characterized by their ^{1}H-NMR and ES-MS spectral data. All the hydroxamate derivatives (**4a–4l**) showed a characteristic peak for the hydroxamate -OH proton between δ 2.0–2.4 ppm as broad singlet, aldehydic =CH proton between δ 8.0–8.4 ppm as a singlet, ketonic $-CH_3$ proton between δ 1.6–1.8 ppm as a singlet and the methyl $-CH_3$ between 2.5–2.9 as a singlet. The structure, physico-chemical and spectral characterization of compounds **3a–3l** and **4a–4l** were presented in Tables 1a and 1b, respectively.

Antimicrobial and anticancer studies

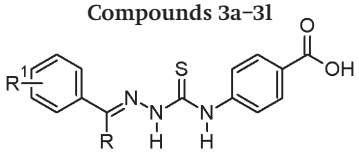
All twelve acid derivatives (**3a–3l**) and all twelve hydroxamate derivatives (**4a–4l**) were evaluated for their antibacterial activity in serial double dilution method against non-pathogenic strains of *Escherichia coli* (NCIM 2068), *Klebsiella pneumoniae* (NCIM 2957), *Staphylococcus aureus* (NCIM 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 *Asprgillus niger*. Only the hydroxamate derivatives (**4a–4l**) were evaluated for possible anticancer activity by MTT assay against HT-29 cell lines.

Hydroxamate derivatives (**4a–4l**) were found to show better antibacterial and antifungal activity than their acid counterpart (**3a–3l**). Compound **4d** was found to have good antibacterial activity against *K. pneumoniae*, *S. aureus*, *V. cholerae*, and *B. subtilis* at a concentration <1.0 μM. Whereas the chloro derivatives **4c** and **4f** showed good antibacterial activity against protease and *E. coli*, respectively. Thus electron pumping groups [-$N(CH_3)_2$] at the R^1 position along with a H at the R position favors antibacterial activity against *K. pneumoniae*, *S. aureus*, *V. cholerae*, and *B. subtilis.* Compounds **4c** and **4f** vary only at the R position. For both the chloro substitution at R^1 was found to be favorable. Whereas -H at the R position favors activity against protease, $-CH_3$ at the same position favors activity against *E. coli*. All the twelve hydroxamates (**4a–4l**) were found to be more active than ciprofloxacin against *B. subtilis*. Four compounds (**4a**, **4b**, **4d** & **4e**) were found to be better than ciprofloxacin against *S. aureus*. Further evaluation of these compounds and their analogues as peptide deformylase inhibitors is in progress.

Compound **4f** (1.29 μM) was found to show good antifungal activity against *C. albicans* and was found to be equivalent to fluconazole (0.98 μM). The compound **4f** was derived from 4-chloro acetophenones and shares similarity with few antifungals on the market (econazole, micanazole). In the case of

Table 1. Physico-chemical & spectral data.

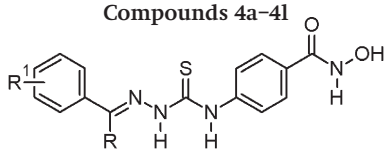
Compounds 3a–3l



3a-3l

Compd.	R	R^1	MF	MW	MP	IR (KBr, cm^{-1})[1]	EA[2]
3a	H	4-OCH_3	$C_{16}H_{15}N_3O_3S$	329	194–196	1680, 2986, 3298	CHNS
3b	H	4-OH	$C_{15}H_{13}N_3O_3S$	316	196–198	1697, 3047, 3182	CHNS
3c	H	4-Cl	$C_{15}H_{12}ClN_3O_2S$	333	206–208	1695, 2990, 3265	CHNS
3d	H	4-$N(CH_3)_2$	$C_{17}H_{18}N_4O_2S$	342	212–214	1695, 2945, 3295	CHNS
3e	CH_3	H	$C_{16}H_{15}N_3O_2S$	313	182–184	1698, 3050, 3195	CHNS
3f	CH_3	4-Cl	$C_{16}H_{14}ClN_3O_2S$	347	208–210	1670, 3020, 3190	CHNS
3g	H	H	$C_{15}H_{13}N_3O_2S$	299	260–262	1690, 2945, 3188	CHNS
3h	H	2-OH	$C_{15}H_{13}N_3O_3S$	315	224–226	1685, 3050, 3258	CHNS
3i	H	4-CH_3	$C_{16}H_{15}N_3O_2S$	313	216–218	1710, 3060, 3155	CHNS
3j	H	3,4-di-OCH_3	$C_{17}H_{17}N_3O_4S$	359	202–204	1735, 3045, 3295	CHNS
3k	CH_3	3-NO_2	$C_{16}H_{14}N_4O_4S$	358	220–222	1693, 2921, 3256	CHNS
3l		Isatin	$C_{16}H_{14}N_4O_4S$	340	246–248	1726, 3092, 3238	CHNS

Compounds 4a–4l

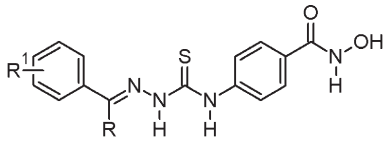


4a-4l

Compd.	R	R^1	MF	MW	MP	^{1}H-NMR (DMSO-d_6, δ ppm)	EI-MS (*m*/*z*)
4a	H	4-OCH_3	$C_{16}H_{16}N_4O_3S$	344	230–232	2.0 (s, 1H, -NHOH), 3.9 (s, 3H, -OCH_3), 6.6 (t, 2H, Ar-H), 7.3 (s, 2H, Ar-H), 7.6 (d, 2H, Ar-H), 7.8 (t, 2H, Ar-H), 8.0 (d, 1H, -NH), 8.2 (s, 1H, =CH), 10.9 (s, 1H, -NH-)	345 $[M + 1]^+$
4b	H	4-OH	$C_{15}H_{14}N_4O_3S$	330	228–230	2.2 (s, 1H, -NHOH), 6.6 (d, 2H, Ar-H), 6.8 (s, 2H, Ar-H), 7.6 (d, 2H, Ar-H), 8.0 (s, 1H, -NH), 8.2 (d, 1H, =CH), 9.6 (s, 1H, Ar-OH), 10.9 (s, 1H, -NH-)	331 $[M + 1]^+$
4c	H	4-Cl	$C_{15}H_{13}ClN_4O_2S$	348	240–242	1.8 (s, 1H, -NHOH), 6.6 (d, 2H, Ar-H), 7.3 (t, 2H, Ar-H), 7.5 (t, 4H, Ar-H), 8.0 (s, 1H, =CH) 8.2 (s, 1H, -NH), 11.2 (s, 1H, -NH-)	349 $[M + 1]^+$
4d	H	4-$N(CH_3)_2$	$C_{17}H_{19}N_5O_2S$	357	242–244	2.2 (s, 1H, -NHOH), 6.4 (d, 2H, Ar-H), 6.8 (s, 2H, Ar-H), 7.8 (t, 4H, Ar-H), 8.0 (s, 1H, -NH), 8.2 (d, 1H, =CH), 2.9 (q, 6H, -$N(CH_3)_2$), 11.2 (s, 1H, -NH-)	358 $[M + 1]^+$
4e	CH_3	H	$C_{16}H_{16}N_4O_2S$	328	226–228	1.8 (s, 3H, -CH_3), 2.2 (s, 1H, -NHOH), 6.5 (s, 2H, Ar-H), 7.3 (d, 1H, Ar-H), 7.5 (t, 4H, Ar-H), 7.8 (q, 2H, Ar-H), 8.2 (s, 1H, -NH), 11.3 (s, 1H, -NH-)	329 $[M + 1]^+$
4f	CH_3	4-Cl	$C_{16}H_{15}ClN_4O_2S$	362	234–236	1.6 (s, 3H, -CH_3), 2.0 (s, 1H, -NHOH), 6.6 (d, 2H, Ar-H), 7.3 (s, 1H, Ar-H), 7.6 (q, 4H, Ar-H), 7.8 (t, 2H, Ar-H), 8.0 (d, 1H, -NH), 11.5 (s, 1H, -NH-)	363 $[M + 1]^+$
4g	H	H	$C_{15}H_{14}N_4O_2S$	314	280–282	2.2 (s, 1H, -NHOH), 4.3 (s, 1H, -NH-Ar), 6.2 (d, 2H, Ar-H), 6.6 (d, 1H, Ar-H), 6.8 (d, 1H, Ar-H), 6.9 (t, 1H, Ar-H), 7.1 (t, 1H, Ar-H), 7.2 (t, 1H, Ar-H), 7.7 (d, 2H, Ar-H), 8.4 (s, 1H, =CH), 9.5 (s, 1H, -NH), 10.9 (s, 1H, =N-NH-)	315 $[M + 1]^+$
4h	H	2-OH	$C_{15}H_{14}N_4O_3S$	330	230–232	2.0 (s, 1H, -NHOH), 4.1 (s, 1H, -NH-Ar), 6.4 (d, 2H, Ar-H), 6.6 (d, 1H, Ar-H), 6.8 (d, 1H, Ar-H), 6.9 (t, 1H, Ar-H), 7.7	331 $[M + 1]^+$

Table 1. (*continued*)

Compounds 4a–4l



4a-4l

Compd.	R	R^1	MF	MW	MP	^{1}H-NMR (DMSO-d_6, δ ppm)	EI-MS (*m*/*z*)
4i	H	4-CH_3	$C_{16}H_{16}N_4O_2S$	328	222–224	(d, 2H, Ar-H), 7.1 (t, 1H, Ar-H), 8.1 (s, 1H, =CH), 8.2 (s, 1H, Ar-OH), 9.5 (s, 1H, -NH-OH), 10.4 (s, 1H, =N-NH-). 2.3 (s, 1H, -NHOH), 2.5 (s, 3H, CH_3), 4.0 (s, 1H, -NH-Ar), 6.2 (d, 2H, Ar-H), 6.6 (d, 1H, Ar-H), 6.8 (d, 1H, Ar-H), 6.9 (d, 1H, Ar-H), 7.1 (d, 1H, Ar-H), 7.7 (d, 2H, Ar-H), 8.2 (s, 1H, =CH), 8.5 (s, 1H, -NH), 10.7 (s, 1H, =N-NH-)	329 $[M + 1]^+$
4j	H	3,4-di-OCH_3	$C_{17}H_{18}N_4O_4S$	374	234–236	2.2 (s, 1H, -NHOH), 4.0 (s, 6H, -OCH_3), 6.7 (d, 1H, Ar-H), 7.5 (s, 2H, Ar-H), 7.6 (d, 2H, Ar-H), 7.8 (t, 2H, Ar-H), 8.0 (d, 1H, -NH), 8.4 (d, 1H, =CH), 10.8 (s, 1H, -NH-)	375 $[M + 1]^+$
4k	CH_3	3-NO_2	$C_{16}H_{15}N_5O_4S$	373	240–242	1.8 (s, 3H, CH_3), 2.2 (s, 1H, -NHOH), 4.3 (s, 1H, -NH-Ar), 6.2 (d, 2H, Ar-H), 6.6 (d, 1H, Ar-H), 6.9 (d, 1H, Ar-H), 6.8 (d, 1H, Ar-H), 7.1 (s, 1H, -NH), 7.7 (d, 2H, Ar-H), 8.4 (s, 1H, Ar-H), 10.7 (s, 1H, =N-NH-)	374 $[M + 1]^+$
4l		Isatin	$C_{16}H_{13}N_5O_3S$	355	260–262	2.4 (s, 1H, NHOH), 5.9 (s, 1H, NH-Ar), 7.0 (m, 8H, Ar-H), 9.2 (s, 1H, NH), 10.8 (s, 1H, NH), 13.9 (s, 1H, NHCO)	356 $[M + 1]^+$

[1] C=O, O–H, N–H stretch respectively, [2] variation between observed and calculated values are within ±0.4%.

A. niger, all twelve hydroxamates (**4a–4l**) were found to be better than fluconazole (>10 μM).

Compound **4h** (0.07 μM) has shown the best anticancer activity against HT-29 cell lines. The compound was six fold less potent than methotrexate (0.012 μM). The compound is of greater interest due to its similarity with **5c** that was reported by our group recently with dual inhibitory activity on HDAC8/RR [18] and **12c** that was reported for its thrombopoietic activity [20] (Fig. 1). Further evaluation of this compound and its derivatives are in progress.

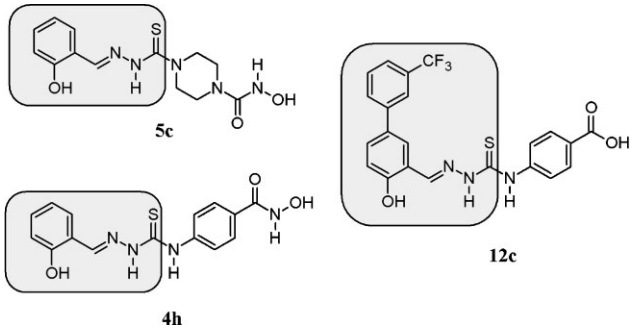


Figure 1. Similarity between compound **4h** with **5c** [18] & **12c** [20]. Compound **5c** has been reported for its dual HDAC8/RR inhibition.

Conclusion

The present investigation provided twelve molecules (**4a–4l**) better than ciprofloxacin against *B. subtilis* and four molecules (**4a**, **4b**, **4d** & **4e**) better than ciprofloxacin against *S. aureus*. Possibly they may inhibit bacterial peptide deformylase and further investigation in this direction is in progress. Compounds **4f** and **4h** were identified as lead molecules for the development of novel antifungal and anticancer candidates, respectively.

Experimental

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 4000–400 cm^{-1} using KBr discs. ^{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 δ were measured in ppm units 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 FAB-MS. The final yields and the physicochemical and spectral data of intermediates **3a–3l** and final compounds **4a–4l** are presented in Table 1.

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

To a cooled solution of potassium hydroxide (0.1 M, 6.6 g/7 mL) was added 2-propanol (7 mL) hydrazine hydrate (85% solution, 0.1 M, 6 mL) with stirring. Ice-cooled carbondisulfide (0.1 M, 10 mL) was added drop wise to the above stirred solution that was maintained <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.

*General procedure for the synthesis of Schiff bases methylhydrazine carbodithioate (**2a–2l**)*

Methyl hydrazinecarbodithioate **1** (0.01 M, 1.22 g) and (un)substituted aromatic aldehydes/ketone (0.012 M) were dissolved in methanol. To this mixture catalytic amount of concentrated sulphuric 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.

*General procedure for the synthesis of 4-[(2-arylidenehydrazyl)-carbothionyl]-aminobenzoic acid (**3a–3l**)*

PABA (0.005 M, 0.685 g) was added to appropriate Schiff base (**2a–2l**, 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.

*General procedure for the synthesis of N-hydroxy-4-[(2-arylidenehydrazinyl)-carbothionyl]-aminobenzamide (**4a–4l**)*

Phenyl chloroformate (0.001 M) and triethylamine (0.001 M) were added to an ice-cooled solution of appropriate benzoic acid derivative (**3a–3l**, 0.001 M) in dry THF and the mixture was stirred for 1 h. The solid obtained was filtered off and to the filtrate was added freshly prepared solution of hydroxylamine. After stirring at room temperature for 3 h, the solid obtained was filtered, dried and recrystallized from suitable solvent.

Antimicrobial study [21]

Antibacterial studies

The antibacterial activity of the test compounds was assayed using serial double dilution method against non-pathogenic strains of *E. coli* (NCIM 2068), *K. pneumoniae* (NCIM 2957), *S. aureus* (NCIM 2079), and *B. subtilis* (NCIM 2921) and pathogenic strains of *V. cholera* and protease in nutrient agar medium by the 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. 25 mL portions of this media were transferred to Petri dishes of 9 cm diameter 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, 4 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 (standard) were dissolved in DMSO (0.5%) and solution ranging between 0.1 and 100 μM were prepared. DMSO control was also maintained. 40 μL of the test compounds and standard 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 plotting a graph between log concentrations and percentage inhibition values. All the studies were performed in triplicate and results were presented in Table 2.

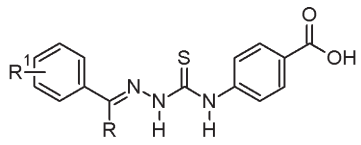
Antifungal studies

The antibacterial activity of the test compounds was 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. 4 cups of suitable diameter were made on the solidified media. The test compounds and fluconazole (standard) were dissolved in DMSO (0.5%) and solution ranging between 0.1 and 100 μM were prepared. DMSO control was also maintained. 40 μL of test compounds and standard 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.

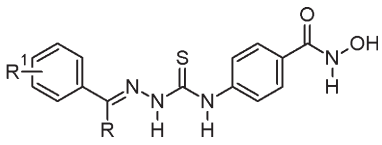
Anticancer studies (MTT assay) [22–24]

Compounds **4a–4l** were evaluated for their anticancer activity on HT-29 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

Table 2. Anti-microbial, anti-fungal & anti-cancer activity of the compounds **3a–3l** and **4a–4l**.



3a-3l



4a-4l

Compd.	R	R^1	IC_{50} (μM)*								
			E. coli	*K. pneumoniae*	*S. aureus*	*B. subtilis*	*V. cholera*	*Protease*	*C. albicans*	*A. niger*	*HT-29*
3a	H	4-OCH_3	1.64	1.45	2.95	1.54	1.44	2.99	3.01	2.96	–
3b	H	4-OH	1.38	1.67	1.72	1.70	1.37	1.60	3.08	3.12	–
3c	H	4-Cl	1.04	1.45	1.81	1.55	1.9	1.74	2.99	1.38	–
3d	H	4-$N(CH_3)_2$	1.56	1.17	1.62	1.63	1.41	2.86	1.34	1.68	–
3e	CH_3	H	1.78	1.38	1.69	1.23	1.66	1.77	3.10	3.13	–
3f	CH_3	4-Cl	1.39	1.45	1.79	1.28	2.81	2.81	2.82	2.84	–
3g	H	H	2.27	2.32	2.42	2.44	NT	2.74	1.73	2.89	–
3h	H	2-OH	1.71	1.89	2.40	1.54	NT	1.76	2.82	2.51	–
3i	H	4-CH_3	2.11	2.13	2.24	2.06	NT	1.94	2.36	1.59	–
3j	H	3,4-di-OCH_3	2.18	2.35	2.20	2.23	NT	2.25	2.45	1.29	–
3k	CH_3	3-NO_2	1.65	1.85	2.29	1.90	NT	1.47	2.31	1.47	–
3l	Isatin		2.08	2.48	2.31	2.14	NT	2.23	2.32	2.05	–
4a	H	4-OCH_3	1.09	1.73	1.09	1.09	0.99	1.36	1.56	2.88	4.20
4b	H	4-OH	1.16	1.25	0.96	1.28	1.21	1.26	2.98	1.55	3.49
4c	H	4-Cl	1.59	1.29	2.18	1.00	0.71	0.91	1.55	1.67	1.32
4d	H	4-$N(CH_3)_2$	1.35	0.89	0.98	0.98	0.67	1.62	1.83	1.69	0.31
4e	CH_3	H	1.14	1.22	1.17	0.86	1.06	1.59	3.00	1.44	0.62
4f	CH_3	4-Cl	0.85	1.10	1.23	0.97	1.6	1.19	1.29	1.67	0.25
4g	H	H	2.07	2.16	2.26	1.97	NT	2.48	1.54	1.45	0.10
4h	H	2-OH	1.36	1.25	1.91	0.98	NT	1.58	2.66	1.75	0.07
4i	H	4-CH_3	1.95	1.98	2.06	2.03	NT	1.76	2.16	1.11	0.14
4j	H	3,4-di-OCH_3	1.92	2.12	1.84	2.04	NT	2.02	1.65	1.10	0.15
4k	CH_3	3-NO_2	1.42	1.79	1.87	1.69	NT	1.24	1.55	0.99	0.13
4l	Isatin		1.53	2.14	2.15	1.94	NT	1.44	1.44	0.82	0.12
CIP			0.025	0.05	1.20	4.70	0.2	0.20			
FLU									0.98	>10	
MTX											0.012

* Mean value of triplicate; MTX: Methotrexate; CIP: Ciprofloxacin; FLU: Fluconazole.

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 2.

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