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Synthesis Antimicrobial and Anticancer Activity of 1-[(arylalkylidene)amino]-3-(4*H*-1,2,4-triazol-4-yl)thiourea

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Abstract: Arylalkylidene derivatives of aminotriazoles (**3a-3j**) were synthesized and tested for their antimicrobial and anticancer activity. Four non-pathogenic bacteria [*E. coli* (NCIM 2068), *K. pneumoniae* (NCIM 2957), *S. aureus* (NCIM 2079), *B. subtilis* (NCIM 2921)] two fungi [*C. albicans*, *A. niger*] and two cancer cell lines [HBL-100 and HT-29] were employed in the study. All the compounds were found to have better antibacterial activity against *B. subtilis* than Ciprofloxacin (standard) and compound **3i** was equivalent to Ciprofloxacin in inhibiting *S. aureas*. Similarly all the compounds inhibited the growth of *A. niger* better than Fluconazole and compound **3c** was equivalent to Fluconazole (standard) in inhibiting *C. albicans*. In case of anticancer activity none of the molecule exhibited activity better than the standard used (Methotrexate), though they have inhibitory concentration at submicromolar level.

Keywords: aminotriazoles; thiosemicarbazones; antimicrobial; antifungal; anticancer

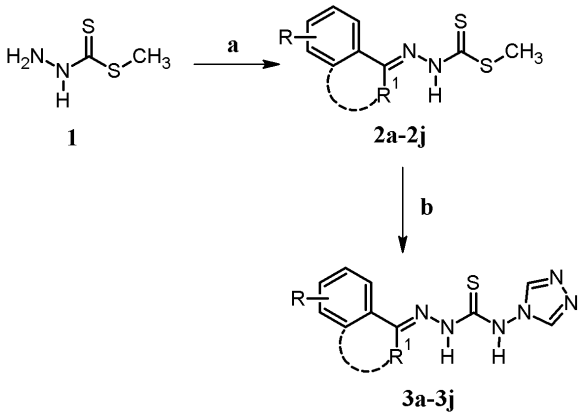
1. Introduction

Many major pathogenic bacteria and parasites have acquired resistance towards currently available antibiotics in the market during the last decade. This has led to the adoption of a resolution on antimicrobial resistance in World Health Assembly during 1998.[1] Development of superbugs has raised fears that infectious diseases may once again become major cause of death in developing/developed countries. Chemotherapeutic agents with novel structure and mode of action should be developed to combat the threat due to the superbugs. Thiosemicarbazones were very well known for their antimicrobial & anticancer property.[2-5] Thiosemicarbazones with free primary N4 amino group were reported for their anticancer property.[6-9] We reported anticancer thiosemicarbazones with secondary and tertiary N4 amino group.[10-13] The presented work elucidated the antimicrobial and anticancer activity of a novel triazol ring containing thiosemicarbazones.

2. Result and Discussion

2.1. Chemistry

The final compounds **3a-3j** was synthesized by following the synthetic route outlined in **Scheme 1**.



Scheme 1. Reagents and conditions: (a) R^1-C_6H_4-CO-R/MeOH, H_2SO_4 [cat], reflux, 6-7 h; (b) 1,2,4-triazol-4-amine/EtOH, reflux.

Methyl hydrazine carbodithioate (**1**) was prepared by the reaction of hydrazine hydrate (85%) with carbon disulfide in the presence of potassium hydroxide.[10, 14] Condensation of **1** with aromatic aldehydes/ketones in the presence of catalytic amount of sulphuric acid in methanol provided **2a–2j**.[10, 14] The final compounds **3a–3j** was synthesized by the reaction of 1,2,4-triazol-4-amine with **2a–2j** in ethanol. The reaction comes to completion when evaluation of methyl mercaptan ceases.[10, 14] The compounds (**3a-3j**) were characterized by their spectral (IR, ^{1}H-NMR & ES-MS) and elemental analysis data. CHNS microanalysis revealed that variation in experimental values compared with calculated values is within ±0.4%. All the thiosemicarbazone derivatives (**3a–3j**) displayed characteristic N-H stretch (between 3028-3266 cm^{-1}), C=N stretch (between 1661-1692 cm^{-1}), C-N stretch (between 1255-1389 cm^{-1}) and C=S stretch (between 1181-1259 cm^{-1}). All the derivatives (**3a–3j**) showed a characteristic peak for the aldehydic proton (=C-H) between δ 7.9– 8.9 ppm as a singlet, ketonic methyl proton (-CH_3) between δ 1.3-2.7 ppm as a singlet and aryl proton between δ 6.0-8.1 ppm as doublets or doublet and singlet, aryl proton of triazole between δ

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7.8-8.6 ppm as singlet, NH proton between δ 1.9-2.3 ppm as signlet, =N-NH proton between δ 11.0-11.5 ppm as singlet. The EI-MS spectra of all the compounds displayed $(M+1)^+$ or $(M-1)^+$ peak. The structure, physico-chemical characterization of compounds **3a–3j** was presented in **Table 1**.

Table 1. Physico-chemical characterization of **3a-3j**

Code	R	R^1	MF	MW	Y (%)	M.P (°C)
3a	4-Cl	H	$C_{10}H_9ClN_6S$	280	58.2	195-197
3b	4-OH	H	$C_{10}H_{10}N_6OS$	262	73.2	212-214
3c	4-OCH_3	H	$C_{11}H_{12}N_6OS$	276	54.4	201-203
3d	3,4-di-OCH_3	H	$C_{12}H_{14}N_6O_2S$	306	52.0	207-209
3e	4-$N(CH_3)_2$	H	$C_{12}H_{15}N_7S$	289	60.2	185-188
3f	3-NO_2	CH_3	$C_{11}H_{11}N_7O_2S$	305	74.0	190-194
3g	4-Cl	CH_3	$C_{11}H_{11}ClN_6S$	294	65.8	210-214
3h	4-OH	CH_3	$C_{11}H_{12}N_6OS$	276	79.7	222-224
3i	4-OCH_3	CH_3	$C_{12}H_{14}N_6OS$	290	73.3	219-221
3j	---Isatin---		$C_{11}H_9N_7OS$	287	50.0	239-241

2.2. Antimicrobial and anticancer studies

All ten thiosemicarbazone derivatives (**3a-3j**) 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 also they were evaluated for their antifungal activity against *Candida albicans* and *Asprgillus niger*.[15] The results were presented in **Table 2**. All the compounds were found to be 3-4 fold potent than standard (Ciprofloxacin) used in the study against *B. subtilis.* Almost all the compounds was found to have antibacterial activity against all the bacteria at a concentration <2.0 μM. Thiosemicarbazones derived from acetophenones (**3g, 3h** and **3i**) were found to be better than those derived from benzaldehydes (**3a, 3b** and **3c**). Among all acetophenone derivatives (**3f-3i**), compound **3i (**p-OCH_3) exhibited better antibacterial activity against *E. coli, K. pneumonia* and *S. aureus* (except *B. subtilis*). Among all benzaldehyde derivatives (**3a-3e**), compound **3b (**p-OH**)** has shown less potent than other derivatives. In the series 4-OH derivative derived from benzaldehyde as well as acetophenone exhibited less antibacterial activity than other compounds substituted with electron donating groups like 4-OCH_3, 3, 4-di-OCH_3 and 4-$N(CH_3)_2$.The compounds were also screened for antifungal activity in serial double dilution method against *C. albicans* and *A. niger*. The results were presented in **Table 2**. They were found to be 5-8-fold potent than standard (Fluconazole) used in the study against *A. niger*. Compound **3c** exhibited potency equivalent to Fluconazole against *C. albicans.* Almost all the compounds were found to have antifungal activity against both the strains at a concentration <2.0 μM. The acetophenone and corresponding benzaldehyde derivatives have shown almost same potency against *A. niger* and *C. albicans* except **3c.**

The compounds **3a-3j** were also evaluated for their anticancer activity against HBL-100 and HT-29 cell lines using MTT assay by serial double dilution method in 96-well plate.[16-18] The results are presented in **Table 2.** All the compounds displayed cytotoxic activity against HBL-100 cell lines at the concentration between 0.15 and 0.33 μM. Compound **3i** (0.15 μM) has shown the best anticancer activity against HBL-100 cell lines within this series and was nearly 4 fold less potent than standard Methotrexate (0.04 μM) used in the study. Thiosemicarbazones derived from acetophenones (**3g, 3h** and **3i**) were found to be better than their corresponding benzaldehyde derivatives (**3a, 3b** and **3c**). The anticancer activity was in the following order: 4-OCH_3>4-OH>4-Cl. In compound **3c** (4-OCH_3; IC_{50}= 0.16 μM) introduced additional substitution offered **3d** (3,4-diOCH_3; IC_{50} = 0.21 μM) and shown less potent. The presence of electron withdrawing group in acetophenone derivatives was found to reduce the potency.

Also all the compounds displayed cytotoxic activity against HT-29 cell lines at the concentration between 0.11 and 0.29 μM. Compound **3d** (0.11 μM) has exhibited the best anticancer activity against HT-29 cell lines within this series and was nearly 5 fold less potent than standard. Thiosemicarbazones derived from benzaldehydes were found to be better than their corresponding acetophenone derivatives (except **3b)**. Further evaluation of this compound and its derivatives are in progress.

3. Experimental

Materials and methods: Melting points were determined using Thermonik Melting Point Apparatus (Campbell electronics, India) by capillary method and are uncorrected. Infrared (IR) spectra were recoded on a Fourier Transform Infrared Spectrophotometer IR-Prestige 21 (Shimatzu Corporation, Japan) from 4000-400 cm^{-1} using KBr. ^{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). Electron Impact (EI) mass spectra were recorded on a VG 7070 H instrument (Micromass, UK) at 70 eV. 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.

3.1. Chemistry

3.1.1. General procedure for 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 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: 40 %; mp: 80-82 °C.[14]

3.1.2. General procedure for synthesis of Schiff's 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

Table 2. Antimicrobial and anticancer activity of **3a-3j**

Code	Antibacterial Activity (IC_{50} in μM)*				Antifungal Activity (IC_{50} in μM)*		MTT Assay (IC_{50} in μM)**	
	B. subtilis	*E. coli*	*K. pneumonia*	*S. aureus*	*C. albicans*	*A. niger*	HBL-100	HT-29
3a	1.82	1.79	1.63	1.38	1.78	1.62	0.27	0.12
3b	1.91	2.09	1.94	1.74	2.38	2.11	0.18	0.20
3c	1.65	1.65	1.18	1.51	0.98	1.82	0.16	0.22
3d	1.63	1.19	1.49	1.33	1.79	1.37	0.21	0.11
3e	1.34	1.34	1.33	1.63	1.58	1.39	0.29	0.23
3f	1.27	1.37	1.71	1.61	1.74	1.37	0.31	0.15
3g	1.32	1.23	1.37	1.42	1.95	1.94	0.21	0.25
3h	1.78	1.65	1.80	1.63	1.82	1.70	0.17	0.14
3i	1.30	0.79	1.16	1.27	1.24	1.23	0.15	0.29
3j	1.83	1.97	2.13	1.87	1.86	1.59	0.33	0.16
CIP	4.70	0.03	0.05	1.20	---	---	---	---
FLU	---	---	---	---	0.98	>10.00	---	---
MTX	---	---	---	---	---	---	0.04	0.02

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

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.

3.1.3. General procedure for synthesis of 2-Arylidene-N-(4H-1,2,4-triazol-4-yl)hydrazine-1-carbothioamide (3a-3j)

1,2,4-triazol-4-amine (0.0056 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 was almost completely ceased. Solvent present in the reaction mixture was evaporated under vacuum. The solid obtained was collected, washed with cold ethanol and further purified by recrystallization from suitable solvent.

*2-(4-Chlorobenzylidene)-N-(4H-1,2,4-triazol-4-yl)hydrazinecarbothioamide (**3a**)*

IR (KBr, cm^{-1}): 3264 (N-H), 1692 (C=N), 1278 (C-N), 1191 (C=S); ^{1}H-NMR (DMSO-d6, δ ppm): 2.0 (s, 1H, NH), 7.2 (d, 2H, Ar-H), 7.4 (d, 2H, Ar-H), 7.9 (s, 1H, CH), 8.6 (s, 2H, Ar-H), 11.0 (s, 1H, =N-NH); EI-MS (m/z): 281$[M+1]^+$; Elemental analysis Found (Calcd.): C, 42.64 (42.78); H, 3.30 (3.23); N, 30.68 (29.94); S, 11.62 (11.42).

*2-(4-Hydroxybenzylidene)-N-(4H-1,2,4-triazol-4-yl)hydrazinecarbothioamide (**3b**)*

IR (KBr, cm^{-1}): 3028 (N- H), 1682 (C=N), 1283 (C-N), 1195 (C=S); ^{1}H-NMR (DMSO-d6, δ ppm): 1.9 (s, 1H, N-H), 6.5 (d, 2H, Ar-H), 7.4 (d, 2H, Ar-H), 8.1 (s, 2H, Ar-H), 8.6 (s, 1H, C-H), 9.7 (s, 1H, OH), 11.5 (s, 1H, =N-NH); EI-MS (m/z): 263 $[M+1]^+$; Elemental analysis Found (Calcd.): C, 45.50 (45.79); H, 3.96 (3.84); N, 32.62 (32.04); S, 12.44 (12.22).

*2-(4-Methoxybenzylidene)-N-(4H-1,2,4-triazol-4-yl)hydrazinecarbothioamide (**3c**)*

IR (KBr, cm^{-1}): 3264 (N-H), 1683 (C=N), 1389 (C-N), 1195 (C=S); ^{1}H-NMR (DMSO-d6, δ ppm): 2.1 (s, 1H, NH), 2.5 (s, 3H, OCH_3), 7.0 (d, 2H, Ar-H), 7.5 (d, 2H, Ar-H), 7.8 (s, 2H, Ar-H), 8.2 (s, 1H, CH), 11.3 (s, 1H, =N-NH); EI-MS (m/z): 275$[M-1]^+$; Elemental analysis Found (Calcd.): C, 48.28 (47.81); H, 4.46 (4.38); N, 30.28 (30.41); S, 11.54 (11.60).

*2-(3,4-Dimethoxybenzylidene)-N-(4H-1,2,4-triazol-4-yl)hydrazinecarbothioamide (**3d**)*

IR (KBr, cm^{-1}): 3266 (N-H), 1673, (C=N), 1270 (C-N), 1183 (C=S); ^{1}H-NMR (DMSO-d6, δ ppm): 2.0 (s, 1H, N-H), 3.6 (s, 6H, OCH_3), 6.0 (d, 1H, Ar-H), 6.6 (d, 1H, Ar-H), 7.0 (s, 1H, Ar-H), 8.0 (s, 2H, Ar-H), 8.9 (s.1H, CH), 11.0 (s,1H, =N-NH); EI-MS (m/z): 305 $[M-1]^+$; Elemental analysis Found (Calcd.): C, 46.90 (47.05); H, 4.72 (4.61); N, 27.08 (27.43); S, 10.74 (10.47).

*2-[4-(Dimethylamino)benzylidene]-N-(4H-1,2,4-triazol-4-yl)hydrazinecarbothioamide (**3e**)*

IR (KBr, cm^{-1}): 3256 (N-H), 1688 (C=N), 1360 (C-N), 1259 (C=S); ^{1}H-NMR (DMSO-d6, δ ppm): 2.1 (s, 1H, N-H), 3.6 (s, 6H, CH_3), 7.3 (d, 2H, Ar-H), 7.6 (d, 2H, Ar-H), 8.2 (s, 2H, Ar-H), 8.5 (s, 1H, C-H), 11.1 (s, 1H, =N-NH); EI-MS (m/z): 290$[M+1]^+$; Elemental analysis Found (Calcd.): C, 51.32 (49.81); H, 5.28 (5.23); N, 34.19 (33.88); S 11.25 (11.08).

*2-[1-(3-Nitrophenyl)ethylidene]-N-(4H-1,2,4-triazol-4-yl)hydrazinecarbothioamide (**3f**)*

IR (KBr, cm^{-1}): 3265 (N-H), 1661 (C=N), 1255 (C-N), 1181 (C=S); ^{1}H-NMR (DMSO-d6, δ ppm): 1.3 (s, 3H, $3CH_3$), 1.9 (s, 1H, N-H), 7.2-7.4 (t, 3H, Ar-H), 7.7 (s, 1H, Ar-H), 8.5 (s, 2H, Ar-H), 11.3 (s, 1H, =N-NH); EI-MS (m/z): 306 $[M+1]^+$; Elemental analysis Found (Calcd.): C, 43.16 (43.27); H, 3.74 (3.63); N, 32.20 (32.11); S, 10.88 (10.50).

*2-[1-(4-Chlorophenyl)ethylidene]-N-(4H-1,2,4-triazol-4-yl)hydrazinecarbothioamide (**3g**)*

IR (KBr, cm^{-1}): 3256 (N-H), 1690 (C=N), 1360 (C-N), 1205 (C=S); ^{1}H-NMR (DMSO-d6, δ ppm): 1.4 (s, 3H, CH_3), 2.3 (s, 1H, N-H), 7.7 (d, 2H, Ar-H), 8.1 (d, 2H, Ar-H), 8.2 (s, 2H, Ar-H), 11.3 (s, 1H, =N-NH); EI-MS (m/z): 295 $[M+1]^+$; Elemental analysis Found (Calcd.): C, 44.76 (44.82); H, 3.88 (3.76); N, 28.94 (28.51); S, 10.98 (10.88).

*2-[1-(4-Hydroxyphenyl)ethylidene]-N-(4H-1,2,4-triazol-4-yl)hydrazinecarbothioamide (**3h**)*

IR (KBr, cm^{-1}): 3264 (N-H), 1683, (C=N), 1283 (C-N), 1210 (C=S); ^{1}H-NMR (DMSO-d6, δ ppm): 2.0 (s, 1H, N-H), 2.7 (s, 3H, CH_3), 6.8 (d, 2H, Ar-H), 7.3 (d, 2H, Ar-H), 8.3 (s, 2H, Ar-H), 9.6 (s, 1H, OH), 11.3 (s, 1H, =N-NH); EI-MS (m/z): 275 $[M-1]^+$; Elemental analysis Found (Calcd.): C, 47.86 (47.81); H, 4.42 (4.38); N, 30.36 (30.41); S, 11.83 (11.60).

*2-[1-(4-Methoxyphenyl)ethylidene]-N-(4H-1,2,4-triazol-4-yl)hydrazinecarbothioamide (**3i**)*

IR (KBr, cm^{-1}): 3265 (N-H), 1682 (C=N), 1283 (C-N), 1201 (C=S); ^{1}H-NMR (DMSO-d6, δ ppm): 1.9 (s,1H, NH), 2.7 (s, 3H, CH_3), 4.3 (s 3H, OCH_3), 7.6 (d, 2H, Ar-H), 7.9 (d, 2H, Ar-H), 8.1 (s, 2H, Ar-H), 11.1 (s, 1H, =N-NH); EI-MS (m/z): 291 $[M+1]^+$; Elemental analysis Found

(Calcd.): C, 49.72 (49.64); H, 5.02 (4.86); N, 29.06 (28.95); S, 11.35 (11.04).

*2-(Isatin-3-ylidene)-N-(4H-1,2,4-triazol-4-yl)hydrazinecarbothioamide (**3j**)*

IR (KBr, cm^{-1}): 3112 (N-H), 1670 (C=N), 1285 (C-N), 1206 (C=S); ^{1}H-NMR (DMSO-d6, δ ppm): 2.0 (s, 1H, NH), 6.7-7.1 (m,4H, Ar-H), 8.0 (s, 2H, Ar-H), 10.0 (s, 1H, NHCO), 11.0 (s, 1H, =N-NH); EI-MS (m/z): 288 $[M+1]^+$; Elemental analysis Found (Calcd.) C, 45.28 (45.99); H, 3.24 (3.16); N, 33.74 (34.13); S, 11.63(11.16).

3.2. Antimicrobial study

3.2.1. Antibacterial studies

The antibacterial activities of the newly synthesized compounds (**3a-3j**) were tested using serial double dilution method[15] against non-pathogenic strains of *E. coli* (NCIM 2068), *K. pneumoniae* (NCIM 2957), *S. aureus* (NCIM 2079) and *B. subtilis* (NCIM 2921) in nutrient agar medium by Cup-plate method. Sterilized media was cooled to 40 °C and 0.5 mL of inoculums 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, 4 cups of suitable diameter were made with a sterile borer. All these procedures were conducted aseptically under laminar air flow workstation (Elite; Elite Scientific and Equipments). The test compounds and Ciprofloxacin 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 of inhibitions (in mm) were measured after incubation and IC_{50} values were 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**.

3.2.2. 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.[15] 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 were dissolved in DMSO (0.5% v/v) 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 were 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**.

3.2.3. Anticancer studies (MTT assay)

The compounds **3a-3j** were evaluated for their anticancer activities on HBL-100 and HT-29 cell lines using MTT assay by serial double dilution method in 96-well plate.[16-18] 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% v/v. 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 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide give the chemical name of the dye (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. 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 2**.

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