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Original Article

Thia-, Oxa-, and Aza Heterocyclic Derivatives Based on Thiochroman- and Benzothiepine 1,1-dioxide Moieties: Syntheses, Characterization, Antimicrobial-, Antituberculosis-, and Anticancer Activity

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Abstract

A series of thia-, oxa-, and aza heterocyclic derivatives based on 3,4-dihydro-1-benzothiepin-5(2*H*)-one 1,1-dioxide, and 2,3-dihydro-4*H*-thiochroman-4-one 1,1-dioxide moieties were prepared from the diketoesters **2** and **11.** All derivatives were evaluated on a few Gram-positive and Gram-negative bacterial strains, as well as the fungal strains. It would also be noticed that the benzodiazepine derivatives **9** and **18** exhibited better antibacterial potentials than the rest. 1,2-Diphenylethane-1,2-diamine substituted thiochromeno- and benzothiepino derivatives **9** and **18** showed good inhibitory activity against MTB ($H_{37}Rv$) at MIC 7.0 and 6.5 µM. Compounds **9** and **18** were found to exhibit higher cytotoxic potency (15, 13 µM and 14, 12 µM) than that of doxorubicin (21 µM and 19 µM) against HeLa cell and HCT116 cell.

Key words: Thiochroman, benzothiepine, antimicrobial, antituberclosis, anticancer

INTRODUCTION

Thiochroman-1,1-dioxide unit is found to be an important core in numerous bioactive compounds with a wide range of biological activity including insecticidal-, antifungal-, herbicidal-, anti-hepatitis-, antitumor-, anti-inflammatory-, anticancer-, anti-HIV-1-, and anti-tubercular [1] properties. Seto *et al*. discovered that replacement of benzocycloheptene ring with 1-benzothiepine-1,1-dioxide unit enhanced the CCR5 antagonistic activity [2]. Pyrazole derivatives are reported to have a broad spectrum of biological activities, such as antitumour-, anticoagulant-, analgesic-, antipyretic-, antimicrobial-, and hypoglycemic activity [3]. These derivatives are useful in drug development process [4]. Isoxazoline derivatives have been reported to possess antifungal-, antibacterial-, anticonvulsant-, anti-inflammatory-, antiviral-, analgesic-, anticancer-, antitubercular-, and antioxidant [5] properties. Pyrimidine unit is involved in a broad spectrum of pharmacophores displaying bactericidal-, fungicidal-, analgesic-, antihypertensive-, and antitumor activity [6]. Among the pyrimidine containing heterocycles, thiouracils are used as anti-inflammatory-, and virucidal agents [7]. Benzodiazepines constitute an important class of biologically active compounds and their synthesis has been receiving much attention in the field of medicinal- and pharmaceutical chemistry owing to their applications as anticonvulsant-, anti-inflammatory-*etc*. They also act as HIV inhibitors and farnesyl transferase inhibitors [8]. It has been known that compounds containing pharmacophores such as CNNC, NCCN, and CS (=O)(=O)C display several bioactivity [9]. In view of the above mentioned facts and in continuation of our interest in the synthesis of sulphur-containing heterocycles [10], we report herein the synthesis, antimicrobial-, antituberculosis-, and anticancer evaluation of structure hybrids incorporating the 3,4-dihydro-1-benzothiepin-5(2H)-one 1,1-dioxide and 2,3-dihydro-4H-thiochromen-4-one 1,1-dioxide moieties with the pyrazole/isoxazole/ pyrimidine/ benzodiazepine ring systems. Earlier we have reported [11] the synthesis and bioactivity of the sulfide counterparts reported in this article.

RESULTS AND DISCUSSION

Chemistry: The strategies adopted for the synthesis of the intermediates and target compounds are depicted in Fig. 1 Scheme 1.

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Compounds **1** and **10** were prepared as per the reported methods [12]. Addition of 1 equiv of 2,3-dihydro-4H-thiochromen-4-one 1,1-dioxide (**1)** or 3,4-dihydro-1-benzothiepin-5(2H)-one 1,1-dioxide (**10**) to diethyl oxalate in ethanol at room temperature in the presence of 2 equiv of a base, afforded the Claisen condensation products **2** and **11** respectively. Subsequent reaction of 1 equiv of the mixture **2** with 1.15 equiv of phenylhydrazine hydrochloride/hydrazine hydrochloride under reflux in ethanol afforded the pyrazole derivatives **3** and **4**. Similarly **12** and **13** were obtained from **11**. The isoxazole moiety in compounds **5** and **14** was constructed from the diketoester **2** and **11** with 1.5 equiv of hydroxylamine hydrochloride under reflux in ethanol for 4h and the pyrimidine derivatives **6**, **7**, **15**, and **16** were prepared from the diketoester **2/11** with 1.5 equiv of urea/thiourea under reflux in acetic acid for 5h. The benzodiazepine derivatives **8**, **9**, **17**, and **18** were prepared from the condensation of the diketoester mixtures **2/11** with 1.5 equiv of *o*-phenylenediamine/1,2-diphenylethane-1,2-diamine. The structures of compounds **3-9** and **12-18** have been derived on the basis of their IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data (*vide* supplementary material). The IR spectra of pyrazole derivatives (**3**, **4**, **12**, and **13**) show the carbonyl absorption band at 1703-17011 cm^{-1} and sulfone absorption bands at 1151-1157 (sym) and 1309-1313 (asym) cm^{-1}. The ^{1}H-NMR spectra of compounds **3**, **4**, **12**, and **13** show a triplet at 1.2-1.3 ppm (-CH_3), a quartet at 4.2-4.4 ppm (-O-CH_2), and a multiplet in the region 6.8-7.8 ppm (aromatic protons). Compounds **3** and **4** have a singlet a 4.5 and 4.6 ppm (-SO_2-CH_2) respectively while compounds **12** and **13** display two triplets at 3.1 (-SO_2-CH_2-C$\underline{H}_2$) and 3.7 (-SO_2-C$\underline{H}_2$-CH_2) ppm respectively. The carbonyl carbon absorbs in the region 160.08-162.54 ppm in the ^{13}C-NMR. Formation of isoxazoline ring in **5** and **14** is confirmed by the appearance of bands in the region 1595 and 1586 due to C=N-O_{str}. Compounds **5** and **14,** display characteristic strong bands at 1702 and 1697 cm^{-1} that are assigned to the stretching of C=O and bands at 1151-1158 (sym) and 1310-1317 (asym) cm^{-1} are due to sulfone absorption. The ^{1}H-NMR of compounds **5** and **14** showed a triplet at 1.2-1.3 ppm (CH_3), a quartet at 4.2-4.3 ppm (-O-CH_2) and a multiplet in the region 6.9-7.8 ppm (aromatic protons). In addition, compound **5** showed a singlet δ 4.6 (-SO_2-CH_2), while compound **14** showed two triplets at 3.2 (-SO_2-CH_2-C$\underline{H}_2$) and 3.8 (-SO_2-C$\underline{H}_2$-CH_2) ppm. The carbonyl carbon absorbs at 166.01 and 167.1 ppm for **5** and **14** respectively in the ^{13}C-NMR. IR spectra of the pyrimidine derivatives (**6**, **7**, **15**, and **16**) showed a strong band in the region 1689-1701 cm^{-1} for the ester carbonyl. Compounds **7** and **16** displayed $\upsilon_{C=S}$ at 1135 and 1151 cm^{-1} respectively and the sulfone absorption bands at 1155-1164 (sym) and 1316-1322 (asym) cm^{-1}. The ^{1}H-NMR spectra of compounds **6**, **7**, **15**, and **16** showed a triplet at 1.2-1.3 ppm (-CH_3), a quartet at 4.2-4.4 ppm (-O-CH_2), and a multiplet in the region 6.5-7.8 ppm (aromatic protons). Whereas compounds **6** and **7** have a singlet at 4.4-4.8 ppm (-SO_2-CH_2), compounds **15** and **16** display two triplets at 3.0-3.3 (-SO_2-CH_2-C$\underline{H}_2$) and 3.7-3.9 (-SO_2-C$\underline{H}_2$-CH_2) ppm. The ester C=O absorbs at 161.07-163.8 ppm, the NHCO at 157.9-158.5 ppm (**6** and **15**), and the C=S (**7** and **16**) absorbs at 180.07-181.21 ppm in the ^{13}C-NMR. IR spectra of the benzodiazepine derivatives (**8**, **9**, **17**, and **18**) showed carbonyl absorption band at 1696-1705 cm^{-1}. The ^{1}H-NMR spectra of compounds **8**, **9**, **17**, and **18** show a triplet at 1.0-1.3 ppm (-CH_3), a quartet at 4.4-4.6 ppm (-O-CH_2), and a multiplet in the region 6.5-7.5ppm (aromatic protons). Compounds **8** and **9** display a singlet at 4.3-4.4 ppm (-SO_2-CH_2), while compounds **17** and **18** display two triplets at 3.1-3.2 (-SO_2-CH_2-C$\underline{H}_2$), and 3.6-3.8 (-SO_2-C$\underline{H}_2$-CH_2) ppm. The methine protons of compounds **9** and **18** resonate in the range 3.2 and 4.8 ppm. The carbonyl carbons of **8**, **9**, **17**, and **18** absorb at 161.3-166.7 ppm in the ^{13}C-NMR.

BIOLOGICAL EVALUATION

Antimicrobial studies: All the fourteen newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus*, and *Streptococcus pneumoniae*, as examples of Gram-positive bacteria and *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* as examples of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *Candida albicans*, *Aspergillus flavus*, and *Aspergillus niger* fungal strains. Agar-diffusion method was used for the determination of the preliminary antibacterial- and antifungal activity. Chloroamphenicol, amikacin, and clotrimazole were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial- or fungal growth around the discs in mm. The minimum inhibitory concentration (MIC) measurement for these compounds showed significant growth inhibition zones (>20 mm) by a two-fold serial dilution method [13]. The MIC (µg/mL) and inhibition zone diameter values are recorded in Table 1.

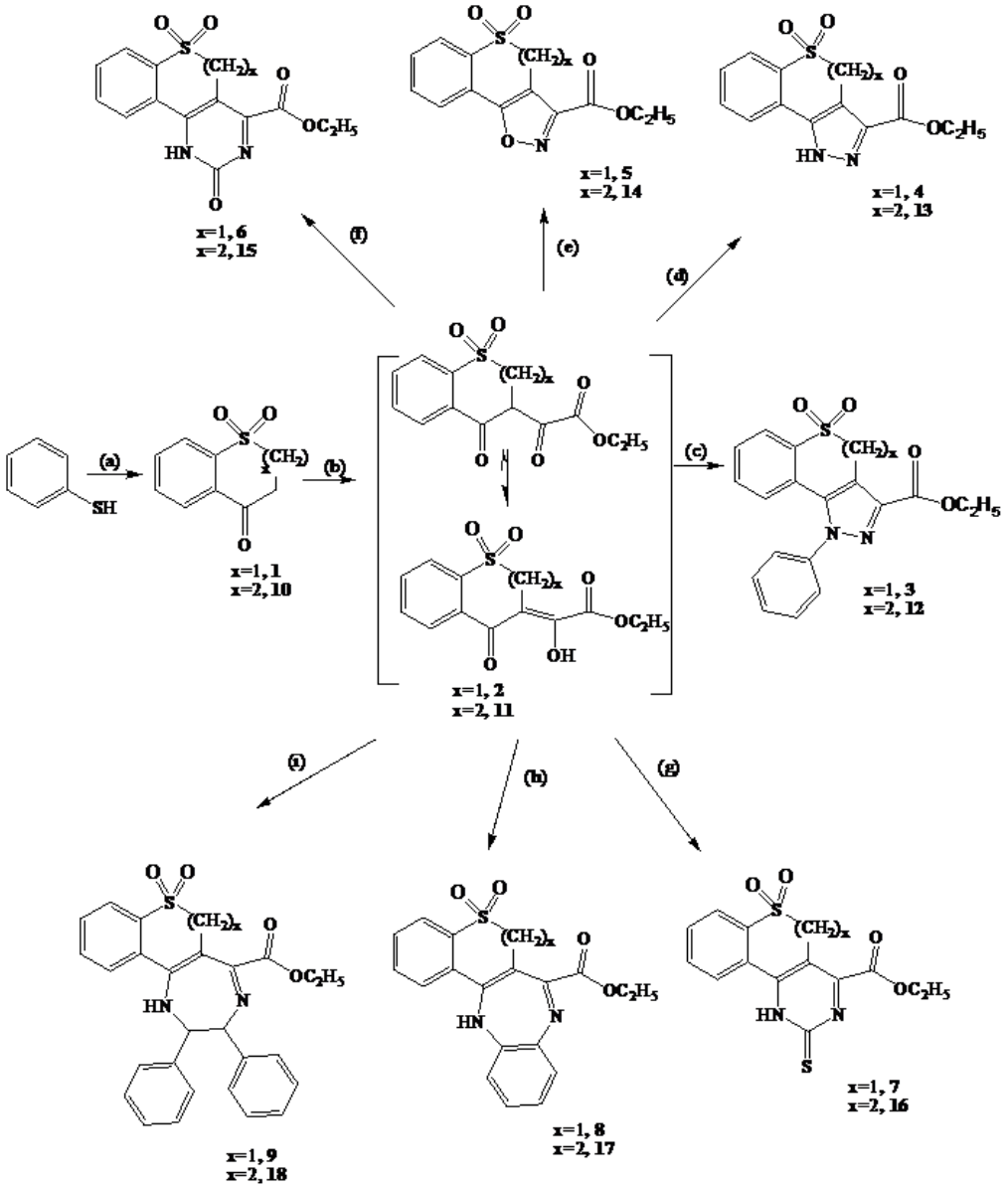

Fig. 1 Scheme 1 Synthetic route for the pyrazole-, isoxazole-, pyrimidine-, and benzodiazepine analogues (**3-9 and 12-18**) containing thiochromeno- and benzothiepino-1,1-dioxide moieties. Reagents and conditions: (a) $Cl(CH_2)_{1or2}COOH/NaOH$; PPA (b) Na, dry EtOH, $(COOEt)_2$ (c) $C_6H_5NHNH_2.HCl$, EtOH (d) $NH_2NH_2.HCl$, EtOH (e) $NH_2OH.HCl$, EtOH (f) urea, CH_3COOH (g) thiourea, CH_3COOH (h) o-phenylenediamine, CH_3COOH (i) 1,2-diphenylethane-1,2-diamine, CH_3COOH

Table 1 Minimal inhibitory concentrations (MIC, µg/mL) and inhibition zone (mm) of compounds **3-9** and **12-18**.

Compound No.	MIC in µg/mL, and zone of inhibition (mm)							
	Bacteria					Fungi		
	Gram-positive bacteria			Gram-negative bacteria				
	Staphylo-coccus aureus	*Strepto-coccus pneumoniae*	*Klebsiella pneumoniae*	*Pseudo-monas aeruginosa*	*Escherichia coli*	*Candida albicans*	*Aspergillus flavus*	*Aspergillus niger*
3	25 (23-25)	25 (20-22)	25(20-22)	12.5 (21-23)	25 (23-25)	25 (23-25)	50(20-22)	25(21-23)
4	25 (26-28)	25 (28-30)	25 (30-32)	12.5 (30-32)	25 (28-30)	12.5 (27-29)	25 (24-26)	25 (26-28)
5	25 (26-28)	25 (26-28)	12.5 (26-28)	12.5 (23-25)	25 (24-26)	25 (22-25)	25 (23-25)	12.5 (24-26)
6	25 (24-26)	12.5 (21-23)	6.25(23-25)	12.5 (26-28)	6.25 (24-26)	6.25 (25-27)	12.5(25-28)	6.25 (26-28)
7	25 (25-27)	12.5 (22-24)	6.25 (24-26)	12.5 (27-29)	6.25 (25-27)	6.25 (26-28)	12.5 (25-27)	6.25 (26-28)
8	25 (24-26)	25 (25-27)	6.25 (25-27)	12.5 (23-25)	6.25 (24-26)	6.25 (27-29)	12.5 (23-25)	6.25 (23-25)
9	6.25 (25-26)	6.25 (26-28)	3.125 (26-28)	6.25 (26-28)	3.125 (23-25)	3.125 (25-27)	6.25 (25-27)	3.125 (23-25)
12	25 (24-26)	25 (21-23)	25(21-23)	12.5 (24-26)	25 (25-27)	25 (23-25)	50(23-25)	25(22-25)
13	25 (26-28)	25 (25-27)	25 (31-33)	12.5 (30-32)	25 (28-30)	12.5 (28-30)	25 (26-28)	25 (26-28)
14	25 (28-30)	25 (28-30)	12.5 (27-29)	12.5 (25-27)	25 (26-28)	25 (23-25)	25 (26-28)	12.5 (26-28)
15	25 (26-28)	12.5 (23-25)	6.25(25-27)	12.5 (28-30)	6.25 (27-29)	6.25 (27-29)	12.5(27-29)	6.25 (27-29)
16	25 (25-27)	12.5 (25-27)	6.25 (26-28)	12.5 (25-27)	6.25 (26-28)	6.25(28-30)	12.5 (28-30)	6.25 (26-28)
17	25 (26-28)	25 (27-30)	6.25 (25-27)	12.5 (27-29)	6.25 (24-26)	6.25 (26-28)	12.5 (25-27)	6.25 (26-28)
18	6.25 (28-30)	6.25 (26-28)	3.125 (26-28)	6.25 (27-30)	3.125 (26-28)	3.125 (27-29)	6.25 (27-29)	3.125 (25-27)
Chloram-phenicol	3.125 (26-28)	6.25 (28-31)	6.25 (28-30)	6.25 (27-29)	6.25 (28-30)	NT	NT	NT
Amikacin	6.25 (28-30)	6.25 (31-33)	3.125 (37-40)	6.25 (28-30)	6.25 (27-29)	NT	NT	NT
Clotrimazole	NT	NT	NT	NT	NT	6.25 (28-30)	6.25 (29-31)	3.125 (27-29)

NT-Not Tested

The results depicted in Table 1 reveal that most of the tested compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains, as well as on the fungal strains. In general, these compounds revealed better activity against the Gram-negative rather than the Gram-positive bacteria. It would also be noticed that the benzodiazepine derivatives (**9** and **18**) exhibited better antibacterial potentials than the rest. Regarding the structure-activity relationship (SAR) of the pyrazolecarboxamides against Gram-negative bacteria, the results reveal that the pyrazole-, isoxazole-, pyrimidine-, and benzodiazepine derivatives exhibited broad spectrum antibacterial profile against the tested organisms. In this view, 1,2-diphenylethane-1,2-diamine substituted thiochromeno- and benzothiepino derivatives **9** and **18** were found to exhibit higher activity (MIC 3.125 µg/mL) than that of chloroamphenicol (MIC 6.25 µg/mL) against *Klebsiella pneumoniae* and *Escherichia coli*. The benzodiazepine derivatives displayed higher activity than amikacin in inhibiting the growth of *Escherichia coli* (MIC 3.125 µg/mL). Regarding the activity of pyrazole-, isoxazole-, pyrimidine-, and benzodiazepine derivatives against antifungal strains, the results revealed that the isoxazole-, and pyrimidine derivatives showed a comparable activity against clotrimazole. Benzodiazepine derivatives are equipotent to clotrimazole in inhibiting the growth of *Candida albicans* (MIC 3.125 µg/mL).

***In vitro* antituberculosis activity:** Recently Hadda and coworkers have reported the synthesis and evaluation of the bioactivity of spirothiochromanone (STC) derivatives against *Trypanosoma cruzi* [14]. These authors have described the importance of spirothichromanone (STC)-pharmacophore sites for the potent antituberculosis activity. This prompted us to screen all the compounds synthesized by us for their *in vitro* antituberculosis activity against MTB ($H_{37}Rv$). The primary screening was carried out by agar-dilution method using two-fold dilution techniques. Isoniazid (INH) was used as the standard. The observed data on the antituberculosis activity of the title compounds and the standard drug are given in Table 2. Ten compounds were found to be active with minimum inhibitory concentrations of 6.5-16 µM. 1,2-Diphenylethane-1,2-diamine substituted thiochromeno- and benzothiepino derivatives **(9** and **18)** showed good inhibitory activity against MTB ($H_{37}Rv$) at MIC 7.0 and 6.5 µM, while compounds **8** and **17** showed moderate inhibitory activity with MIC 9.2-9.0 µM (Table 2).

Table 2 Antituberculosis activity and anticancer activity of compounds **3-9** and **12-18**

Compound No.	Antituberculosis activity MIC (µM)	Anticancer activity IC_{50}(µM)[a]	
		HeLa cell	HCT116
3	14.8	33	32
4	13.2	34	33
5	12.5	26	24
6	14	27	26
7	8.8	30	29
8	13.5	23	22
9	6.7	13	11
12	13.5	31	29
13	11.5	32	31
14	11	26	25
15	13	25	24
16	8.0	27	25
17	10	21	20
18	6.2	13	11
INH	8.5	---[b]	---[b]
Doxorubicin[c]	---[b]	20	18

Negative control DMSO, no activity; [a] The IC_{50} value is defined as the concentration at which 50% survival of cells was observed; [b]Not Tested; [c] Used as a positive control.

***In vitro* antitumor activity:** The newly synthesized compounds **3-9** and **12-18** were initially screened at a single concentration of two-fold dilution using the colorimetric MTT to test their *in vitro* cytotoxicity against HeLa (cervical cancer cells) and HCT116 (colon cancer cells). Doxorubicin was used as the reference drug in this study. The cytotoxicity of the tested compounds was estimated in terms of percent growth inhibition compared to untreated control cells. All the compounds effected >70% inhibition and were retested by a two-fold dilution from 6.25 to 100 µM. The results are expressed as IC_{50} (inhibitory concentration 50%), the concentration of the compound which inhibits the tumor cell growth by 50%, and the data are presented in Table 2 and Figs. 2 and 3. Cell growth inhibition was analyzed by MTT assay and the results show that the compounds **3-9** and **12-18** exhibit an inhibitory effect on the proliferation of HeLa and HCT116 cells in a dose-dependent manner (Table 2). Compounds **9** and **18** were found to exhibit higher cytotoxic potency (15, 13 µM and 14, 12 µM) than that of doxorubicin (21 µM and 19 µM) against HeLa cell and HCT116 cell. Of note is that, the 1,2-diphenylethane-1,2-diamine substituted thiochromeno- and benzothiepino derivatives **9** and **18** possess higher activity than the rest on both the cancer cells. Maya *et al* have reported recently the antitumor activity of some benzothiepino derivatives [15] on HCT-15. But similar derivatives (**9** and **18**) prepared by us exhibited better IC_{50} values. The o-phenylenediamine substituted compounds **8** and **17** showed comparable IC_{50} values than the other substituted compounds on both the cells. In general, many of the IC_{50} values for HCT116 cells are lower than those for the corresponding HeLa cells. The sulfone analogues displayed better antimicrobial-, antituberculosis-, and antitumor activity against the sulfide analogues [17]. Compounds **9** and **18** exhibited better bioactivity than other derivatives. These compounds contain the benzodiazepine scaffold as the previleged structure [18].

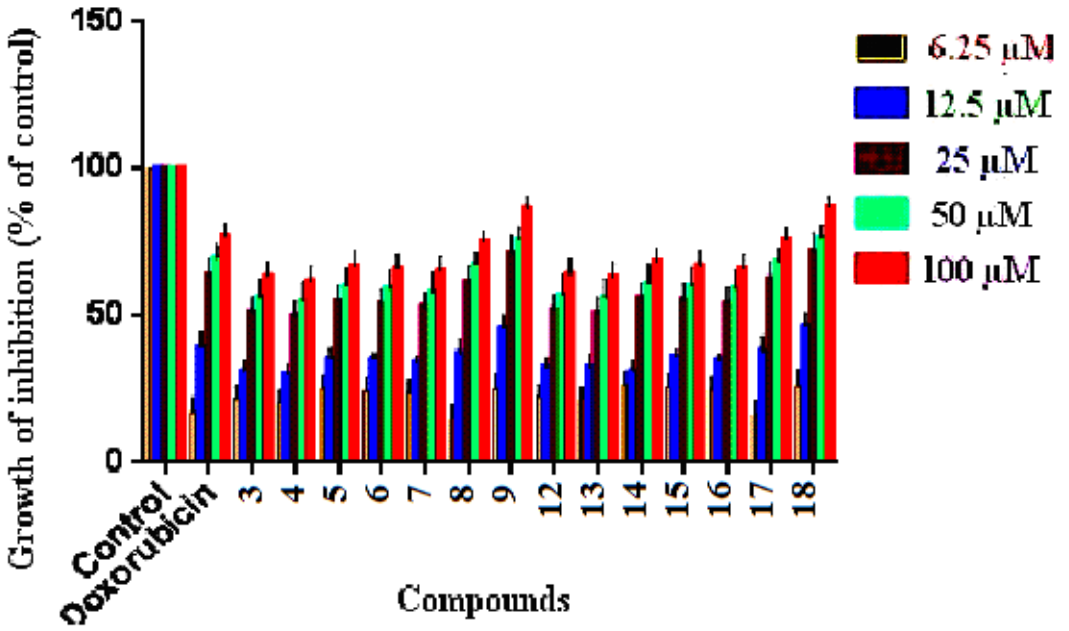

Fig. 2 Effect of different concentrations of the compounds **3-9** and **12-18** on the growth inhibition of HeLa cell

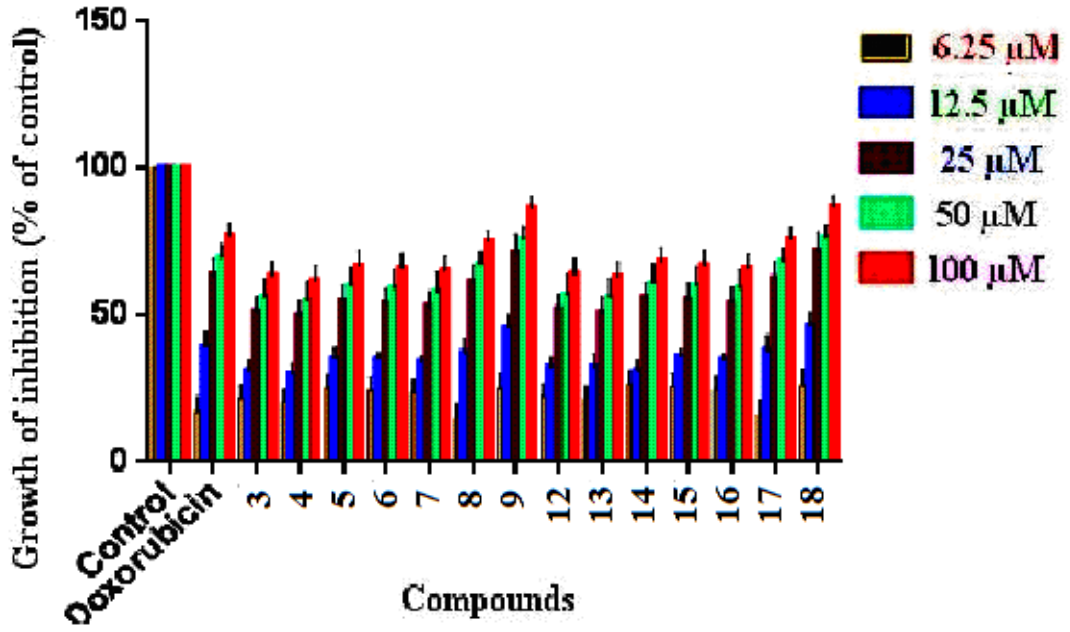

Fig. 3 Effect of different concentrations of the compounds **3-9** and **12-18** on the growth inhibition of HCT 116 cell

CONCLUSION

We have synthesized and investigated the antimicrobial-, antituberculosis-, and anticancer activity of some new pyrazole-, isoxazole-, pyrimidine-, and benzodiazepine derivatives containing thiochromeno- and benzothiepino dioxide moieties. Results obtained revealed that the 1,2-diphenylethane-1,2-diamine substituted thiochromeno- and benzothiepino compounds **9** and **18** displayed better antimicrobial-, antituberculosis-, and antitumor activity as against other derivatives. In general, the benzothiepine analogues exhibited higher activity than the thiochroman analogues.

EXPERIMENTAL

Analysis and instruments: Melting points were obtained on a TECHNICO melting point apparatus and are uncorrected. IR spectra were recorded as thin films (for oils) on NaCl plates or as KBr discs (for solids) with a JASCO FT- IR spectrophotometer and are expressed in cm^{-1}. All NMR spectra were taken on a Brucker Advance 400 FT-NMR spectrometer with ^1H and ^{13}C being observed at 400 MHz. Chemical shifts for ^1H and ^{13}C-NMR spectra are reported in ppm downfield from TMS [$(CH_3)_4Si$]. Multiplicities are recorded as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), dd (double doublets), and m (multiplet). ESI mass spectra were obtained on an Agilent 1100 series LC/MSD spectrometer. All reactions involving air- or moisture-sensitive compounds were performed under nitrogen atmosphere. Separation of compounds was carried out by column chromatography using silica gel. Fungal- and bacterial strains were purchased from Genei, Bangalore. Unless otherwise specified, all materials, solvents, and reagents were obtained commercially.

Synthesis

Compounds **3-18** were synthesized as per the reported method [16].

Ethyl 1,4-dihydro-1-phenylthiochromeno[4,3-c]pyrazole-3-carboxylate 5,5-dioxide (3): Yellow solid (0.86g, 57%), mp 172-175 ^0C; FT-IR 1713 cm^{-1}; ^1H-NMR ($CDCl_3$) δ ppm 1.3 (t, 3H,-CH_3), 4.3 (q, 2H, -O-CH_2), 4.4 (s, 2H, Ar-SO_2-CH_2), 6.9-7.5 (m, 9H, aromatic); ^{13}C-NMR ($CDCl_3$) δ ppm 14.3, 42.2, 61.3, 118.6, 120.2, 120.7, 125.5, 126.7, 127.6, 128.2, 129.4, 137.7, 139.2, 143.2, 149.8, 160.2 (CO). ESI-MS m/z: 368.06 and Anal. Calcd for $C_{19}H_{16}N_2O_4S$; C, 61.94; H, 4.38; N, 7.60; S, 8.70, Found: C, 61.79; H, 4.28; N, 7.51; and S, 8.61%.

Ethyl 1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxylate 5,5-dioxide (4): Colourless solid (0.55g, 51%), Rf = 0.61 (petroleum ether/EtOAc, 8:2); mp 169-172 ^0C; FT-IR 1708 cm^{-1}; ^1H-NMR ($CDCl_3$) δ ppm 1.1 (t, 3H,-CH_3), 4.2 (q, 2H, -O-CH_2), 4.4 (s, 2H, Ar-SO_2-CH_2), 7.2-7.5 (m, 4H, aromatic); ^{13}C-NMR ($CDCl_3$) δ ppm 14.3, 41.6, 61.3, 117.2, 119.7, 125.2, 127.7, 129.5, 137.9 (C=N-N), 144.4 (=C-NH-N), 160.8 (CO). ESI-MS m/z: 294.04 and Anal. Calcd for $C_{13}H_{12}N_2O_4S$; C, 53.42; H, 4.14; N, 9.58; S, 10.97, Found: C, 53.34; H, 4.09; N, 9.43; and S, 10.86%.

Ethyl 4H-thiochromeno[3,4-d]isoxazole-3-carboxylate 5,5-dioxide (5): Colourless solid (0.51g, 49%), Rf = 0.61(petroleum ether/EtOAc, 8:2); mp 170-173 ^0C; FT-IR 1716 cm^{-1}; ^1H-NMR ($CDCl_3$) δ ppm 1.3 (t, 3H, -CH_3), 4.2 (q, 2H, -O-CH_2), 4.4 (s, 2H, Ar-SO_2-CH_2), 7.2-7.6 (m, 4H, aromatic); ^{13}C-NMR ($CDCl_3$) δ ppm 14.9, 43.2, 62.5, 100.6, 124.8, 126.2, 128.9, 136.3, 137.9, 153.6 (C=N), 168.6 (=C-O-N), 167.3 (CO). ESI-MS m/z: 293.02 and Anal. Calcd for $C_{13}H_{11}NO_5S$; C, 53.24; H, 3.78; N, 4.78; S, 10.93, Found: C, 53.12; H, 3.62; N, 4.63; and S, 10.84%.

Ethyl 2,5-dihydro-2-oxo-1H-thiochromeno[4,3-d]pyrimidine-4-carboxylate 5,5-dioxide (6): Colourless solid (0.41g, 38%), Rf = 0.59 (petroleum ether/EtOAc, 6:4); mp 121-123 ^0C; FT-IR 1709 cm^{-1}; ^1H-NMR ($CDCl_3$) δ ppm 1.3 (t, 3H, -CH_3), 4.2 (q, 2H, -O-CH_2), 4.8 (s, 2H, Ar-SO_2-CH_2), 7.2-7.5 (m, 4H, aromatic), 8.7 (bs, 1H, -NH) ; ^{13}C-NMR ($CDCl_3$) δ ppm 12.3, 41.9, 61.4, 99.9, 124.6, 125.9, 127.4, 134.6, 135.8, 139.8, 156.4 (NCON), 162.3 (C=N), 163.4 (CO). ESI-MS m/z: 320.03 and Anal. Calcd for $C_{14}H_{12}N_2O_5S$; C, 52.49; H, 3.78; N, 8.75; S, 10.01, Found: C, 52.31; H, 3.62; N, 8.63; and S, 9.91%.

Ethyl 2,5-dihydro-2-thioxo-1H-thiochromeno[4,3-d]pyrimidine-4-carboxylate 5,5-dioxide (7): Colourless solid (0.40g, 37%), Rf = 0.60 (petroleum ether/EtOAc, 6:4); mp 212-215 ^0C; FT-IR 1712 ($\upsilon_{C=O}$) and 1148 ($\upsilon_{C=S}$) cm^{-1}; ^1H-NMR ($CDCl_3$) δ ppm 1.3 (t, 3H, -CH_3), 4.2 (q, 2H, -O-CH_2), 4.0 (s, 2H, Ar-SO_2-CH_2), 7.1-7.8 (m, 4H, aromatic), 2.1 (bs, 1H, -NH) ; ^{13}C-NMR ($CDCl_3$) δ ppm 13.4, 42.5, 60.3, 99.7, 124.9, 125.8, 127.4, 134.4, 145.6, 163.4 (C=N), 164.2 (CO), 179.5 (C=S). ESI-MS m/z: 336.04 and Anal. Calcd for $C_{14}H_{12}N_2O_4S_2$; C, 49.99; H, 3.60; N, 8.33; S, 19.06, Found: C, 49.82; H, 3.51; N, 8.22; and S, 18.91%.

Ethyl 1,4-dihydro-1H-thiochromeno[5,4-c] benzodiazepine-4-carboxylate 5,5-dioxide (8): Colourless solid (0.53g, 58%), Rf = 0.61 (petroleum ether/EtOAc, 6:4); mp 231-233 ^0C; FT-IR 1708 cm^{-1}; ^1H-NMR ($CDCl_3$) δ ppm 1.3 (t, 3H, -CH_3), 4.2 (bs, 1H, NH), 4.0 (q, 2H, -O-CH_2), 4.7 (s, 2H, Ar-SO_2-CH_2), 6.7-7.9 (m, 8H, aromatic); ^{13}C-NMR ($CDCl_3$) δ ppm 12.2, 41.8, 61.2, 99.9, 121.2, 124.5, 125.2, 126.8, 128.9, 133.8, 137.7, 138.5, 145.7, 156.3, 164.2 (C=N), 163.7 (C=O), ESI-MS m/z: 368.06 and Anal. Calcd for $C_{19}H_{16}N_2O_4S$; C, 61.94; H, 4.38; N, 7.60; S, 8.70, Found: C, 61.84; H, 7.23; N, 7.58; and S, 8.58%.

Ethyl 2,3-diphenyl-2,3-dihydro-1H-thiochromeno [5,4-c]diazepine-5-carboxylate 5,5-dioxide (9): Colourless solid (0.86g, 61%), Rf = 0.62 (petroleum ether/EtOAc, 6:4); mp 292-295 ^0C; FT-IR 1702 cm^{-1}; ^1H-NMR ($CDCl_3$) δ ppm 1.3 (t, 3H, -CH_3), 2.1 (d, 1H, NH), 3.2 (d, 1H, N-CH), 4.2 (q, 2H, -O-CH_2), 4.4 (dd, 1H, NH-CH,), 4.7 (s, 2H, Ar-SO_2-CH_2), 6.6-7.9 (m, 14H, aromatic); ^{13}C-NMR ($CDCl_3$) δ ppm 13.2, 42.5, 60.3, 99.6, 125.6,

126.7, 127.1, 127.8, 128.5, 128.5, 128.5, 133.8, 139.4, 142.2, 143.7, 149.3, 162.5 (C=N), 163.3 (CO). ESI-MS m/z: 472.13 and Anal. Calcd for $C_{27}H_{24}N_2O_4S$; C, 68.62; H, 5.12; N, 5.93; S, 6.79, Found: C, 68.51; H, 5.03; N, 5.86; and S, 6.64%.

Ethyl 1-phenyl-4,5-dihydro-1H-[1] benzothiepino [5,4-c]pyrazole-3-carboxylate 6,6-dioxide (12):

Yellow solid (1.03g, 61%), Rf = 0.60 (petroleum ether/EtOAc, 8:2); mp 182-185 ^{0}C; FT-IR 1153, 1309, 1708 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 1.3 (t, 3H, -CH_3), 3.1 (t, 2H, -SO_2-CH_2-C$\underline{H}_2$), 3.8 (t, 2H, -SO_2-C$\underline{H}_2$-CH_2), 4.2 (q, 2H, -O-CH_2), 6.5-7.6 (m, 9H, aromatic); ^{13}C-NMR ($CDCl_3$) δ ppm 13. 5, 16.5, 52.3, 60.5, 119.4, 120.7, 120.8, 124.3, 126.5, 127.8, 129.5, 129.8, 137.6, 139.5, 143.5 (C=N), 149.6 (=C-N-N), 160.1 (CO). ESI-MS m/z: 382.09 and Anal. Calcd for $C_{20}H_{18}N_2O_4S$; C, 62.81; H, 4.74; N, 7.33; S, 8.38, Found: C, 62.72; H, 4.63; N, 7.26; and S, 8.24%.

Ethyl 4,5-dihydro-1H-[1]benzothiepino[5,4-c] pyrazole-3-carboxylate 6,6-dioxide (13):

Colourless solid (1.01g, 58%), Rf = 0.58 (petroleum ether/EtOAc, 8:2); mp 181-183 ^{0}C; FT-IR 1150, 1312, 1713 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 1.3 (t, 3H, -CH_3), 3.1 (t, 2H, -SO_2-CH_2-C$\underline{H}_2$) 3.7 (t, 2H -SO_2-C$\underline{H}_2$-CH_2), 4.3 (q, 2H, -O-CH_2), 7.1-7.8 (m, 4H, aromatic); ^{13}C-NMR ($CDCl_3$) δ ppm 14.3, 16.3, 52.4, 60.5, 117.8, 118.6, 124.6, 127.7, 128.7, 137.6, 138.6 (C=N), 144.8 (=C-NH-N), 160.8 (CO). ESI-MS m/z: 306.05 and Anal. Calcd for $C_{14}H_{14}N_2O_4S$; C, 54.89; H, 4.61; N, 9.14; S, 10.47 Found: C, 54.76; H, 4.53; N, 9.02; and S, 10.36%.

Ethyl 4,5-dihydro[1]benzothiepino[4,5-d]isoxazole -3-carboxylate 6,6-dioxide (14):

Colourless solid (0.82g, 54%), Rf = 0.62 (petroleum ether/EtOAc, 8:2); mp 183-185 ^{0}C; FT-IR 1152, 1313, 1586, 1706 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 1.3 (t, 3H, -CH_3), 3.1 (t, 2H, -SO_2-CH_2-C$\underline{H}_2$), 3.8 (t, 2H, -SO_2-C$\underline{H}_2$-CH_2), 4.3 (q, 2H, -O-CH_2), 7.0-7.6 (m, 4H, aromatic); ^{13}C-NMR ($CDCl_3$) δ ppm 15.3, 16.4, 52.4, 61.5, 101.5, 126.8, 128.6, 129.8, 134.7, 137.4, 150.4 (C=N), 166.3 (=C-O-N), 167.5 (CO). ESI-MS m/z: 307.04 and Anal. Calcd for $C_{14}H_{13}NO_5S$; C, 54.71; H, 4.26; N, 4.56; S, 10.43, Found: C, 54.62; H, 4.13; N, 4.48; and S, 10.35%.

Ethyl 2-oxo-1,2,5,6-tetrahydro[1]benzothiepino [5,4-d]pyrimidine-4-carboxylate 6,6-dioxide (15):

Colourless solid (0.45g, 38%), Rf = 0.55 (petroleum ether/EtOAc, 6:4); mp 173-175 ^{0}C; FT-IR 1152, 1315, 1701 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 1.1 (t, 3H, -CH_3), 2.5 (t, 2H, -SO_2-CH_2-C$\underline{H}_2$), 3.5 (t, 2H, -SO_2-C$\underline{H}_2$-CH_2), 3.7 (q, 2H, -O-CH_2), 7.1-7.8 (m, 4H, aromatic), 8.4 (b, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ ppm 13.5, 16.5, 52.7, 60.8, 100.5, 125.7, 126.2, 126.4, 128.9, 133.4, 137.6, 156.4 (NH-C=O), 163.5 (C=N), 164.5 (CO). ESI-MS m/z: 334.04 and Anal. Calcd for $C_{15}H_{14}N_2O_5S$; C, 53.88; H, 4.22; N, 8.38; S, 9.59, Found: C, 53.76; H, 4.13; N, 8.29; and S, 9.46%.

Ethyl 2-thioxo-1,2,5,6-tetrahydro[1]benzothiepino [5,4-d]pyrimidine-4-carboxylate 6,6-dioxide (16):

Colourless solid (0.38g, 35%), Rf = 0.61 (petroleum ether/EtOAc, 6:4); mp 211-1214 ^{0}C; FT-IR 1151, 1354, 1702 ($\upsilon_{C=O}$) and 1143 ($\upsilon_{C=S}$) cm^{-1} ; ^{1}H-NMR ($CDCl_3$) δ ppm 0.8 (t, 3H, -CH_3), 2.0 (b, 1H, -NH), 2.4 (t, 2H, -SO_2-CH_2-C$\underline{H}_2$), 3.4 (t, 2H, -SO_2-C$\underline{H}_2$-CH_2), 3.7 (q, 2H, -O-CH_2), 7.0-7.9 (m, 4H, aromatic); ^{13}C-NMR ($CDCl_3$) δ ppm 12.6, 16.3, 52.4, 61.3, 99.7, 124.3, 125.4, 126.8, 127.6, 133.9, 138.5, 138.7, 164.3(CO), 165.3 (C=N), 180.3 (C=S). ESI-MS m/z: 350.02 and Anal. Calcd for $C_{15}H_{14}N_2O_4S_2$; C, 51.41; H, 4.03; N, 7.99; S, 18.30, Found: C, 51.36; H, 3.94; N, 7.86; and S, 18.24%.

Ethyl 1,4-dihydro-1H-benzothiepino[5,4-c] benzodiazepine-4-carboxylate 6,6-dioxide (17):

Colourless solid (0.53g, 51%), Rf = 0.64 (petroleum ether/EtOAc, 6:4); mp 239-241 ^{0}C; FT-IR 1154, 1356, 1704 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 1.3 (t, 3H, -CH_3), 2.4 (t, 2H, -SO_2-CH_2-C$\underline{H}_2$), 3.4 (t, 2H, -SO_2-C$\underline{H}_2$-CH_2), 3.8 (b, 1H, NH), 4.4 (q, 2H, -O-CH_2), 6.5-7.7 (m, 8H, aromatic); ^{13}C-NMR ($CDCl_3$) δ ppm 13.3, 16.8, 51.7, 60.5, 99.7, 121.4, 123.8, 125.3, 126.9, 127.3, 133.5, 137.3, 138.8, 138.6, 144.5, 157.4, 164.3 (C=N), 165.3 (C=O). ESI-MS m/z: 382.08 and Anal. Calcd for $C_{20}H_{18}N_2O_4S$; C, 62.81; H, 4.74; N, 7.33; S, 8.38, Found: C, 62.75; H, 4.63; N, 7.26; and S, 8.34%.

Ethyl 2,3-diphenyl-2,3-dihydro-1H- benzothiepino [5,4-c]diazepine-5-carboxylate 6,6-dioxide (18):;

Colourless solid (0.79g, 56%), Rf = 0.62 (petroleum ether/EtOAc, 6:4); mp 318-321 ^{0}C; FT-IR 1698 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 0.98 (t, 3H, -CH_3), 1.9 (s, 1H, NH), 2.3 (t, 2H, -SO_2-CH_2-C$\underline{H}_2$), 3.2 (t, 2H, -SO_2-C$\underline{H}_2$-CH_2), 3.3 (d, 1H, N-C$\underline{H}$), 4.4 (dd, 1H, NH-C$\underline{H}$), 4.6 (q, 2H, -O-CH_2), 6.5-7.3 (m, 14H, aromatic); ^{13}C-NMR ($CDCl_3$) δ ppm 12.2, 17.4, 52.8, 61.3, 99.7, 125.5, 127.3, 127.7, 128.7, 128.8, 129.5, 129.8, 133.6, 138.7, 140.7, 143.5, 149.4, 163.4 (C=N), 164.3 (C=O). ESI-MS m/z: 486.14 and Anal. Calcd for $C_{28}H_{26}N_2O_4S$; C, 69.11; H, 5.39; N, 5.76; S, 6.59, Found: C, 69.02; H, 5.21; N, 5.34; and S, 6.43%.

ANTIMICROBIAL EVALUATION

Standard sterilized filter paper discs (5 mm diameter) impregnated with a solution of the test compound in DMSO (1 µg/mL) was placed on an

agar plate seeded with the test organism in triplicates. The utilized test organisms were *Staphylococcus aureus, Streptococcus pneumoniae* (Gram-positive bacteria) *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Escherichia coli* (Gram-negative bacteria). They were also evaluated for their *in vitro* antifungal potential against *Candida albicans, Aspergillus flavus,* and Aspergillus *Niger* strains. Amikacin and chloroamphenicol were used as standard antibacterial agents and clotrimazole, as a standard antifungal agent. DMSO alone was used as control at the above mentioned concentration. The plates were incubated at 37 ^{0}C for 24h for bacteria and 28 ^{0}C for 48h for fungi. Compounds that showed significant growth inhibition zones (>20 mm) using the two-fold serial dilution technique were further evaluated for their minimal inhibitory concentrations (MICs).

Minimal inhibitory concentration (MIC) measurement: Broth dilution test was used to determine 'Minimal Inhibitory Concentration (MIC)' of the above mentioned samples [18, 19]. The micro dilution susceptibility test was used for the determination of antibacterial- and antifungal activity. Stock solutions of the tested compounds and the standareds, amikacin, chloroamphenicol, and clotrimazole were prepared in DMSO at concentrations of 1000 µg/mL followed by a two-fold dilution at concentrations of 500, 250… 3.125 µg/mL. All the plates were incubated at 37 ^{0}C for 24h for bacteria and at 28 ^{0}C for 48h for fungi and the minimal inhibitory concentrations (MIC) were determined. Control experiments were also carried out.

Antituberculosis activity: All the compounds were screened for their *in vitro* antimycobacterial activity against MTB using the resazurin microplate assay (REMA) [20, 21]. MTB $H_{37}Rv$ was grown in Middlebrook 7H11 broth medium supplemented with 10% OADC (oleic acid, albumin, dextrose, and catalase; 1, 10, 100 µg /L). After incubation at 37 ºC for 7 days, 15 µL of 0.01% resazurin (Sigma, St. Louis. MO, USA) solution in sterile water was added to the first growth control wells and incubated for 24h. While the first set of growth controls turned pink, the dye solution was added to the second set of growth controls and the test wells, and incubated for 24h at 37 ºC. Blue color in the wells containing the test compounds indicates inhibition of growth and pink color indicates lack of inhibition of growth of *M. tuberculosis*. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound required to 99.9% inhibition of bacterial growth.

Anticancer activity: The *in vitro* anticancer activity was analyzed by MTT assay method [22, 23]. The human cervical cancer cell line (HeLa) and colon cancer cell (HCT116) were obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagle's Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). The cells were maintained at 37 ^{0}C, 95% air, 5% CO_2, and 100% relative humidity. The culture maintenance was passaged weekly, and the culture medium changed twice a week.

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid(EDTA) to make single cell suspensions. Using a hemocytometer the viable cells were counted and diluted with a medium containing 5% FBS to give a final density of $1x10^5$ cells/mL. One hundred microlitres per well of cell suspension were seeded into 96-well plates at a plating density of 10,000 cells/well and incubated to allow for cell attachment at 37 ^{0}C, 95% air, 5% CO_2, and 100% relative humidity. After 24h, the cells were treated with serial concentrations of the test samples. They were initially dissolved in pure dimethylsulfoxide (DMSO) to prepare the stock (200 mM) and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted twice the desired final maximum test concentration with serum free medium. Additional three, two-fold serial dilutions were made to provide a total of four drug concentrations. Aliquots of 100 µL of these different drug dilutions were added to the appropriate wells containing 100 µL of medium, resulting in the required final drug concentrations. After the drug addition, the plates were incubated for an additional 48h at 37 ^{0}C, 5% CO_2, 95% air, and 100% relative humidity. The medium without samples served as control and a triplicate was maintained for all these concentrations. After 48h of incubation, 15 µL of MTT (5mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37 ^{0}C for 4h. The medium with MTT was flicked off and the formazan crystals formed were solubilized in 100 µL of DMSO. The absorbance was measured at 570 nm using a micro plate reader. The % cell inhibition was determined using the following formula.

% Cell inhibition = 100-Abs (sample)/Abs (control) x100

Nonlinear regression graph was plotted between % cell inhibition and $\log_{10}$ concentration and IC_{50} was determined using Graph Pad Prism software.

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