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Tricyclic Pyrazoles: Synthesis, Characterization, Antimicrobial, Antituberculosis, and Antitumor Activity of *N*,1-Diphenyl-1,4-dihydrothiochromeno[4,3-*c*]pyrazole-3-carboxamide-5,5-dioxide Derivatives

Pandaram Palanisamy[1] and Sudalaiandi Kumaresan[2*]

[1]Department of Chemistry, Manonmaniam Sundaranar University, Tirunelveli-627 012, Tamilnadu, India.
[2]Department of Chemistry, Noorul Islam University, Kumaracoil, Thuckalay-629 180, Kanyakumari Dist, Tamilnadu, India.

Authors' contributions

This work was prepared in the research group of author SK. He proposed the work and drafted the manuscript. Author PP participated in the design and presiding the experiments, collected data, and drafted the manuscript. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: A series of *N*,1-diphenyl-1,4-dihydrothiochromeno[4,3-*c*]pyrazole-3-carboxamide 5,5-dioxide derivatives (**6a-m**) were synthesized and evaluated for anticancer, antibacterial, and antifungal activity.
Methodology: Reaction of 2,3-dihydro-4H-thiochromen-4-one 1,1-dioxide **2** with diethyl oxalate in ethanol in the presence of a base afforded the Claisen condensation product **3**. Subsequent reaction of **3** with phenylhydrazine hydrochloride at reflux in ethanol afforded ethyl 1-phenyl-1,4-dihydrothiochromeno[4,3-*c*]pyrazole-3-carboxylate 5,5-dioxide (**4**). Alkaline hydrolysis of **4** furnished the corresponding 1-phenyl-1,4-dihydrothiochromeno[4,3-*c*]pyrazole-3-carboxylic acid 5,5-dioxide **5**. The pyrazole acid **5** was converted into the corresponding acid chloride followed by treatment with an excess of the appropriate amine to give **6a-m**.

**Corresponding author: Email: skumarmsu@yahoo.com;*

Results: Compound **6k** showed better activity than chloroamphenicol against *Klebsiella pneumoniae* and *Escherichia coli* and equipotent to clotrimazole in inhibiting the growth of *Candida albicans* (MIC 3.125 µg/mL). All compounds were screened for their cytotoxic activity against two tumor cell lines, namely, human colon tumor cell line (HCT116) and human cervical cancer cell line (HeLa) using the colorimetric MTT assay. Most of the tested compounds exhibited potent antitumor activity. Particularly, compound **6k** displayed the highest activity among the tested compounds with IC_{50} equal to 17 µM (HeLa) and 15 µM (HCT116) respectively. Among the tested compounds, **6k** was found to be more active against *M. tuberculosis,* (H37Rv) with minimum inhibitory concentration (7.8 µM).
Conclusion: The chloro- (6b and 6c), 2-aminobenzothiazole- (6l), and 4-aminoantipyrine- (6k) linkages exhibited better antimicrobial activity than their counterparts. Compound 6k was found to possess comparatively more antimicrobial, antituberculosis, and antitumor activity against the other derivatives.

Keywords: N,1-diphenyl-1;4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxamide 5,5-dioxide; antimicrobial; antimycobacterial; anticancer activity.

1. INTRODUCTION

Many natural products possess the pyrazole unit as the basic moiety [1]. Pyrazole derivatives are reported to have the broad spectrum of biological activities, such as antitumour [2], anticoagulant [3], antihyperglycemic, analgesic, antipyretic, antimicrobial, and hypoglycemic activity [4–8]. They are also known to exhibit a wide range of biological properties such as cannabinoid type-1 (CB1) receptor antagonists [9, 10]. These derivatives have the applications in drug development [11]. Recent literature shows that some arylpyrazoles were reported to have non-nucleoside HIV-1 reverse transcriptase inhibitory activity [12]. Li et al. have synthesized a series of *N*-1,3-triphenyl-1H-pyrazole-4-carboxamide derivatives which exhibited potent antiproliferative activities against HTC116 and MCF-7 cells and Aurora-A kinase inhibitory activities [13]. Recently, Ding et al. [14] reported the syntheses of novel 1-arylmethyl-3-aryl-1H-pyrazole-5-carboxamide derivatives which inhibited much more proliferation of A549 cell. The azole group of heterocyclic compounds possesses significant pharmacokinetic property and lipophilicity that influence the ability of drug to reach the target by transmembrane diffusion, and show promising activity against resistant TB by inhibiting the biosynthesis of lipids [15,16]. Ahsan et al. reported [17] the antimycobacterial activity of 4-dihydro-3H-indeno[1,2-c]pyrazole-2-carboxamide analogues [18,19].

The synthesis and *in vitro* antitumour activity of several benzo[b]thiophenesulfonamide 1,1-dioxide derivatives have been reported [20]. These compounds were found to inhibit the tumour-associated NADH oxidase (tNOX) activity of the plasma membrane induced in human leukaemia CCRF-CEM cells, a typical process of apoptosis that included cell shrinkage, chromatin condensation, phosphatidylserine translocation to cell surface, mitochondrial dysfunction, and internucleosomal DNA degradation [21,22]. Benzo[b]thiophene-4-carboxamide 1,1-dioxide derivatives have been described in the literature as preventive for various inflammatory and neoplastic diseases caused by an abnormal production of interleukin-6 or interleukin-12 [23]. Sagardoy et al. [24] have designed and synthesized some benzo[b]thiophene-6-carboxamide 1,1-dioxide derivatives

(BTC) structurally related to benzo[b]thiophenesulfonamide1,1-dioxide derivatives (BTS), which display growth inhibition of HTB-54, CCRF-CEM, and HeLa tumour cells.

In view of the above mentioned facts and in continuation of our interest in the synthesis of *N*,1-diphenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxamides, to identify new compounds that may exhibit potent, selective, and less toxic antimicrobial, antituberculosis, and anticancer agents, we report herein the synthesis and bioactivity of some *N*,1-diphenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxamide-5,5-dioxides. Nevertheless, these dioxide analogues were found to exhibit better biological activity than the previously reported [25] *N*,1-diphenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxamide analogues.

2. EXPERIMENTAL DETAILS

2. 1 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) or KBr (for solids) on NaCl plates 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 ^{1}H and ^{13}C being observed at 300 MHz. Chemical shifts for ^{1}H and ^{13}C-NMR spectra were reported in δ or ppm downfield from TMS [$(CH_3)_4Si$]. Multiplicities are recorded as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), dd (doublet of 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. Unless otherwise specified, all materials, solvents, and reagents were obtained from commercial suppliers.

2.2 Methods of Preparation

2.2.1 Synthesis of ethyl 1-phenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxylate 5,5-dioxide (4)

A stirred mixture of the diketoester **3** (1.0 equiv, 4 mmol, 1.47g) and phenylhydrazine hydrochloride (1.15 equiv) in EtOH (30 mL) was heated under reflux for 4h. The reaction was allowed to cool to room temperature and the insoluble material was collected by filtration and washed with a small volume of ice-cold ethanol. Purification by column chromatography afforded the analytically pure product ethyl 1-phenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxylate 5,5-dioxide (**4**) as a yellow solid (1.05g, 62%). Rf = 0.64 [petroleum ether (40-60ºC) /EtOAc 8:2]; mp 181-183ºC. FT-IR (KBr) 1714 (C=O), 1305 (SO_2-asym), 1152(SO_2-sym) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 1.2 (t, 3H, -CH_3,*J=7.2*), 4.2 (q, 2H, -OCH_2, *J=5.2*), 4.7 (s, 2H, -SO_2-CH_2), 7.4-7.9 (m, 9H, aromatic); ^{13}C-NMR ($CDCl_3$) δ ppm 14.56, 50.25, 60.47, 119.63, 120.54, 120.79, 125.64, 126.63, 127.56, 129.76, 129.86, 137.24, 139.42, 143.65, 149.36, 161.25 (CO). ESI-MS m/z: 368.05 and Anal. Calcd for $C_{19}H_{16}N_2O_4S$; C, 68.83; H, 4.95; O, 17.47; N, 7.70; S, 8.75, Found: C, 68.25; H, 4.29; *N*, 7.57; and S, 8.64%.

2.2.2 Synthesis of 1-phenyl-1,4-dihydrothiochromeno[4,3-C]pyrazole-3-carboxylic Acid 5,5-dioxide (5)

To a mixture of the ester **4** (1.0 equiv, 5 mmol, 1.7g) in methanol (25 mL) was added to a solution of potassium hydroxide (2.0 equiv) in methanol (18 mL). The resulting mixture was heated under reflux 12h. The mixture was cooled to room temperature and then poured onto water and acidified with 1N hydrochloric acid. The precipitate was filtered, washed with water, and air-dried to yield the analytically pure product of 1-phenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxylic acid 5,5-dioxide (**5**) as a colorless solid (1.87g, 95.2%). Rf = 0.54 ($CHCl_3$/MeOH 9:1); mp 155-158 ^{0}C; FT-IR (KBr) 1654 cm^{-1}, 1155 (SO_2-sym) and 1306 (SO_2-asym) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 4.76 (s, 2H, -SO_2-CH_2), 7.3-7.9 (m, 9H, aromatic);^{13}C-NMR ($CDCl_3$) δ ppm 46.64, 119.34, 120.38, 120.42, 125.41, 126.34, 127.64, 127.65, 129.68, 129.96, 137.36, 139.84, 143.26, 149.63, 162.45(CO). ESI-MS m/z: 340.03. Anal. Calcd for $C_{17}H_{12}N_2O_4S$; C, 59.99; H, 3.55; *N*, 8.23; O, 18.80; S, 9.42, Found: C, 59.56; H, 3.29; *N*, 8.14; and S, 9.36%.

2.2.3 General syntheses of carboxamide 5,5-dioxides (6)

A mixture of the appropriate 1-phenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxylic acid 5,5-dioxide **5** (1 equiv, 4.0 mmol, 1.36g) and thionyl chloride (3.0 equiv, 0.87ml) in toluene (30 mL) was refluxed for 30 min. The solvent and the excess $SOCl_2$ were removed under reduced pressure and the resulting dark solid dissolved in CH_2Cl_2 (15 mL) was dropwise added to a solution of requisite amine (1.5 equiv) and Et_3N, (1.5 equiv) in CH_2Cl_2 (15 mL) at 0ºC. The mixture was refluxed for 3–4h (Table 1), taken in a separatory funnel and washed with brine. The aqueous layer was separated and extracted with CH_2Cl_2. The combined organic layers were washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. The analytically pure product was isolated by an appropriate method of purification as indicated below in each case.

2.2.3.1 N,1-Diphenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxamide 5,5-dioxide (6a)

The mixture was separated by column chromatography [petroleum ether (40-60 °C)/EtOAc (8:2)] to afford **6a** as a colorless solid (1.58g, 73%). FT-IR (KBr) 1654, 3165, 1309, 1154 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 4.86 (s, 2H, -SO_2-CH_2), 7.2-7.9 (m, 14H, aromatic), 10.2 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ ppm 46.68, 117.35, 120.52, 120.94, 121.34, 121.68, 124.46, 125.63, 126.45, 129.87, 134.82, 135.54, 136.24, 137.54, 138.65, 141.82, 149.65, 162.12 (CO). ESI-MS m/z: 415.07. Anal. Calcd for $C_{23}H_{17}N_3O_3S$; C, 66.49; H, 4.12; *N*, 10.11; O, 11.55; S, 7.72 Found: C, 66.25; H, 4.02; *N*, 9.86; and S, 7.61%.

2.2.3.2 N-(3-Chlorophenyl)-1-phenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxamide 5,5-dioxide (6b)

The mixture was separated by column chromatography [petroleum ether (40-60 °C)/EtOAc (8:2)] to furnish **6b** as a colorless solid (1.64 g, 80%). FT-IR (KBr) 1662, 3190, 1152, 1305 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 4.8 (s, 2H, (s, 2H, -SO_2-CH_2), 7.1-7.9 (m, 13H, aromatic), 10.31(s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ ppm 48.32, 117.53, 120.46, 123.56, 126.45, 128.36, 129.56, 129.65, 129.86, 133.47, 133.81, 134.68, 137.42, 139.35, 141.25, 149.68, 162.35 (CO). ESI-MS m/z: 449.04. Anal. Calcd for $C_{23}H_{16}ClN_3O_3S$; C, 61.40; H, 3.58; Cl, 7.88; *N*, 9.34; O, 10.67; S, 7.13 Found: C, 66.13; H, 3.31, *N*, 9.03, and S, 6.97%.

2.2.3.3 N-(4-Chlorophenyl)-1-phenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxamide 5,5-dioxide (6c)

Separation of mixture was done through column chromatography [petroleum ether (40-60ºC)/EtOAc (8:2)] to afford **6c** as a colorless solid (1.54g, 65%). FT-IR 1652, 3160, 1154, 1309 cm^{-1}; ^{1}H-NMR ($CDCl_3$) ppm δ 4.8 (s, 2H, (s, 2H, -SO_2-CH_2),), 7.1-7.9 (m, 13 H, aromatic), 10.28 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ ppm 47.35, 117.64, 119.54, 120.14, 121.35, 122.46, 124.35, 126.54, 129.46, 129.68, 129.84, 130.46, 134.84, 137.86, 139.54, 141.35, 149.64, 162.34 (CO). ESI-MS m/z: 449.02. Anal. Calcd for $C_{23}H_{16}ClN_3O_3S$; C, 61.40; H, 3.58; Cl, 7.88; *N*, 9.34; O, 10.67; S, 7.13, Found: C, 61.28; H, 3.31, *N*, 9.08, and S, 7.04%.

2.2.3.4 N-(3-Nitrophenyl)-1-phenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxamide 5,5-dioxide (6d)

The mixture was separated by column chromatography [petroleum ether (40-60ºC)/EtOAc (8:2)] to give **6d** as a colorless solid (1.24g, 59%). FT-IR 1656, 3190, 1151, 1307 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 4.8 (s, 2H, -SO_2-CH_2), 7.0-7.9 (m, 13H, aromatic), 10.42 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ ppm 47.68, 114.35, 117.45, 119.46, 120.56, 126.45, 127.36, 129.68, 129.89, 134.64, 136.24, 137.68, 139.61, 141.55, 149.35, 149.35, 162.42 (CO). ESI-MS m/z: 460.04. Anal. Calcd for $C_{23}H_{16}N_4O_5S$; C, 59.99; H, 3.50; *N*, 12.17; O, 17.37; S, 6.96, Found: C, 59.68; H, 3.33; *N*, 12.10 and S, 6.89 %.

2.2.3.5 N-(4-Nitrophenyl)-1-phenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxamide 5,5-dioxide (6e)

The mixture was separated through column chromatography [petroleum ether (40-60ºC)/EtOAc (1:1)] to afford **6e** (1.05 g, 57%) as a colorless solid. FT-IR 1665, 3184, 1150, 1303 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 4.78, (s, 2H, -SO_2-CH_2), 7.1.7.9 (m, 13 H, aromatic) and 10.36 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$) δ ppm 48.15, 117.34, 120.47, 120.48, 122.67, 122.84, 124.68, 126.35, 129.45, 129.65, 129.85, 134.68, 137.64, 139.46, 141.54, 143.63, 149.57, 162.35(CO). ESI-MS m/z: 460.06. Anal. Calcd for $C_{23}H_{16}N_4O_5S$; C, 59.99; H, 3.50; *N*, 12.17; O, 17.37; S, 6.96 Found: C, 59.68, H, 3.41, *N*, 12.03, and S, 6.61%.

2.2.3.6 N-(3-Methylphenyl)-1-phenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxamide 5,5-dioxide (6f)

The mixture was separated through column chromatography [petroleum ether (40-60ºC)/EtOAc (8:2)] to furnish **6f** (1.32 g, 64%) as a white solid. FT-IR 3195, 1663, 1306, 1152 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 2.3 (3H, s, CH_3), 4.4.7 (s, 2H, -SO_2-CH_2), 7.1-7.9 (m, 13 H, aromatic), 10.36 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ ppm 34.36, 47.65, 117.61, 118.63, 119.45, 120.58, 121.54, 124.27, 126.54, 129.63, 129.84, 134.86, 135.42, 137.48, 138.42, 139.84, 141.66, 149.36, 162.45 (CO). ESI-MS m/z: 429.02. Anal. Calcd for $C_{24}H_{19}N_3O_3S$; C, 67.12; H, 4.46; *N*, 9.78; O, 11.18; S, 7.47; S, 8.07 Found: C, 67.03; H, 4.28; *N*, 9.45; and S, 7.11%.

2.2.3.7 N-(4-Methylphenyl)-1-phenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxamide 5,5-dioxide (6g)

The mixture was separated through column chromatography [petroleum ether (40-60ºC)/EtOAc (8:2)] to obtain **6g** (1.38 g, 61%) as a colorless solid. FT- IR 3178, 1655, 1307,

1151 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 2.3 (3H, s, CH_3), 4.78 (s, 2H, -SO_2-CH_2), 7.1-7.9 (m, 13 H, aromatic), 10.36 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$) δ ppm 24.86, 47.84, 117.25, 119.46, 120.56, 121.57, 124.27, 126.76, 128.64, 129.68, 132.54, 134.46, 134.68, 137.44, 139.68, 141.34, 149.35, 162.45 (CO). ESI-MS m/z: 429.05. Anal. Calcd for $C_{24}H_{19}N_3O_3S$; C, 67.12; H, 4.46; *N*, 9.78; O, 11.18; S, 7.47 Found: C, 67.03; H, 4.39; *N*, 9.67; S, 7.38%.

2.2.3.8 N-(2-Aminophenyl)-1-phenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxamide 5,5-dioxide (6h)

Separation through column chromatography [petroleum ether (40-60ºC)/EtOAc (8:2)] afforded **6h** (1.38 g, 63%) as a yellow solid. FT-IR 3194, 1658, 1305, 1152 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 4.82 (s, 2H, -SO_2-CH_2), 5.14(s, 2H, NH_2), 7.1-7.9 (m, 13 H, aromatic), 10.32 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ ppm 51.23, 115.95, 117.62, 119.42, 120.42, 120.64, 122.62, 125.48, 129.46, 129.86, 134.23, 134.68, 137.45, 139.52, 141.25, 141.68, 149.35, 162.46 (CO). ESI-MS m/z: 430.09. Anal. Calcd for $C_{23}H_{18}N_4O_3S$; C, 64.17; H, 4.21; *N*, 13.01; O, 11.15; S, 7.45. Found: C, 64.01, H, 4.10, *N*, 12.89, and S, 7.24%.

2. 2. 3. 9 1-Phenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carbohydrazide 5,5-dioxide (6i)

The mixture was separated by column chromatography [petroleum ether (40-60ºC)/EtOAc (8:2)] to obtain **6i** (1.45 g, 67%) as a colourless solid. FT-IR 3186,1665, 1307, 1150 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 4.28 (s, 2H, NH_2), 4.78 (s, 2H, -SO_2-CH_2),), 7.4-7.9 (m, 9H, aromatic), 9.8 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ ppm 53.42, 117.34, 120.46, 126.52, 129.43, 129.68, 129.84, 134.24, 137.62, 137.26, 139.74, 141.34, 149.65, 161.54 (CO). ESI-MS m/z: 354.02. Anal. Calcd for $C_{17}H_{14}N_4O_3S$; C, 57.62; H, 3.98; *N*, 15.81; O, 13.54; S, 9.05, Found: C, 57.55, H, 3.76, *N*, 15.64, and S, 8.88%.

2.2.5.10 N',1-diphenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carbohydrazide 5,5-dioxide (6j)

Separation through column chromatography [petroleum ether (40-60ºC)/EtOAc (8:2)] afforded **6j** (1.24 g, 69%) as a colourless solid. FT-IR 3194, 1655, 1304, 1153 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 3.19 (s, 1H, PhNH), 4.8 (s, 2H, -SO_2-CH_2),), 7.1-7.9 (m, 14 H, aromatic), 10.54 (s, 1H, -CONH); ^{13}C-NMR ($CDCl_3$) δ ppm 50.36, 113.64, 113.65, 117.48, 119.46, 119.65, 120.42, 126.35, 129.42, 129.67, 129.86, 134.56, 137.58, 139.42, 141.42, 149.36, 151.23, 161.42 (CO). ESI-MS m/z: 430.06. Anal. Calcd for $C_{23}H_{18}N_4OS$; C, 69.32; H, 4.55; *N*, 14.06; O, 4.02; S, 8.05, Found: C, 69.35, H, 4.50, *N*, 14.03, and S, 8.08%.

2.2.3.11 N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-1-phenyl-1,4-dihydro - thiochromeno [4,3-c]pyrazole-3-carboxamide 5,5-dioxide (6k)

Column chromatography [petroleum ether (40-60ºC)/EtOAc (8:2)] afforded **6k** (1.3 g, 60%) as a colorless solid. IR 3117, 1632, 1306, 1152 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 1.6 (s, 3H, CH_3), 2.2 (s, 3H, CH_3), 4.3 (s, 2H, -SO_2-CH_2),), 6.9-7.8 (m, 14 H, aromatic), 9.3 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ ppm 12.05, 22.86, 39.54, 103.24, 113.54, 117.27, 119.57, 120.35, 120.58, 125.36, 126.54, 127.56, 129.67, 133.25, 136.42, 137.64, 139.46, 141.26, 149.45, 160.45(CONH), 161.021(CON). ESI-MS m/z: 525.12. Anal. Calcd for $C_{28}H_{23}N_5O_2S$; C, 63.99; H, 4.41; *N*, 13.33; O, 12.18; S, 6.10 Found: C, 68.78, H, 4.24, *N*, 13.08, and S, 6.59 %.

2.2.3.12 N-(Benzo[d]thiazol-2-yl)-1-phenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carbox -amide 5,5-dioxide (6l)

Column chromatography [petroleum ether (40-60ºC) /EtOAc (8:2)] furnished **6l** (1.30 g, 64%) as a pale yellow solid. FT-IR 3154, 1628, 1308, 1152 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 4.3 (s, 2H, $-SO_2-CH_2$), 6.8-7.5 (m, 13H, aromatic), 10.4 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ ppm 21.98, 117.26, 119.32, 120.65, 120.87, 121.65, 124.64, 125.21, 125.76, 127.36, 127.84, 129.12, 129.54, 129.86, 137.41, 139.46, 141.26, 149.26, 149.62, 162.04(CO), 174.21(C=N). ESI-MS m/z: 472.02. Anal. Calcd for $C_{24}H_{16}N_4OS_2$; C, 65.43; H, 3.66; *N*, 12.72; O, 3.63; S, 14.56 Found: C, 65.49, H, 3.49, *N*, 12.33, and S, 14.59%.

2.2.5.13 N-(Naphthalen-1-yl)-1-phenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxami -de 5,5-dioxide (6m)

The mixture was separated by column chromatography [petroleum ether (40-60ºC)/EtOAc (8:2)] to afford **6m** (1.24 g, 68%) as a colourless solid. IR 3134, 1635, 1305,1153 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm 4.3 (s, 2H, $-SO_2-CH_2$), 6.8-7.9 (m, 16 H, aromatic), 10.42 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ ppm 21.65, 117.65, 118.45, 119.32, 120.42, 121.56, 122.67, 123.24, 124.34, 125.56, 126.35, 127.43, 129.14, 129.46, 129.82, 139.21, 140.32, 142.21, 143.97, 151.21, 161.35 (CO). ESI-MS m/z: 465.08. Anal. Calcd for $C_{27}H_{19}N_3OS$; C, 74.80; H, 4.42; *N*, 9.69; O, 3.69; S, 7.40 Found: C, 74.65; H, 4.17; *N*, 9.52; and S, 7.21%.

2.3 Antimicrobial Evaluation

Standard sterilized filter paper discs (5 mm diameter) impregnated with a solution of the test compound in DMSO (1 mg/mL) was placed on an agar plate seeded with the appropriate test organism in triplicates. The utilized test organisms were *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* strains. Amikacin and chloroamphenicol were used as standard antibacterial agents and clotrimazole was used as a standard antifungal agent. DMSO alone was used as a control at the above mentioned concentration. The plates were incubated at 37ºC for 24h for bacteria and 28ºC 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).

2.3.1 Minimal inhibitory concentration (MIC) measurement

Broth dilution test was used to determine 'Minimal Inhibitory Concentration (MIC)' of the above mentioned samples [26,27]. The micro dilution susceptibility test was used for the determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds, and those of amikacin, chloroamphenicol, and clotrimazole were prepared in DMSO at concentrations of 1000 mg/mL followed by a two-fold dilution at concentrations of 500, 250, ….. 3.125 mg/mL. All the plates were incubated at 37ºC for 24h for bacteria and at 28ºC for 48h for fungi and the minimal inhibitory concentrations (MIC) were determined. Control experiments were also done.

2.4 Antimycobacterial Activity

All the compounds were screened for their *in vitro* antimycobacterial activity against MTB. The antimycobacterial activity of the compounds was tested by 'resazurin microplate assay (REMA)' as per Martin et al. [28,29]. 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 37ºC 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. Once 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 37ºC. Blue color in the wells containing the test compounds would indicate inhibition of growth and pink would indicate lack of inhibition of growth of *M. tuberculosis*. The minimum inhibitory concentration (MIC) was defined as the minimum concentration of compound required to 99.9% inhibition of bacterial growth.

2.5 Anticancer Activity

The *in vitro* anticancer activity was analyzed by MTT assay method [30, 31]. 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 37ºC, 5% CO_2, 95% air, and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid(EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with a medium containing 5% FBS to give the final density of 1x105 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 the cell attachment at 37ºC, 5% CO_2, 95% air, and 100% relative humidity. After 24h, the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) to prepare the stock (200 mM) and stored frozen prior to use. At the time of drug addition, an aliquot of the 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 already containing 100 µL of the medium, resulting in the required final drug concentrations. Following the drug addition, the plates were incubated for an additional 48h at 37ºC, 5% CO_2, 95% air, and 100% relative humidity. The medium without samples served as control and a triplicate was maintained for all concentrations.

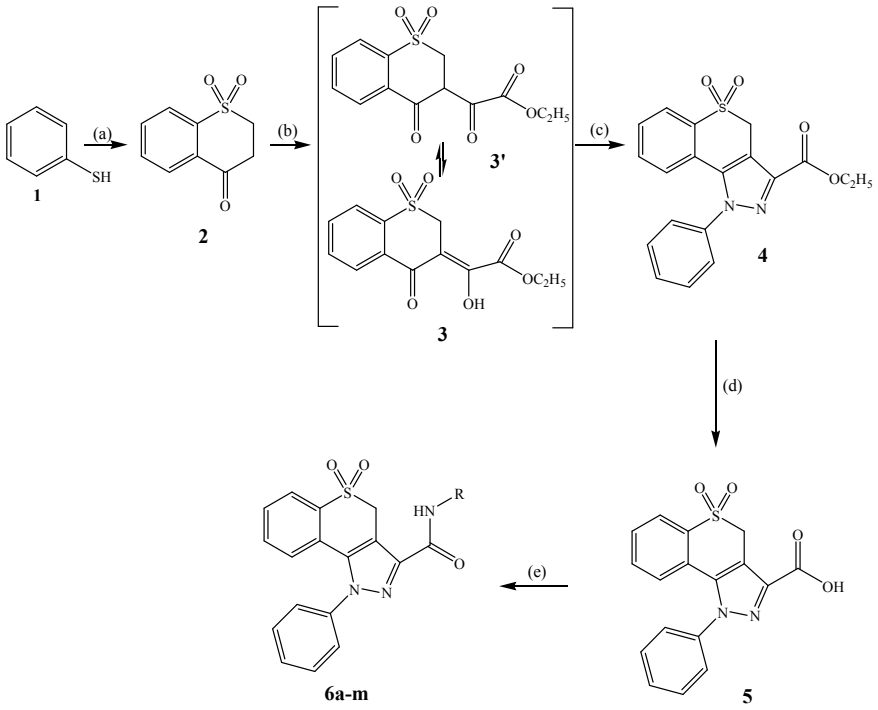
MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48h of incubation, 15µL 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 then flicked off and the precipitated formazan crystals were solubilized in 100 µL of DMSO and the absorbance measured at 570 nm using micro plate reader.

The % cell inhibition was determined using the following formula.
% cell Inhibition = 100-Abs (sample)/Abs (control) x100

3. RESULTS AND DISCUSSION

3.1 Chemistry

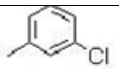
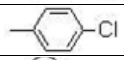
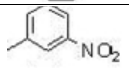
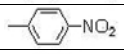
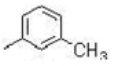
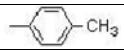
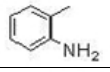
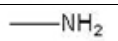
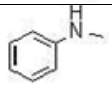
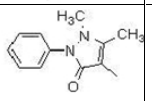
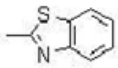
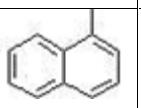
The strategies adopted for the synthesis of the intermediates and target compounds are depicted in Scheme 1 and Table 1. Compound **2** was prepared as per the reported methods [32,33]. Addition of 1 equiv of 2,3-dihydro-4H-thiochromen-4-one 1,1-dioxide (**2)** to diethyl oxalate in ethanol at room temperature in the presence of 2 equiv of a base, afforded the Claisen condensation product **3** [34] in which a single carbonyl group partially tautomerized.



Scheme 1. Synthetic route for the pyrazolecarboxamide analogues (6a-m). Reagents and conditions: (a) $ClCH_2CH_2COOH/NaOH$; PPA, H_2O_2/CH_3COOH (b) Na, dry EtOH, $(COOEt)_2$, (c) $C_6H_5NHNH_2$. HCl, EtOH (d) KOH, MeOH; Δ (e) i) $C_6H_5CH_3$, $SOCl_2$ ii) CH_2Cl_2, TEA, R-NH_2

Scheme 1 Synthetic route for the pyrazolecarboxamide analogues (**6a-m**). Reagents and conditions: (a) $ClCH_2CH_2COOH/NaOH$; PPA, H_2O_2/CH_3COOH (b) Na, dry EtOH, $(COOEt)_2$, (c) $C_6H_5NHNH_2$. HCl, EtOH (d) KOH, MeOH; Δ (e) i) $C_6H_5CH_3$, $SOCl_2$ ii) CH_2Cl_2, TEA, R-NH_2.

Table 1. Pyrazolecarboxamide analogues (6a-m) from 1-phenyl-1,4-dihydrothiochro - meno[4,3-c]pyrazole-3-carboxylic acid 5,5-dioxide (5) and amines

S.No	R	Compound (6)	6a-6m		
			Time (h)	M.pt (^oC)	Yield (%)
1.		a	3.5	268-271	69
2.		b	3.5	276-279	73
3.		c	3.5	275-278	65
4.		d	4.5	287-290	59
5.		e	4.5	278-281	57
6.		f	3	277-280	65
7.		g	3	276-279	61
8.		h	3	289-292	63
9.		i	3.5	283-286	67
10.		J	3.5	289-292	69
11.		k	3	294-297	68
12.		l	3.5	292-295	65
13.		m	4	296-299	61

Subsequent reaction of 1 equiv of this mixture with 1.15 equiv of phenylhydrazine hydrochloride at reflux in ethanol afforded the ethyl 1-phenyl-1,4-dihydrothiochromeno[4,3-c]pyrazole-3-carboxylate 5,5-dioxide (**4**). The IR spectrum of **4** showed absorption bands at 3089 and 1705 cm^{-1} due to NH and C=O groups, respectively and sulfone absorption bands were observed at 1309(asym) and 1152 (sym) cm^{-1}. The ^{1}H-NMR spectrum showed a triplet at 1.2 ppm (-C<u>H</u>$_3$), a quartet at 4.2 ppm (-O-C<u>H</u>$_2$), a singlet at 4.7(-SO_2-CH_2) and a multiplet in the region at 7.4-7.9 ppm (aromatic protons). The mass spectrum revealed a molecular ion peak at m/z = 368.05.

Alkaline hydrolysis of **4** afforded the corresponding 1-phenyl-1,4-dihydrothiochromeno[4,3-*c*]pyrazole-3-carboxylic acid 5,5-dioxide **5**. The IR spectrum showed a strong band at 1668 (conjugated carbonyl) and the sulfone absorption bands were found at 1306 cm^{-1}(asym) and 1150 (sym) cm^{-1}. The ^{1}H-NMR spectrum showed a singlet at 4.7 (-SO_2-CH_2) and a multiplet

in the region 7.3-7.9 ppm (aromatic protons). The mass spectrum revealed a molecular ion peak at m/z = 340.03.

The pyrazole acid **5** was converted to the corresponding acid chloride followed by treatment with an excess of the appropriate amine (Table 1) to give **6a-m**. Among all the analogues, nitro substituted compound **6d** and **6e** were obtained in low yields (57-59%) and the chloro and 4-aminoantipyrine substituted compounds were in high yields (68-73%). The toludine and 4-aminoantipyrine compounds readily reacted with the acid chloride of **5**.

The structures of compounds **6a-m** have been elucidated on the basis their IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectral data (*vide* supplementary materials). Compounds **6a-m** displayed characteristic absorption bands in the IR spectra around 3117-3196 and 1632-1657 cm^{-1} due to N-H and C=O functional groups respectively and the sulfone absorption bands were found at 1149-1158 and 1298-1310 cm^{-1}. ^{1}H-NMR spectra of **6a-m** exhibited a singlet at 4.1-4.7 ($-SO_2-CH_2$) ppm, and a broad singlet at δ 10.2-10.5 ppm due to CONH proton. Molecular ion peaks at *m/z* 415.07, 449.04, 449.02, 460.04, 460.06, 429.02, 429.05, 430.09, 354.02, 430.06, 525.12, 472.02, and 465.08 corresponded to **6a**, **6b, 6c, 6d, 6e, 6f, 6g, 6h, 6i, 6j, 6k, 6l,** and **6m** respectively. Elemental analyses were satisfactory and confirmed the elemental compositions and purities of the newly synthesized compounds **6a-m**.

3.2 Pharmacology

3.2.1 Antimicrobial evaluation

All the fifteen newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *S. aureus,* and *S. pneumoniae,* as examples of Gram-positive bacteria and *K. pneumonia, P. aeruginosa,* and *E. coli* as examples of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *C. albicans, A. flavus* and *A. niger* fungal strains. Agar-diffusion method was used for the determination of the preliminary antibacterial- and antifungal activity. Amikacin, chloroamphenicol, 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 minimal inhibitory concentration (MIC) measurement for these compounds showed significant growth inhibition zones (>20 mm) by a two-fold serial dilution method [35]. The MIC (µg/mL) and inhibition zone diameter values are recorded in Table 2.

The results depicted in Table 2 revealed that most of the tested compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains, and also against antifungal 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 intermediates **4** and **5** exhibited better antibacterial potentials than the pyrazolecarboxamides **(6a-m)** (Scheme 1, Table 1).

Regarding the pyrazole carboxamides against Gram-negative bacteria, the results revealed that compounds **6b**, **6c**, **6k**, and **6l** exhibited broad spectrum antibacterial profile against the tested organisms. Pyrazoles with electron withdrawing groups such as chloro, 2-aminobenzothizole, and 4-aminoantipyrine recorded higher activity. In this view, compound **6k** was 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*. Compounds **6b**, **6c,** and **6l** displayed higher activity on amikacin in inhibiting the growth of *Escherichia coli* (MIC 3.125 µg/mL).

Table 2. Minimal inhibitory concentrations (MIC µg/mL) and inhibition zone (mm) of compounds 4, 5, and 6a-m

Compound no.	MIC in mg/mL, and zone of inhibition (mm)							
	Bacteria					Fungi		
	Gram-positive bacteria		Gram-negative bacteria					
	Staphylococcus aureus	***Streptococcus pneumoniae***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Candida albicans***	***Aspergillus flavus***	***Aspergillus niger***
4	>100 (24-27)	50 (21-24)	---[a]	12.5 (20-23)	50 (25-28)	50 (22-25)	---	25(26-29)
5	50 (21-24)	25 (24-27)	25 (25-28)	12.5 (24-27)	50 (26-29)	25 (25-28)	50 (24-27)	25 (24-27)
6a	25 (24-27)	25 (25-28)	12.5 (26-29)	12.5 (28-31)	25 (26-29)	25 (27-30)	25 (25-28)	12.5 (29-32)
6b	25 (31-33)	12.5 (32-35)	6.25 (30-33)	6.25 (29-32)	3.125 (33-36)	6.25 (29-32)	6.25(31-34)	3.25 (25-28)
6c	25 (32-35)	12.5 (36-39)	6.25 (34-37)	6.25 (32-35)	3.125 (35-38)	6.25 (30-33)	6.25 (34-37)	3.25 (27-30)
6d	25 (28-31)	25 (25-28)	12.5 (27-30)	12.5 (25-28)	25 (28-31)	25 (29-32)	12.5 (25-28)	12.5 (29-32)
6e	25(27-30)	25 (28-31)	12.5 (29-32)	.5 (23-25)	25 (25-28)	25 (28-31)	12.5 (24-27)	12.5 (27-30)
6f	12.5(32-35)	12.5 (33-36)	25 (34-37)	12.5 (33-35)	12.5 (31-33)	25 (27-30)	12.5 (30-33)	25 (32-35)
6g	12.5 (33-35)	12.5(35-38)	25(32-35)	12.5 (32-35)	12.5 (31-34)	25 (28-31)	12.5 (30-33)	25 (31-34)
6h	25 (25-28)	25 (29-32)	12.5 (25-28)	12.5 (23-25)	25 (26-29)	25 (28-31)	25 (25-28)	12.5 (26-29)
6i	25 (27-30)	25 (27-30)	12.5 (28-29)	25 (25-28)	12.5 (30-33)	12.5 (22-25)	12.5 (24-27)	12.5 (25-28)
6j	12.5 (25-28)	25 (29-32)	12.5 (25-28)	25 (22-25)	12.5 (24-27)	12.5 (25-28)	12.5 (26-29)	6.25 (28-31)
6k	6.25 (38-41)	6.25 (37-40)	3.125 (31-33)	6.25 (35-38)	3.125 (37-40)	3.125 (30-33)	6.25 (31-34)	3.125 (32-34)
6l	12.5 (34-37)	6.25 (31-33)	6.25 (30-33)	6.25 (22-25)	3.125 (30-33)	6.25 (28-31)	6.25 (30-33)	3.125 (27-30)
6m	25(21-24)	25 (27-30)	12.5 (25-28)	12.5 (23-27)	12.5 (25-28)	25 (26-29)	25 (21-24)	12.5 (23-24)
Chloramphenicol	3.125 (38-41)	6.25 (38-41)	6.25 (32–35)	6.25 (36–39)	6.25 (34-37)	NT[b]	NT	NT
Amikacin	6.25 (35-38)	6.25 (34-37)	3.125 (37-40)	6.25 (29–32)	6.25 (36–39)	NT	NT	NT
Clotrimazole	NT	NT	NT	NT	NT	6.25 (29-32)	6.25 (28-31)	3.125 (27-30)

[a] *(---): totally inactive (no inhibition zone)* [b] *NT: Not tested.*

Compounds **6a**, **6d**, **6e**, **6f**, **6h**, **6i**, **6j,** and **6m** exhibited weak growth inhibitory activity against Gram-positive bacteria and moderate growth inhibitory activity against Gram-negative bacteria as revealed from their MIC values (12.5-25 µg/mL). Among these compounds **6f**, **6g**, **6j**, and **6l** showed relatively good growth inhibitory profiles against *Staphylococcus aureus* (MIC 12.5 µg/mL) compared to chloroamphenicol and amikacin. Compounds **6b**, **6c**, **6f**, and **6g** displayed relatively moderate growth inhibitory profiles against *Streptococcus pneumoniae* (MIC 12.5 µg/mL). Compounds **4**, **5**, **6a**, **6d**, **6e**, **6f**, **6g**, and **6m** displayed moderate growth inhibitory profiles against *Pseudomonas aeruginosa* and compounds **6f**, **6g**, **6i**, **6j**, and **6m** showed relatively good growth inhibitory profiles against *Escherichia coli* (MIC 12.5 µg/mL) compared to chloroamphenicol and amikacin.

Regarding the activity of pyrazole ester **4,** pyrazole acid **5,** and pyrazole carboxamides **6a-m**, against antifungal strains, the results revealed that compounds **6i**, and **6j** exhibited relatively good growth inhibitory profiles against *Candida albicans,* compounds **6a**, **6d**, **6e**, **6h**, **6i**, and **6m** against *Aspergillus flavus* and compounds **6a**, **6d**, **6e**, **6h**, **6i**, and **6m** against *Aspergillus niger* (MIC 12.5 µg/mL) compared to clotrimazole. Compounds **6b**, **6c**, and **6l** showed a comparable activity against clotrimazole. Compound **6k** was equipotent to clotrimazole in inhibiting the growth of *Candida albicans* (MIC 3.125 µg/mL).

3.2.2 *In vitro* antituberculosis activity

All the compounds were screened 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 a standard drug. The pyrazole carboxamides **6a-m** displayed better antituberculosis activity compared to their predecessors, the pyrazole ester (**4**) and pyrazole acid (**5**). The observed data on the anti-tuberculosis activity of the title compounds (**6a-m**) and the standard drug are given in Table 3. Thirteen compounds were found to be active with minimum inhibitory concentrations of 7.8-27 µM. The chloro-, 2-aminobenzothiazole-, and 4-aminoantipyrine-substituted compounds produced more inhibitory activity whereas the nitro-, phenyl-, and naphthalene-substituted compounds showed less inhibitory activity. Compound **6k** showed good inhibitory activity against MTB at MIC 7.8 µM, while compounds **6b**, **6c,** and **6l** displayed moderate inhibitory activity against MTB at MIC 8.2-8.8 µM (Table 3).

3.2.3 *In vitro* antitumor activity

The newly synthesized compounds **6a-m** were initially screened at the 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 100µM. 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 3 and Fig. 1 and Fig. 2.

Table 3. Antimycobacterial activity and antitumor activity of compounds 4, 5, and 6a-m

Compounds	Antitumor activity IC_{50}(μM)[a]		Antituberculosis activity MIC (μM)
	HeLa cell	HCT116	
4	>100	>100	72
5	>100	>100	63
6a	72	68	27
6b	23	21	8.8
6c	22	20	8.6
6d	62	63	37
6e	60	65	31
6f	54	52	26
6g	44	46	28
6h	58	48	33
6i	56	52	24
6j	65	64	28
6k	17	15	7.8
6l	22	20	8.4
6m	64	66	37
INH	---[b]	---[b]	8.2
Doxorubicin[c]	20	18	---[b]

[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. Negative control DMSO, no activity.*

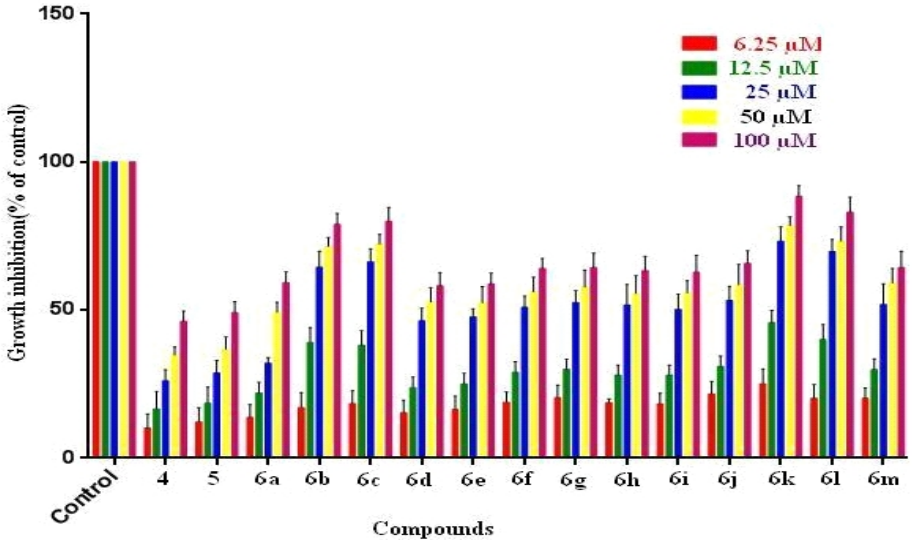


Fig. 1. Effect of the compound 4, 5, and 6a-m on growth inhibition based on concentration of HeLa cell

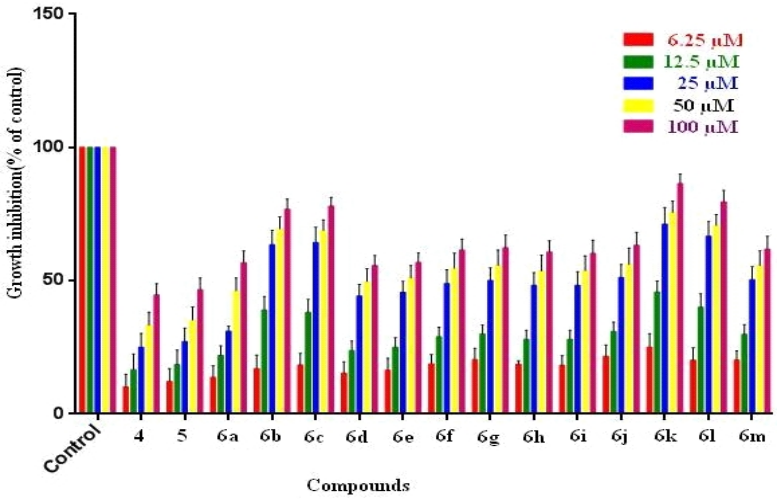


Fig. 2. Effect of the compound 4, 5, and 6a-m on growth inhibition based on concentration of HCT116 cell

Cell growth inhibition was analyzed by MTT assay and the results showed that the compounds **4**, **5**, and **6a–m** exhibited an inhibitory effect on the proliferation of HeLa and HCT116 cells in a dose-dependent manner (Table 3). Compound **6k** was found to exhibit higher cytotoxic potency (17µM and 15 µM) than that of doxorubicin (20µM and 18 µM) against HeLa cell and HCT116 cell. Of note is that the 4-aminoantipyrinepyrazole carboxamide derivative (**6k**) has higher potency than the remaining derivatives on both the cancer cells. The m- and p-chloro substituted compounds (**6b** and **6c**), and 2-aminobenzothiazole substituted compound (**6l**) showed comparable IC_{50} values than the other 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.

4. CONCLUSION

A new series of *N*,1-diphenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxamide 5,5-dioxide derivatives (**6a-m)** was synthesized and their bioactivity was investigated. Results obtained clearly revealed that the chloro (**6b** and **6c**), 2-aminobenzothiazole (**6l**), and 4-aminoantipyrine (**6k**) linkages exhibited better antimicrobial activity than their counterparts. Similarly, compound **6k** was found to possess comparatively more antimicrobial, antituberculosis, and antitumor activity against the other derivatives.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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