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Analogues of *N*,1-diphenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxamide and *N*,1-diphenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxamide-6,6-dioxide: syntheses, characterization, antimicrobial, antituberculosis, and antitumor activity†

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A series of *N*,1-diphenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxamides (**11a–m**) and *N*,1-diphenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxamide-6,6-dioxides (**12a–m**) were synthesized by varying the active part (carboxamide group) of the pyrazole and were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, mass spectral data, and elemental analyses. All compounds were evaluated for their antibacterial and antifungal activity. Compounds **11k** and **12k** showed higher activity than chloroamphenicol against *Klebsiella pneumonia* and *Escherichia coli*. Compounds **11b**, **11c**, **11l**, **12b**, **12c**, and **12l** displayed higher activity towards amikacin in inhibiting the growth of *Escherichia coli* (MIC 3.125 mg mL^{-1}). Compounds **11k** and **12k** were equipotent to clotrimazole in inhibiting the growth of *Candida albicans* (MIC 3.125 mg mL^{-1}). All compounds were screened for their cytotoxic activity against two tumor cell lines, namely the human colon tumor cell line (HCT116) and human cervical cancer cell line (HeLa). Most of the test compounds exhibited potent antitumor activity, especially compounds **11k** and **12k**, which displayed the highest activity among the test compounds with an IC_{50} equal to 18 and 12 μM for HeLa cells, and 16 and 10 μM for HCT116 cells, respectively. All the synthesized compounds showed low to moderate inhibitory activities against *M. tuberculosis* (MTB) H_{37}Rv, whereas **11k** and **12k** were found to be more active against *M. tuberculosis*, with MIC values of 8.2 and 7.8 μM, compared to other analogues.

1 Introduction

The pyrazole moiety is present in many natural products.[1] Its derivatives are reported to have a broad spectrum of biological activities, such as antitumour,[2] anticoagulant,[3] antihyperglycemic, analgesic, antipyretic, antimicrobial, and hypoglycemic activity.[4–8] These derivatives have applications in drug development.[9] Arylpyrazoles are important in medicinal and pesticidal chemistry.[10,11] Some arylpyrazoles were reported to have non-nucleoside HIV-1 reverse transcriptase inhibitory activity.[12] Li *et al.* have reported a series of *N*,1,3-triphenyl-1*H*-pyrazole-4-carboxamide derivatives that exhibited potent antiproliferative activities against HTC116, MCF-7 cells, and Aurora-A kinase inhibitory activities.[13] Pyrazolo[3,4-*d*]pyrimidines are of considerable chemical and pharmacological importance because of their structural similarities with purine, and many derivatives of pyrazolo[3,4-*d*]pyrimidines have been reported as antitumoragents[14–23] and cannabinoid type-1 (CB1) receptor antagonists.[24,25] Ding *et al.* reported a series of novel 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carboxamide derivatives which inhibited the proliferation of A549 cells.[26] The azole group of heterocyclic compounds possesses a significant pharmacokinetic property, and lipophilicity that influences the ability of a drug to reach the target by transmembrane diffusion, and shows promising activity against resistant TB by inhibiting the biosynthesis of lipids.[27,28] Ahsan *et al.* have reported the antimycobacterial activity of 4-dihydro-3*H*-indeno[1,2-*c*]pyrazole-2-carboxamide analogues.[29–31]

The synthesis and *in vitro* antitumour activity of several benzo[*b*]thiophenesulfonamide-1,1-dioxide derivatives have been reported.[32] Benzo[*b*]thiophene-4-carboxamide-1,1-dioxide derivatives have been described in the literature as preventives for various inflammatory and neoplastic diseases caused by an abnormal production of interleukin-6 or interleukin-12.[33] Alonso[34] and Sagardoy[35] and co-workers

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synthesized a series of benzo[*b*]thiophene-6-carboxamide-1,1-dioxide derivatives which exhibited growth inhibition of HTB-54, CCRF-CEM, and HeLa tumour cells.

In view of the above-mentioned facts to identify new candidates that may be valued as potent and less toxic antimicrobial, antimycobacterial and antitumor agents, we report herein the syntheses, characterization, and biological evaluation of a series of pyrazole carboxamides.

2 Results and discussion

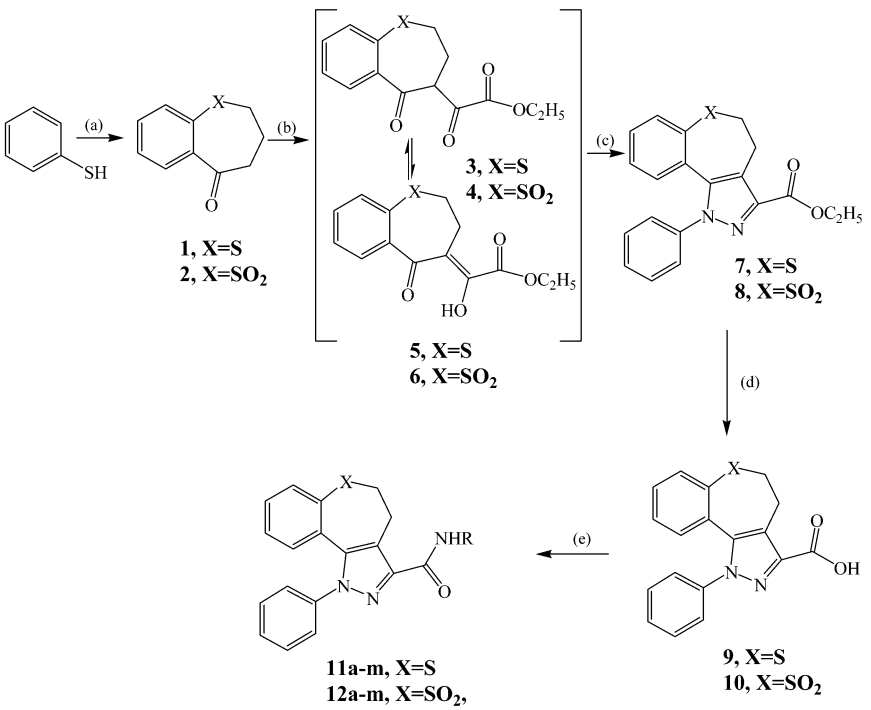
2.1 Chemistry

The strategies adopted for the synthesis of the intermediates and target compounds are depicted in Scheme 1 and Table 1. Compound **1** and **2** were prepared as per the reported methods.[36] Addition of 1 equiv. of the requisite thiochroman-4-one (**1**) and 3,4-dihydro-1-benzothiepin-5(2*H*)-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 products **3**/**5** and **4**/**6** respectively.[37]

Subsequent reaction of 1 equiv. of a mixture of **3**/**5** and **4**/**6** with 1.15 equiv. of the phenylhydrazine hydrochloride at reflux in ethanol afforded the ethyl 1-phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxylate (**7**) and ethyl 1-phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxylate-6,6-dioxide (**8**) respectively. The IR spectrum of **7** showed a carbonyl absorption band at 1707 cm^{-1}. The ^{1}H-NMR spectrum showed a triplet at δ 1.2 ppm (–CH_3 group), two more triplets at δ 2.9 and 3.0 ppm (–S–CH_2–CH_2 and –S–CH_2–CH_2 group), a quartet at δ 4.2 ppm (–O–CH_2 group) and a multiplet in the region of δ 6.8–7.5 ppm (aromatic protons). The mass spectrum revealed a molecular ion peak at m/z = 350.11. The IR spectrum of compound **8** showed a carbonyl absorption band at 1696 cm^{-1} and sulfone absorption bands at 1156 (sym) and 1308 (asym) cm^{-1}. The ^{1}H-NMR spectrum showed a triplet at δ 1.1 ppm (–CH_3 group), two more triplets at δ 3.1 and 3.8 ppm (–SO_2–CH_2–CH_2 and –SO_2–CH_2–CH_2 group), a quartet at δ 4.3 ppm (–O–CH_2 group) and a multiplet in the region of δ 6.9–7.6 ppm (aromatic protons). The mass spectrum of **8** revealed a molecular ion peak at m/z = 382.08.

Alkaline hydrolysis of **7** and **8** afforded the corresponding 1,4-dihydro-1-phenylthiochromeno[4,3-c]pyrazole-3-carboxylic acid (**9**) and 1-phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxylic acid-6,6-dioxide (**10**), respectively. The IR spectrum of compound **9** showed a strong band at 1688 cm^{-1}, which was due to the conjugated carbonyl. The ^{1}H-NMR spectrum showed two triplets at δ 2.9 and 3.0 ppm (–S–CH_2 and –CH_2 group), and a multiplet in the region of 6.8–7.7 ppm (9H, aromatic). The mass spectrum displayed a molecular ion peak at m/z = 322.10. The IR spectrum of compound **10** displayed a strong band at 1668 cm^{-1}, which was due to the conjugated carbonyl, and the sulfone absorption bands were at 1152 (sym) and 1303 cm^{-1}(asym). The ^{1}H-NMR spectrum showed two triplets at δ 3.1 and 3.8 ppm (–SO_2–CH_2–CH_2 and –SO_2–CH_2–CH_2 group), and a multiplet in the region of 6.9–7.9 ppm (9H, aromatic). The mass spectrum displayed a molecular ion peak at m/z = 354.05.

The pyrazole acid **9** and **10** were converted to the corresponding acid chloride followed by treatment with an



Scheme 1 Synthetic route for the pyrazole carboxamide analogues (**11a–m** and **12a–m**). Reagents and conditions: (a) $ClCH_2CH_2CH_2COOH$/NaOH; PPA (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

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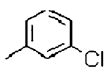
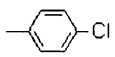
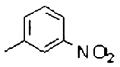
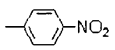
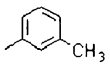
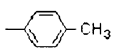
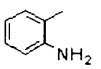
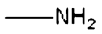
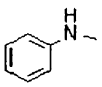
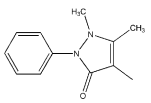
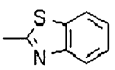
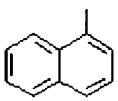
Table 1 Pyrazole carboxamide analogues (**11a–m** and **12a–m** from 1-phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxylic acid and amines

			11a–m			**12a–m**		
No	R	Compound **11** and **12**	Time (h)	M. p. (°C)	Yield (%)	Time (h)	M. p.(°C)	Yield (%)
1		**a**	3.0	238–241	71	3.5	268–271	51
2		**b**	3.5	246–249	75	3.5	275–278	52
3		**c**	3.5	245–248	68	4.0	276–279	54
4		**d**	4.0	257–260	62	4.0	286–289	49
5		**e**	4.0	258–261	60	4.0	286–289	46
6		**f**	2.5	247–250	68	3.0	246–249	56
7		**g**	2.5	246–249	64	3.0	245–248	54
8		**h**	2.5	259–262	66	3.0	287–290	52
9		**i**	3.0	253–256	70	3.0	282–286	54
10		**j**	3.0	259–262	72	3.0	287–290	56
11		**k**	2.5	264–267	72	3.0	292–292	56
12		**l**	3.0	262–265	68	3.0	290–293	52
13		**m**	3.5	266–269	64	4.0	293–296	54

excess of the appropriate amine (Table 1) to give **11a–m** and **12a–m**. The sulfone derivatives **12a–m** were obtained in lower yield compared to **11a–m**. The nitro substituted compounds **12d** and **12e** were obtained in moderate yield (46–48%) and the chloro- and 4-aminoantipyrine substituted compounds were obtained in good yields (72–75%). The toludine- and 4-aminoantipyrine-substituted compounds readily reacted with the corresponding acid chlorides.

The structures of compounds **11a–m** and **12a–m** have been elucidated on the basis of their IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data (ESI†). Compounds **11a–m** displayed characteristic absorption bands in the IR spectra around 3117–3196 and 1632–1657 cm^{-1}, which were due to the N–H and C=O stretching, respectively. The ^{1}H-NMR spectra of **11a–m** exhibited a triplet at δ 2.9 and 3.0 ppm (–S–CH_2–CH_2 and –S–CH_2–CH_2 group), and a broad singlet at δ 9.5–10.5, which was due to the CONH proton. The mass spectra of products **11a–m** agree well with the structures, with molecular ion peaks at *m*/*z* 397.51, 431.07, 431.10, 442.13, 442.10, 411.12, 411.16, 412.12, 336.12, 412.16, 507.15, 454.11, and 447.12, which correspond to **11a**, **11b**, **11c**, **11d**, **11e**, **11f**, **11g**, **11h**, **11i**, **11j**, **11k**, **11l**, and **11m** respectively. Elemental analyses were

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satisfactory, which confirmed the elemental compositions and purities of the newly synthesized compounds **11a–m**.

Compounds **12a–m** displayed characteristic absorption bands in the IR spectra at around 3124–3211 and 1624–1661 cm^{-1}, due to N–H and C=O stretching, respectively, and the sulfone absorption bands were found at 1150–1156 and 1300–1308 cm^{-1}. The ^{1}H-NMR spectra of **12a–m** exhibited two triplets at δ 3.0–3.1 and 3.7–3.8 ppm (–SO_2–CH_2–CH_2 and –SO_2–CH_2–CH_2 group), and a broad singlet at δ 9.7–10.9, which was due to the CONH proton. The mass spectra of products **12a–m** agree well with the structures, with molecular ion peaks at *m*/*z* 429.19, 463.06, 463.05, 474.08, 474.06, 443.12, 443.10, 444.10, 368.09, 444.12, 539.14, 486.06, and 479.10, which correspond to **12a**, **12b**, **12c**, **12d**, **12e**, **12f**, **12g**, **12h**, **12i**, **12j**, **12k**, **12l**, and **12m** respectively. Elemental analyses confirmed the elemental compositions of **12a–m**.

3 Pharmacology

3.1 Antimicrobial evaluation

All 15 newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus*, and *Streptococcus pneumonia*, as examples of Gram-positive bacteria and *Klebsiella pneumonia*, *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. The 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 minimum inhibitory concentration (MIC) measurement for these compounds showed significant growth inhibition zones (>20 mm) by a two fold serial dilution method.[38] The MIC (mg mL^{-1}) 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 the fungal strains. In general, these compounds revealed better activity against the Gram-negative rather than the Gram-positive bacteria. It was found that the intermediates **7**, **8**, **9** and **10** exhibited better antibacterial potentials than the pyrazole carboxamides **11a–m** and **12a–m**. (Scheme 1 and Table 1)

Regarding the structure activity relationship (SAR) of the pyrazole carboxamide against Gram-negative bacteria, the results revealed that the chloro-, 4-aminoantipyrine-, and 2-aminobenzothiazole substituted pyrazole compounds exhibited a broad spectrum antibacterial profile against the tested organisms. In this view, 4-aminoantipyrine-substituted pyrazole was found to exhibit higher activity (MIC 3.125 mg mL^{-1}) than that of chloroamphenicol (MIC 6.25 mg mL^{-1}) against *K. pneumonia* and *E. coli*. The chloro- and 2-aminobenzothiazole derivatives displayed higher activity than amikacin in inhibiting the growth of *E. coli* (MIC 3.125 mg mL^{-1}).

The toludine- and phenylhydrazine-substituted pyrazoles showed reasonably good growth inhibitory profiles against *S. aureus* (MIC 12.5 mg mL^{-1}) compared to chloroamphenicol and amikacin. The chloro- and toludine-pyrazole analogues displayed relatively moderate growth inhibitory profiles against *S. pneumonia* (MIC 12.5 mg mL^{-1}). Regarding the activity of the pyrazole ester, pyrazole carboxylic acid, and pyrazole carboxamides, against antifungal strains, the results revealed that the chloro- and 2-aminobenzothiazole derivatives showed a comparable activity against clotrimazole. 4-Aminoantipyrine analogues are equipotent to clotrimazole in inhibiting the growth of *C. albicans* (MIC 3.125 mg mL^{-1}).

3.2 *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 *via* the agar dilution method using two fold dilution techniques. Isoniazid (INH) was used as a standard drug. The pyrazole carboxamides (**11a–m** and **12a–m**) displayed better antituberculosis activity compared to their predecessors, the pyrazole ester (**7** and **8**) and pyrazole acids (**9** and **10**). The observed data on the antituberculosis activity of the title compounds and the standard drug are given in Table 3. Thirty compounds were found to be active with minimum inhibitory concentrations of 7.8–26 μM. The chloro-, 2-aminobenzothiazole-, and 4-aminoantipyrine-substituted compounds produced more inhibitory activity than the nitro-, phenyl-, and naphthyl-substituted systems. 4-Aminoantipyrine substituted pyrazole carboxamide (**11k** and **12k**) showed good inhibitory activity against MTB at MIC 8.2 and 7.8 μM, while compounds **11b–c**, **12b–c**, **11l** and **12l** showed moderate inhibitory activity against MTB at MIC 8.9–9.8 (Table 3).

3.3 *In vitro* antitumor activity

The newly synthesized compounds **11a–m** and **12a–m** 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 re-tested 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 3 and Fig. 1 and Fig. 2.

Cell growth inhibition was analyzed by the MTT assay and the results show that the compounds **7**, **8**, **9**, **10**, **11a–m**, and **12a–m** exhibit an inhibitory effect on the proliferation of HeLa and HCT116 cells in a dose-dependent manner (Table 3). Compounds **11k** and **12k** were found to exhibit a higher cytotoxic potency (18, 12 μM and 16, 10 μM) than that of doxorubicin (21 μM and 19 μM) against HeLa cells and HCT116 cells. Of note is that the 4-aminoantipyrinepyrazole-carboxamide derivatives (**11k** and **12k**) have a higher ability than the rest towards both the cancer cells. The *m*- and

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Table 2 Minimal inhibitory concentrations (MIC, mg mL^{-1}) and inhibition zone (mm) of compounds.**7**, **8 9**, **10**, **11a–m**, and **12a–m**

	MIC in mg mL^{-1}, and zone of inhibition/mm							
	Bacteria					Fungi		
	Gram-positive bacteria		Gram-negative bacteria					
Compound no.	*Staphylococcus aureus*	*Streptococcus pneumonia*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*	*Aspergillus flavus*	*Aspergillus niger*
7	>100 (26–29)	50 (25–28)	—[a]	12.5 (23–26)	50 (27–30)	50 (25–28)	—	25(28–31)
9	50 (25–28)	25 (27–30)	25 (29–32)	12.5 (28–31)	50 (29–32)	25 (28–31)	50 (29–30)	25 (27–30)
11a	25 (29–32)	25 (28–31)	12.5 (31–34)	12.5 (32–35)	25 (31–34)	25 (32–35)	25 (29–32)	12.5 (31–34)
11b	25 (39–42)	12.5 (38–41)	6.25 (36–39)	6.25 (33–36)	3.125 (37–39)	6.25 (31–34)	6.25(36–39)	3.25 (29–32)
11c	25 (38–41)	12.5 (39–42)	6.25 (36–39)	6.25 (34–37)	3.125 (37–40)	6.25 (32–35)	6.25 (36–39)	3.25 (29–32)
11d	25 (30–33)	25 (28–31)	12.5 (30–33)	12.5 (28–31)	25 (30–33)	25 (32–35)	12.5 (28–31)	12.5 (32–35)
11e	25(31–34)	25 (29–32)	12.5 (31–34)	12.5 (27–30)	25 (29–32)	25 (31–33)	12.5 (29–32)	12.5 (33–36)
11f	12.5(37–40)	12.5 (38–41)	25 (36–39)	12.5 (37–40)	12.5 (34–37)	25 (29–32)	12.5 (32–35)	25 (36–39)
11g	12.5 (38–41)	12.5(39–42)	25(35–38)	12.5 (36–39)	12.5 (35–38)	25 (30–33)	12.5 (33–36)	25 (34–37)
11h	25 (28–31)	25 (31–33)	12.5 (29–32)	12.5 (26–29)	25 (29–32)	25 (30–33)	25 (27–30)	12.5 (28–31)
11i	25 (30–33)	25 (29–32)	12.5 (28–31)	25 (27–30)	12.5 (33–36)	12.5 (25–28)	12.5 (28–31)	12.5 (27–30)
11j	12.5 (28–31)	25 (32–35)	12.5 (27–30)	25 (26–29)	12.5 (26–29)	12.5 (27–30)	12.5 (28–31)	6.25 (31–34)
11k	6.25 (40–43)	6.25 (39–42)	3.125 (34–37)	6.25 (38–41)	3.125 (40–43)	3.125 (33–36)	6.25 (34–37)	3.125 (33–36)
11l	12.5 (39–42)	6.25 (35–38)	6.25 (33–36)	6.25 (24–25)	3.125 (33–36)	6.25 (31–34)	6.25 (33–36)	3.125 (29–32)
11m	25(24–27)	25 (30–33)	12.5 (27–30)	12.5 (25–28)	12.5 (28–31)	25 (29–32)	25 (24–27)	12.5 (26–29)
8	>100 (22–25)	50(26–29)	>100 (23–25)	12.5(28–31)	25(26–29)	25(28–31)	>100(24–27)	12.5(23–26)
10	50(28–31)	25 (31–33)	25(28–31)	12.5 (28–31)	50(30–33)	25 (29–31)	50 (30–33)	25 (29–31)
12a	25 (33–36)	25 (32–35)	12.5 (33–36)	12.5 (33–36)	25 (32–35)	25 (33–36)	25 (30–33)	12.5 (32–35)
12b	25 (40–43)	12.5 (39–42)	6.25 (38–41)	6.25 (34–37)	3.125 (37–39)	6.25 (33–37)	6.25(38–41)	3.25 (31–34)
12c	25 (41–43)	12.5 (41–44)	6.25 (39–42)	6.25 (38–41)	3.125 (39–42)	6.25 (35–38)	6.25 (38–41)	3.25 (31–34)
12d	25 (32–35)	25 (30–33)	12.5 (32–35)	12.5 (29–32)	25 (32–35)	25 (33–36)	12.5 (30–33)	12.5 (34–37)
12e	25(33–36)	25 (31–34)	12.5 (33–37)	12.5 (29–32)	25 (33–35)	25 (33–35)	12.5 (30–33)	12.5 (35–38)
12f	12.5(39–42)	12.5 (40–43)	25 (39–42)	12.5 (40–43)	12.5 (37–40)	25 (31–34)	12.5 (34–37)	25 (39–42)
12g	12.5 (38–41)	12.5(39–42)	25(35–38)	12.5 (36–39)	12.5 (35–38)	25 (30–33)	12.5 (33–36)	25 (34–37)
12h	25 (32–35)	25 (34–37)	12.5 (32–35)	12.5 (29–32)	25 (30–33)	25 (33–36)	25 (30–33)	12.5 (29–32)
12i	25 (33–36)	25 (31–34)	12.5 (31–34)	25 (30–33)	12.5 (37–39)	12.5 (27–30)	12.5 (30–33)	12.5 (29–32)
12j	12.5 (28–31)	25 (32–35)	12.5 (27–30)	25 (26–29)	12.5 (26–29)	12.5 (27–30)	12.5 (28–31)	6.25 (31–34)
12k	6.25 (43–46)	6.25 (40–43)	3.125 (36–40)	6.25 (39–42)	3.125 (43–46)	3.125 (34–37)	6.25 (35–38)	3.125 (35–38)
12l	12.5 (40–43)	6.25 (38–41)	6.25 (36–39)	6.25 (26–29)	3.125 (36–39)	6.25 (35–38)	6.25 (37–40)	3.125 (32–35)
12m	25(25–28)	25 (33–36)	12.5 (30–33)	12.5 (28–31)	12.5 (32–35)	25 (32–35)	25 (27–30)	12.5 (29–32)
Chloramphe-nicol	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.

p-chloro substituted compounds (**11b–c** and **12b–c**), and 2-aminobenzothiazole substituted compounds (**11l** and **12l**) show comparable IC_{50} values than the other substituted compounds on both the cells. In general, many of the IC_{50} values for the HCT116 cells are lower than those for the corresponding HeLa cells.

4 Conclusion

The objective of the present study was to synthesize and investigate the antimicrobial activities of some new *N*,1-diphenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxamides (**11a–m**) and carboxamide-6,6-dioxides (**12a–m**) with the hope of discovering their bioactivity. Results obtained clearly revealed that the chloro-, 2-aminobenzothiazole-, and 4-aminoantipyrine-linkages exhibited better antimicrobial activity than their counterparts. Similarly, compounds **11k** and **12k** displayed higher antimicrobial, antituberculosis, and antitumor activity compared to the other derivatives. Compounds **12a–m** exhibited higher activity than the **11a–m** analogues.

5 Experimental

5.1 Analysis and instruments

Melting points were obtained on 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 400 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 (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 a nitrogen atmo-

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Table 3 Antimycobacterial activity and antitumor activity of the title compounds **7**, **8**, **9**, **10**, **11a–m** and **12a–m**[a]

Compound	Antitumor activity IC_{50}(μM)[b]		Antituberculosis activity MIC (μM)
	HeLa cell	HCT116	
7	>100	>100	64
9	>100	>100	54
11a	68	62	26
11b	24	23	9.8
11c	23	24	9.6
11d	60	58	38
11e	64	62	34
11f	58	54	28
11g	48	46	26
11h	56	50	30
11i	58	54	28
11j	62	60	24
11k	18	16	8.2
11l	22	20	9.4
11m	64	60	34
8	>100	>100	60
10	95	90	50
12a	60	57	20
12b	20	20	9.0
12c	21	21	8.9
12d	52	54	30
12e	54	56	28
12f	53	50	26
12g	42	40	24
12h	50	45	26
12i	52	50	22
12j	68	54	16
12k	12	10	7.8
12l	20	18	9.0
12m	58	54	30
INH	—[c]	—[c]	8.6
Doxorubicin[d]	21	19	—[c]

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

sphere. The separation of compounds was carried out by column chromatography using silica gel. Unless otherwise specified, all materials, solvents, and reagents were obtained commercially.

5.2 Synthesis

5.2.1 Ethyl 1-phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxylate (7). A stirred mixture of the diketoester (**3**/**5**) (1.0 equiv., 4 mmol) and the phenylhydrazine hydrochloride (1.15 equiv.) in EtOH (28 mL) was heated under reflux for 3.5 h. 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-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxylate (**7**) (1.12 g, 71%) as a yellow solid. R_f 0.64 (petroleum ether (40–60 °C)/EtOAc, 8 : 2); mp 156–159 °C (triturated with petroleum ether); FT-IR 1707, 1591, 1508, 1369, 1211 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.2 (t, 3H, $-CH_3$), 2.9 and 3.0 (t, 2H, $-S-CH_2-CH_2$ and $-S-CH_2-CH_2$), 4.2 (q, 2H, $-O-CH_2$), 6.8–7.5 (m, 9H, aromatic); ^{13}C-NMR ($CDCl_3$) δ 14.25 (CH_3), 23.34 (C_5), 35.86 (CH_3-CH_2), 60.54 (C_4), 119.42–149.64 (aromatic and olefinic carbon), 160.12 (CO). ESI-MS calcd for *m*/*z*: 350.11. Anal. Calcd for $C_{20}H_{18}N_2O_2S$: C, 68.55; H, 5.18; N, 7.99; O, 9.13; S, 9.15%. Found: C, 68.25; H, 5.16; N, 7.77; S, 9.11%.

5.2.2 1-(6,6-Dioxido-1-phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazol-3-yl)butan-1-one (8). A similar procedure for the preparation of **7** was followed for the preparation of **8**, with refluxing for 4 h. Compound **8** (0.92 g, 51%) was obtained as a yellow solid. R_f 0.64 (petroleum ether/EtOAc, 8 : 2); mp 186–189 °C (triturated with petroleum ether); FT-IR 1698, 1571, 1502, 1358, 1214, 1156, 1308 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.1 (t, 3H, $-CH_3$), 3.1 and 3.8 (t, 2H, $-SO_2-CH_2-CH_2$ and $-SO_2-CH_2-CH_2$), 4.3 (q, 2H, $-O-CH_2$), 6.9–7.6 (m, 9H, aromatic); ^{13}C-NMR ($CDCl_3$) δ 11.36 (C_5), 21.36 (CH_3), 34.72 ($-CH_3-CH_2$), 68.32 (C_4), 119.36–149.76 (aromatic and olefinic carbon), 160.35 (CO). ESI-MS calcd for *m*/*z*: 382.08. Anal. Calcd for $C_{20}H_{18}N_2O_4S$: C, 62.81; H, 4.74; N, 7.33; O, 16.73; S, 8.38%. Found: C, 62.57; H, 4.56; N, 7.17; S, 8.31%.

5.2.3 1-Phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxylic acid (9). To a mixture of ester **7** (1.0 equiv., 5 mmol) in methanol (25 mL), was added a solution of potassium hydroxide (2.0 equiv., 10mmol) in methanol (18 mL). The resulting mixture was heated under reflux for 3.5 h, cooled overnight, poured into water, and acidified with 1 N hydrochloric acid. The precipitate was filtered, washed with water, and air-dried to yield the analytically pure product of 1-phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxylic acid (**9**) (1.87g, 95.2%) as a colorless solid. R_f 0.54 ($CHCl_3$/MeOH 9 : 1); mp 261–263 °C; FT-IR 1688 (conjugated carbonyl), 1517, 1431, 1379, 1200 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.9 and 3.0 (t, 2H, $-S-CH_2-CH_2$ and $-S-CH_2-CH_2$), 6.8–7.5 (m, 9H, aromatic); ^{13}C-NMR ($CDCl_3$) δ 22.35 (C_4), 35.68 (C_5), 119.17–149.55 (aromatic and olefinic carbon), 159.454 (CO). ESI-MS calcd for *m*/*z*: 322.10. Anal. Calcd for $C_{18}H_{14}N_2O_2S$: C, 67.06; H, 4.38; N, 8.69; O, 9.93; S, 9.95%. Found: C, 66.95; H, 4.29; N, 8.57; S, 9.65%.

5.2.4 1-Phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxylic acid 6,6-dioxide (10). A similar procedure for the preparation of **7** was followed for the preparation of **8**, with refluxing for 4 h. Compound **10** (1.56g, 78%) was obtained as a colorless solid. R_f 0.54 ($CHCl_3$/MeOH 9 : 1); mp 291–293 °C; FT-IR 1688 (conjugated carbonyl), 1517, 1431, 1379, 1200, and sulfone absorption bands at 1152 and 1303 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 3.1 and 3.8 (t, 2H, $-SO_2-CH_2-CH_2$ and $-SO_2-CH_2-CH_2$), 7.1–7.5 (m, 9H, aromatic); ^{13}C-NMR ($CDCl_3$) δ 14.35 (C_4), 60.35 (C_5), 117.35–149.75 (aromatic and olefinic carbon), 160.45 (CO). ESI-MS calcd for *m*/*z*: 354.05. Anal. Calcd for $C_{18}H_{14}N_2O_4S$: C, 61.01; H, 3.98; N, 7.90; O, 18.06; S, 9.05%. Found: C, 60.95; H, 3.69; N, 7.57; S, 8.95%.

5.2.5 General syntheses of carboxamides (11 and 12). A mixture of the appropriate 1-phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxylic acid (**9**) or 1-phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxylic acid-6,6-dioxide (**10**) (1 equiv., 4.0 mmol) and thionyl chloride (3.0 equiv.) 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

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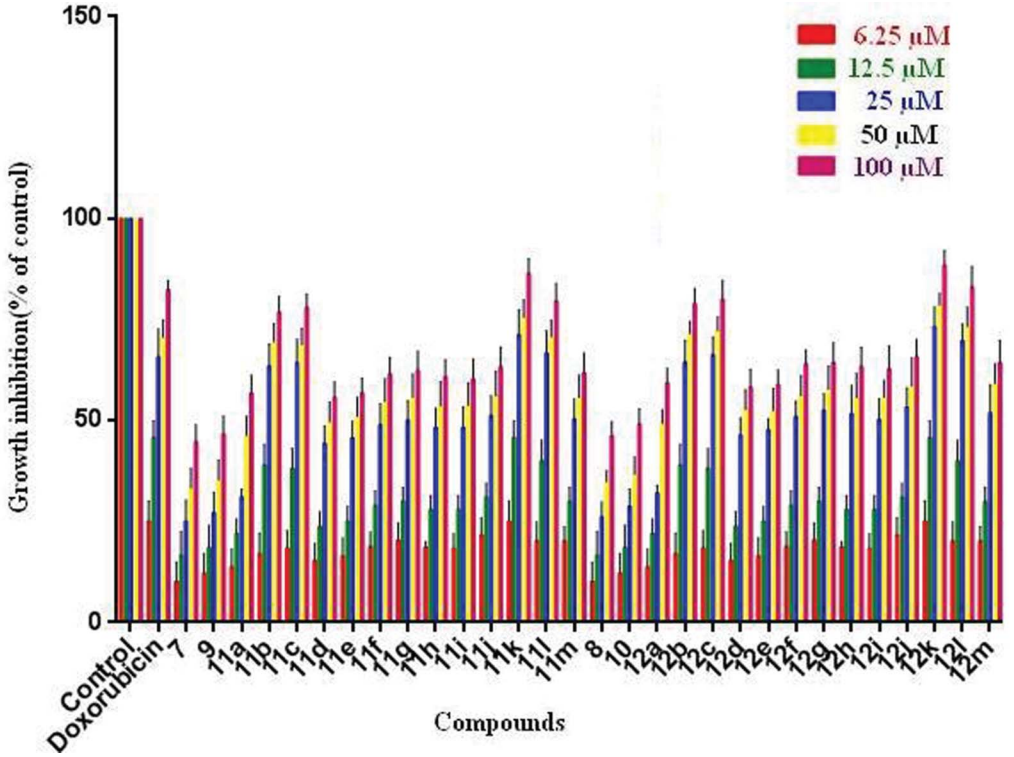

Fig. 1 Growth of inhibition of compounds **7–11a–m** and **12a–m**, based on the concentration in the HeLa cells

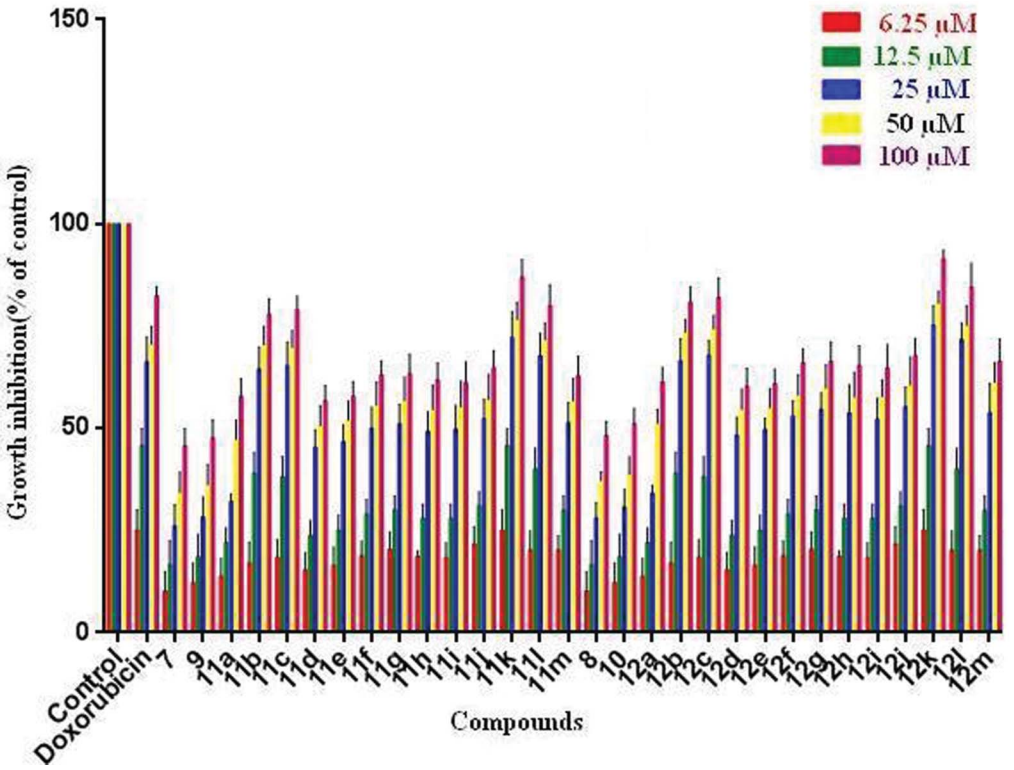

Fig. 2 Growth of inhibition of compounds **7–11a–m** and **12a–m**, based on the concentration in the HCT116 cells

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mL) was dropwise added to a solution of the corresponding amine (1.5 equiv.) and Et_3N (1.5 equiv.) in CH_2Cl_2 (15 mL) at 0 °C. The mixture was refluxed for 3–4 h (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.

*5.2.5.1 N,1-Diphenyl-4,5-dihydro-1H-[1]benzothiepino[5,4-c]pyrazole-3-carboxamide (**11a**).* The mixture was separated by column chromatography [petroleum ether/EtOAc, (1 : 1)] to afford **11a** (1.42 g, 71%) as a colorless solid. IR 1657, 3175 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.9 and 3.0 (t, 2H, –S–CH_2–CH_2 and –S–CH_2–CH_2), 6.7–7.4 (m, 14 H, aromatic), 9.9 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 22.34 (C_4), 35.86 (C_5), 117.25–149. 65 (aromatic and olefinic carbon), 160.25 (CO). ESI-MS calcd for *m*/*z*: 397.51. Anal. Calcd for $C_{24}H_{19}N_3OS$: C, 72.52; H, 4.82; N, 10.57; O, 4.03; S, 8.07%. Found: C, 72.48; H, 4.78; N, 10.55; S, 8.03%.

*5.2.5.2 N-(3-Chlorophenyl)-1-phenyl-4,5-dihydro-1H-[1]benzothiepino[5,4-c]pyrazole-3-carboxamide (**11b**).* The mixture was separated by column chromatography [petroleum ether/EtOAc, (1 : 1)] to furnish **11b** (1.64 g, 80%) as a colorless solid. IR 1654, 3180cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.9 and 3.0 (t, 2H, –S–CH_2–CH_2 and –S–CH_2–CH_2), 6.7–7.4 (m, 13H, aromatic), 9.9 (s,1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 23.46 (C_5), 35.98 (C_4), 116.86–149.68 (aromatic and olefinic carbon), 160.89 (CO). ESI-MS calcd for *m*/*z*: 431.07. Anal. Calcd for $C_{24}H_{18}ClN_3OS$: C, 66.74; H, 4.20; Cl, 8.21; N, 9.73; O, 3.70; S, 7.42%. Found: C, 66.70; H, 4.18; N, 9.68; S, 7.38%.

*5.2.5.3 N-(4-Chlorophenyl)-1-phenyl-4,5-dihydro-1H-[1]benzothiepino[5,4-c]pyrazole-3-carboxamide (**11c**).* The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to afford **11c** (1.43 g, 75%) as a colorless solid. IR 1656, 3180 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.9 and 3.0 (t, 2H, –S–CH_2–CH_2 and –S–CH_2–CH_2), 6.7–7.5 (m, 13H, aromatic), 9.8 (s,1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 23.26 (C_5), 35.98 (C_4), 117.89–150.75 (aromatic and olefinic carbon), 161.14 (CO). ESI-MS calcd for *m*/*z*: 431.11. Anal. Calcd for $C_{24}H_{18}ClN_3OS$: C, 66.74; H, 4.20; Cl, 8.21; N, 9.73; O, 3.70; S, 7.42%. Found: C, 66.70; H, 4.18; N, 9.68; S, 7.38%.

*5.2.5.4 N-(3-Nitrophenyl)-1-phenyl-4,5-dihydro-1H-[1]benzothiepino[5,4-c]pyrazole-3-carboxamide (**11d**).* The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to give **11d** (1.34 g, 63%) as a colorless solid. IR 1651, 3180 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.9 and 3.0 (t, 2H, –S–CH_2–CH_2 and –S–CH_2–CH_2), 7.1–7.8 (m, 13H, aromatic), 10.31 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 22.85 (C_5), 36.01 (C_4), 117.24–150.11 (aromatic and olefinic carbon), 161.01 (CONH). ESI-MS calcd for *m*/*z*: 442.10. Anal. Calcd for $C_{24}H_{18}N_4O_3S$: C, 65.14; H, 4.10; N, 12.66; O, 10.85; S, 7.25%. Found: C, 65.10; H, 4.06; N, 12.58; S, 7.19%.

*5.2.5.5 N-(4-Nitrophenyl)-1-phenyl-4,5-dihydro-1H-[1]benzothiepino[5,4-c]pyrazole-3-carboxamide (**11e**).* The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to afford **11e** (1.35 g, 65%) as a colorless solid. IR 1655, 3194 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.9 and 3.0 (t, 2H, –S–CH_2–CH_2 and –S–CH_2–CH_2), 7.1–7.8 (m, 13H, aromatic), 9.6 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 22.98 (C_5), 35.98 (C_4), 117.36–150.12 (aromatic and olefinic carbon), 161.96 (CO). ESI-MS calcd for *m*/*z*: 442.10. Anal. Calcd for $C_{25}H_{21}N_3OS$: C, 65.14; H, 4.10; N, 12.66; O, 10.85; S, 7.25%. Found: C, 65.04; H, 4.02; N, 12.48; S, 7.10%.

*5.2.5.6 1-Phenyl-N-m-tolyl-4,5-dihydro-1H-[1]benzothiepino[5,4-c]pyrazole-3-carboxamide (**11f**).* The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to furnish **11f** (1.33 g, 68%) as a colorless solid. IR 1653, 3185 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.7 (s, 3H, CH_3), 2.9 and 3.0 (t, 2H, –S–CH_2–CH_2 and –S–CH_2–CH_2), 6.8–7.6 (m, 13H, aromatic), 9.6 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 21.16 (CH_3), 25.12 (C_5), 36.12 (C_4), 117.36–150.65 (aromatic and olefinic carbon), 161.34 (CONH). ESI-MS calcd for *m*/*z*: 411.12. Anal. Calcd for $C_{25}H_{21}N_3OS$: C, 72.97; H, 5.14; N, 10.21; O, 3.89; S, 7.79%. Found: C, 72.36; H, 5.04; N, 10.12; S, 7.40%.

*5.2.5.7 1-Phenyl-N-p-tolyl-14,5-dihydro-1H-[1]benzothiepino[5,4-c]pyrazole-3-carboxamide (**11g**).* The mixture was separated by column chromatography [petroleum ether/EtOAc (1;1)] to obtain **11g** (1.58 g, 71%) as a colorless solid. IR 1650, 3180 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.4 (s, 3H, CH_3), 2.9 and 3.0 (t, 2H, –S–CH_2–CH_2 and –S–CH_2–CH_2), 6.8–7.7 (m, 13H, aromatic), 9.8 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 21.96 (CH_3), 27.43 (C_5), 36.25 (C_4), 118.14–150.46 (aromatic and olefinic carbon), 162.12 (CO). ESI-MS calcd for *m*/*z*: 411.16. Anal. Calcd for $C_{25}H_{21}N_3OS$: C, 72.97; H, 5.14; N, 10.21; O, 3.89; S, 7.79%. Found: C, 72.46; H, 5.10; N, 10.08; S, 7.64%.

*5.2.5.8 N-(2-Aminophenyl)-1-phenyl-4,5-dihydro-1H-[1]benzothiepino[5,4-c]pyrazole-3-carboxamide (**11h**).* The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] afforded **11h** (1.38 g, 67%) as a yellow solid. IR 1658, 3194 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.9 and 3.0 (t, 2H, –S–CH_2–CH_2 and –S–CH_2–CH_2), 5.8 (s, 2H, NH_2), 6.8–7.6 (m, 13H, aromatic), 10.02 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 22.34 (C_5), 31.12 (C_4), 116.42–149.87 (aromatic and olefinic carbon), 161.85 (CO). ESI-MS calcd for *m*/*z*: 412.12. Anal. Calcd for $C_{24}H_{20}N_4OS$: C, 69.88; H, 4.89; N, 13.58; O, 3.88; S, 7.77%. Found: C, 69.58; H, 4.59; N, 13.33; S, 7.59%.

*5.2.5.9 1-Phenyl-4,5-dihydro-1H-[1]benzothiepino[5,4-c]pyrazole-3-carbohydrazide (**11i**).* The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to obtain **11i** (1.45 g, 71%) as a colorless solid. IR 1655, 3196 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.9 and 3.0 (t, 2H, –S–CH_2–CH_2 and –S–CH_2–CH_2), 4.7 (s, 2H, NH_2), 7.2–7.8 (m, 9H, aromatic), 9.5 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 21.45 (C_5), 36.28 (C_4), 117.34–149.84 (aromatic and olefinic carbon), 161.21 (CO). ESI-MS calcd for *m*/*z*: 336.12. Anal. Calcd for $C_{18}H_{16}N_4OS$: C, 64.26; H, 4.79; N, 16.65; O, 4.76; S, 9.53,%. Found: C, 64.11; H, 4.64; N, 16.48; S, 9.41%.

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*5.2.5.10 N′,1-Diphenyl-4,5-dihydro-1H-[1]benzothiepino[5,4-c]pyrazole-3-carbohydrazide (**11j**).* The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to obtain **11j** (1.54 g, 71%) as a colorless solid. IR 1645, 3184 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.9 and 3.0 (t, 2H, –S–CH_2–CH_2 and –S–CH_2–CH_2), 4.2 (s, 1H, NH), 7.2–7.8 (m, 13H, aromatic), 10.31 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 21.89 (C_5), 36.01 (C_4), 113.11–151.46 (aromatic and olefinic carbon), 160.75 (CO). ESI-MS calcd for *m*/*z*: 412.16. Anal. Calcd for $C_{24}H_{20}N_4OS$: C, 69.88; H, 4.89; N, 13.58; O, 3.88; S, 7.77%. Found: C, 69.55; H, 4.60; N, 13.48; S, 7.61%.

*5.2.5.11 N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-1-phenyl-4,5-dihydro-1H-[1]benzothiepino[5,4-c]pyrazole-3-carboxamide (**11k**).* The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to obtain **11k** (1.3 g, 60%) as a colorless solid. IR 1632, 3117 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.9 and 3.0 (t, 2H, –S–CH_2–CH_2 and –S–CH_2–CH_2), 2.8 and 3.2 (s, 3H, CH_3), 6.8–7.6 (m, 14H, aromatic), 9.4 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ12.46 (CH_3), 22.49 (C_5), 36.12 (C_4), 39.79 (CH_3), 103.32–149.56 (aromatic and olefinic carbon), 160.45 (CO), 161.32 (CO). ESI-MS calcd for *m*/*z*: 507.15. Anal. Calcd for $C_{29}H_{25}N_5O_2S$: C, 68.62; H, 4.96; N, 13.80; O, 6.30; S, 6.32%. Found: C, 68.58; H, 4.90; N, 13.68; S, 6.24%.

*5.2.5.12 N-(1,3-Benzothiazol-2-yl)-1-phenyl-4,5-dihydro-1H-[1]benzothiepino[5,4-c]pyrazole-3-carboxamide (**11l**).* The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to obtain **11l** (1.30 g, 64%) as a pale yellow solid. IR 1631, 3154 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.9 and 3.0 (t, 2H, –S–CH_2–CH_2 and –S–CH_2–CH_2), 6.8–7.5 (m, 13H, aromatic). ^{13}C-NMR ($CDCl_3$) δ 21.86 (C_5), 37.42 (C_4), 117.48–149.86 (aromatic and olefinic carbon), 162.51 (CO), 174.23 (C=N). ESI-MS calcd for *m*/*z*: 454.11. Anal. Calcd for $C_{25}H_{18}N_4OS_2$: C, 66.06; H, 3.99; N, 12.33; O, 3.52; S, 14.11%. Found: C, 65.98; H, 3.59; N, 12.03; S, 14.09%.

*5.2.5.13 N-(Naphthalen-1-yl)-1-phenyl-4,5-dihydro-1H-[1]benzothiepino[5,4-c]pyrazole-3-carboxamide (**11m**).* The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to afford **11m** (1.24 g, 68%) as a colorless solid. IR 1635, 3134 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.9 and 3.0 (t, 2H, –S–CH_2–CH_2 and –S–CH_2–CH_2), 6.8–7.8 (m, 12H, aromatic); and δ 10.43 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 22.34 (C_5), 35.98 (C_4), 117.46–151.24 (aromatic and olefinic carbon), 160.98 (CO). ESI-MS calcd for *m*/*z*: 447.12. Anal. Calcd for $C_{28}H_{21}N_3OS$: C, 75.14; H, 4.73; N, 9.39; O, 3.57; S, 7.16%. Found: C, 75.02; H, 4.57; N, 9.22; S, 7.08%.

5.2.6.1 *N*,1-Diphenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxamide 6,6-dioxide (12a). The mixture was separated by column chromatography [petroleum ether/EtOAc, (1 : 1)] to afford **12a** (0.96 g, 51%) as a colorless solid. IR 1638, 3155, 1148, 1298 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 3.1 and 3.8 (t,2H, –SO_2–CH_2–CH_2 and –SO_2–CH_2–CH_2), 6.8–7.5 (m, 14H, aromatic), 10.13 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 13.46 (C_5), 57.86 (C_4), 117.25–149.45 (aromatic and olefinic carbon), 162.35 (CO). ESI-MS calcd for *m*/*z*: 429.19. 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, 67.08; H, 4.40; N, 9.71; S, 7.40%.

5.2.6.2 *N*-(3-Chlorophenyl)-1-phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxamide 6,6-dioxide (12b). The mixture was separated by column chromatography [petroleum ether/EtOAc, (1 : 1)] to furnish **12b** (0.98 g, 52%) as a colorless solid. IR 1651, 3160, 1151, 1305 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 3.1–3.8 (t, 2H, t, 2H, –SO_2–CH_2–CH_2 and –SO_2–CH_2–CH_2), 6.8–7.5 (m, 13H, aromatic), 9.9 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 14.36 (C_5), 58.63 (C_4), 116.34–150.36 (aromatic and olefinic carbon), 161.54 (CO). ESI-MS calcd for *m*/*z*: 463.0. Anal. Calcd for $C_{24}H_{18}ClN_3O_3S$: C, 62.13; H, 3.91; Cl, 7.64; N, 9.06; O, 10.35; S, 6.91%. Found: C, 62.04; H, 3.85; N, 9.01; S, 3.81%.

5.2.6.3 *N*-(4-Chlorophenyl)-1-phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxamide 6,6-dioxide (12c). The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to afford **12c** (0.93 g, 54%) as a colorless solid. IR 1652, 3170, 1152, 1304 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 3.1–3.8 (t, 2H, –SO_2–CH_2–CH_2 and –SO_2–CH_2–CH_2), 6.9–7.6 (m, 13H, aromatic), 9.9 (s,1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 13.96 (C_5), 58.69 (C_4), 117.52–150.34 (aromatic and olefinic carbon), 162.45 (CO). ESI-MS calcd for *m*/*z*: 463.05. Anal. Calcd for $C_{24}H_{18}ClN_3O_3S$: C, 62.13; H, 3.91; Cl, 7.64; N, 9.06; O, 10.35; S, 6.91%. Found: C, 62.03; H, 3.86; N, 9.01; S, 6.86%.

5.2.6.4 *N*-(3-Nitrophenyl)-1-phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxamide 6,6-dioxide (12d). The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to give **12d** (0.81 g, 49%) as a colorless solid. IR 1656, 3175, 1152, 1304 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 3.1 and 3.8 (t, 2H, –SO_2–CH_2–CH_2 and –SO_2–CH_2–CH_2), 7.1–7.9 (m, 13H, aromatic), 10.46 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 14.36 (C_5), 58.36 (C_4), 117.24–151.32 (aromatic and olefinic carbon), 163.52 (CONH). ESI-MS calcd for *m*/*z*: 474.08. Anal. Calcd for $C_{24}H_{18}N_4O_5S$: C, 60.75; H, 3.82; N, 11.81; O, 16.86; S, 6.76%. Found: C, 60.62; H, 3.68; N, 11.75; S, 6.67%.

5.2.6.5 *N*-(4-Nitrophenyl)-1-phenyl-4,5-dihydro-1*H*-[1] benzothiepino[5,4-*c*]pyrazole-3-carboxamide 6,6-dioxide (12e). The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to afford **12e** (0.76 g, 45%) as a colorless solid. IR 1650, 3184, 1155, 1306 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 3.1 and 3.8 (t, 2H, –SO_2–CH_2–CH_2 and –SO_2–CH_2–CH_2), 7.3–7.9 (m, 13H, aromatic), 10.36 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 14.52 (C_5), 56.96 (C_4), 117.52–150.63 (aromatic and olefinic carbon), 162.58 (CO). ESI-MS calcd for *m*/*z*: 474.06. Anal. Calcd for $C_{24}H_{18}N_4O_5S$: C, 60.75; H, 3.82; N, 11.81; O, 16.86; S, 6.76%. Found: C, 60.75; H, 3.75; N, 11.76; S, 6.65%.

5.2.6.6 1-Phenyl-*N*-*m*-tolyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxamide 6,6-dioxide (12f). The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to furnish **12g** (0.92 g, 56%) as a colorless solid. IR 1656, 3195, 1155, 1306 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.7 (s, 3H, CH_3), 3.1 and 3.8 (t, 2H, –SO_2–CH_2–CH_2 and –SO_2–CH_2–CH_2), 7.1–7.7 (m, 13H, aromatic), 9.8 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 14.63(CH_3), 25.24 (C_5), 36.12 (CH_3), 60.35 (C_4), 117.25–151.32 (aromatic and olefinic carbon), 162.48 (CO). ESI-MS calcd for *m*/*z*: 443.12. Anal. Calcd for $C_{25}H_{21}N_3O_3S$: C, 67.70; H, 4.77; N, 9.47; O, 10.82; S, 7.23%. Found: C, 67.66; H, 4.70; N, 9.36; S, 7.16%.

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5.2.6.7 1-Phenyl-*N*-*p*-tolyl-14,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxamide 6,6-dioxide (12g). The mixture was separated by column chromatography [petroleum ether/EtOAc (1;1)] to obtain **12g** (0.96 g, 54%) as a colorless solid. IR 1650, 3180, 1155, 1306 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.8 (s, 3H, CH_3), 3.1 and 3.8 (t, 2H, –SO_2–CH_2–CH_2 and –SO_2–CH_2–CH_2), 7.1–7.7 (m, 13H, aromatic), 9.8 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 27.64 (CH_3), 35.63 (CH_3), 15.36 (C_5), 63.75 (C_4), 118.75–150.75 (aromatic and olefinic carbon), 162.75 (CO). ESI-MS calcd for *m*/*z*: 443.10. Anal. Calcd for $C_{25}H_{21}N_3O_3S$: C, 67.70; H, 4.77; N, 9.47; O, 10.82; S, 7.23%. Found: C, 67.63; H, 4.68; N, 9.40; S, 7.14%.

5.2.6.8 *N*-(2-Aminophenyl)-1-phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxamide6,6-dioxide (12h). The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to afford **12h** (0.86 g, 52%) as a yellow solid. IR 1651, 3186, 1152 and 1304 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 3.1 and 3.8 (t, 2H, –SO_2–CH_2–CH_2 and –SO_2–CH_2–CH_2), δ 5.9 (s, 2H, NH_2), 7.1–7.7 (m, 13H, aromatic), 10.20 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 14.63 (C_5), 57.86 (C_4), 116.35–149.36 (aromatic and olefinic carbon), 162.58 (CO). ESI-MS calcd for *m*/*z*: 444.10. Anal. Calcd for $C_{24}H_{20}N_4OS$: C, 69.88; C, 64.85; H, 4.54; N, 12.60; O, 10.80; S, 7.21%. Found: C, 64.69; H, 4.39; N, 12.56; S, 7.19%.

5.2.6.9 1-Phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carbohydrazide 6,6-dioxide (12i). The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to obtain **12i** (0.84 g, 54%) as a colorless solid. IR 1651, 3185, 1152, 1304 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 3.1 and 3.8 (t, 2H, –SO_2–CH_2–CH_2 and –SO_2–CH_2–CH_2), 4.8 (s, 2H, NH_2), 7.1–7.8 (m, 9H, aromatic), 9.8 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 14.36 (C_5), 57.36 (C_4), 117.45–149.46 (aromatic and olefinic carbon), 162.45 (CO). ESI-MS calcd for *m*/*z*: 368.09. Anal. Calcd for $C_{18}H_{16}N_4O_3S$: C, 58.68; H, 4.38; N, 15.21; O, 13.03; S, 8.70; S, 9.53%. Found: C, 58.59; H, 4.34; N, 15.18; S, 9.43%.

5.2.6.10 *N*′,1-Diphenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carbohydrazide 6,6-dioxide (12j). The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to afford **12j** (0.96 g, 56%) as a colorless solid. IR 1648, 3191, 1152, 1304 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 3.1 and 3.8 (t, 2H, –SO_2–CH_2–CH_2 and –SO_2–CH_2–CH_2), 4.3 (s, 1H, NH), 6.9–7.6 (m, 13H, aromatic), 10.23 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$), δ 14.36 (C_5), 57.36 (C_4), 113.42–151.36 (aromatic and olefinic carbon), 161.52 (CO). ESI-MS calcd for *m*/*z*: 444.10. Anal. Calcd for $C_{24}H_{20}N_4O_3S$: C, 64.85; H, 4.54; N, 12.60; O, 10.80; S, 7.21%. Found: C, 64.76; H, 4.46; N, 12.54; S, 7.11%.

5.2.6.11 *N*-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-1-phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxamide 6,6-dioxide (12k). The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to afford **12k** (0.99 g, 56%) as a colorless solid. IR 1644, 3124, 1154, 1308 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.7 and 3.7 (t, 2H, –SO_2–CH_2–CH_2 and –SO_2–CH_2–CH_2), 2.1 and 2.9 (s, 3H, CH_3), 7.1–7.7 (m, 14H, aromatic), 9.5 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 22.49 (CH_3), 36.12 (CH_3), 11.32 (C_5), 60.35 (C_4), 103.74–149.36 (aromatic and olefinic carbon), 161.54 (CO), 162.54 (CO). ESI-MS calcd for *m*/*z*: 539.14. Anal. Calcd for $C_{29}H_{25}N_5O_4S$: C, 64.55; H, 4.67; N, 12.98; O, 11.86; S, 5.94%. Found: C, 64.48; H, 4.60; N, 12.78; S, 5.86%.

5.2.6.12 *N*-(1,3-Benzothiazol-2-yl)-1-phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxamide 6,6-dioxide (12l). The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to furnish **12l** (0.96 g, 52%) as a pale yellow solid. IR 1636, 3164, 1154, 1308 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 3.1 and 3.8 (t, 2H, –SO_2–CH_2–CH_2 and –SO_2–CH_2–CH_2), 6.9–7.6 (m, 13H, aromatic); ^{13}C-NMR ($CDCl_3$) δ 14.35 (C_5), 56.78 (C_4), 117.36–150.42 (aromatic and olefinic carbon), 163.45 (CO), 175.63 (C=N). ESI-MS calcd for *m*/*z*: 486.06. Anal. Calcd for $C_{25}H_{18}N_4O_3S_2$: C, 61.71; H, 3.73; N, 11.51; O, 9.86; S, 13.18%. Found: C, 61.63; H, 3.62; N, 11.46; S, 13.02%.

5.2.6.13 *N*-(Naphthalen-1-yl)-1-phenyl-4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole-3-carboxamide 6,6-dioxide(12m). The mixture was separated by column chromatography [petroleum ether/EtOAc (1 : 1)] to afford **12m** (0. 93 g, 54%) as a colorless solid. IR 1644, 3164, 1152, 1305 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 3.1 and 3.8 (t, 2H, –SO_2–CH_2–CH_2 and –SO_2–CH_2–CH_2), 6.9–7.8 (m, 12 H, aromatic), 10.43 (s, 1H, –CONH). ^{13}C-NMR ($CDCl_3$) δ 15.12 (C_5), 57.38 (C_4), 117.28–152.36 (aromatic and olefinic carbon), 161.86 (CO). ESI-MS calcd for *m*/*z*: 479.10. Anal. Calcd for $C_{28}H_{21}N_3O_3S$: C, 70.13; H, 4.41; N, 8.76; O, 10.01; S, 6.69%. Found: C, 70.06; H, 4.39; N, 8.69; S, 6.58%.

6 Antimicrobial evaluation

Standard sterilized filter paper discs (5 mm diameter) impregnated with a solution of the test compound in DMSO (1 mg mL^{-1}) was placed on an agar plate seeded with the appropriate test organism in triplicate. The utilized test organisms were *S. aureus*, and *S. pneumonia*, 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* strains. Amikacin and chloroamphenicol were used as a standard antibacterial agent and clotrimazole was used as a standard antifungal agent. DMSO alone was used as the control at the above-mentioned concentration. The plates were incubated at 37 °C for 24 h for bacteria and 28 °C for 48 h 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).

6.1 Minimal inhibitory concentration (MIC) measurement

The broth dilution test was used to determine the Minimum Inhibitory Concentration (MIC) of the above mentioned samples.[39,40] The micro dilution susceptibility test was used for the determination of antibacterial and antifungal activity. Stock solutions of the tested compounds, amikacin, chloroamphenicol, and clotrimazole were prepared in DMSO at concentrations of 1000 mg mL^{-1}, followed by two fold dilution at concentrations of 500, 250, ... 3.125 mg mL^{-1}. All the plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48

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h for fungi and the MICs were determined. Control experiments were also carried out.

7 Antimycobacterial activity

All the compounds were screened for their *in vitro* antimycobacterial activity against MTB. The antimicrobacterial activity of the compounds was tested by the resazurin microplate assay (REMA) following the method of Martin *et al.*[41,42] MTB $H_{37}Rv$ was grown in Middlebrook 7H11 broth medium supplemented with 10% OADC (oleic acid, albumin, dextrose, and catalase, 1, 10, 100 mg L^{-1}). After incubation at 37 °C 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 24 h. 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 24 h at 37 °C. A 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 MIC was defined as the minimum concentration of the compound required to achieve 99.9% inhibition of bacterial growth.

8 Anticancer activity

The *in vitro* anticancer activity was analyzed by the MTT assay method.[43,44] The human cervical cancer cell line (HeLa) and colon cancer cell line (HCT116) were obtained from National Centre for Cell Science (NCCS), Pune, and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). The cells were maintained at 37 °C, 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 a final density of 1×10^5 cells mL^{-1}. 100 μL per well of cell suspension were seeded into 96-well plates at a plating density of 10 000 cells per well and incubated to allow for cell attachment at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. After 24 h, 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 were stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted twice to the desired final maximum test concentration with serum free medium. An 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 medium, resulting in the required final drug concentrations. Following the drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. The medium without samples served as a 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, 15 μL of MTT (5 mg mL^{-1}) in phosphate buffered saline (PBS) was added to each well and incubated at 37 °C for 4h. The medium with MTT was then flicked off and the formed formazan crystals 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:

$$\%\ \text{cell inhibition} = 100 - \text{Abs (sample)/Abs (control)} \times 100$$

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

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References

1 J. Elguero, in *Comprehensive Heterocyclic Chemistry*, vol. 7, ed. A. R. Katrinsky, C. W. Rees and E. F. V. Scriven, Pergamon, Oxford, 1996, p. 1.
2 F. Wei, B.-X. Zhao, B. Huang, L. Zhang, C.-H. Sun, W.-L. Dong, D.-S. Shin and J.-Y. Miao, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 6342–6347.
3 Y. Xia, Z.-W. Dong, B.-X. Zhao, X. Ge, N. Meng, D.-S. Shin and J.-Y. Miao, *Bioorg. Med. Chem.*, 2007, **15**, 6893–6899.
4 B. Cottineau, P. Toto, C. Marot, A. Pipaud and J. Chenault, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 2105–2108.
5 K. Y. Lee, J. M. Kim and J. N. Kim, *Tetrahedron Lett.*, 2003, **44**, 6737–6740.
6 Z. J. Jia, Y. Wu, W. Huang, P. Zhang, Y. Song, J. Woolfrey, U. Sinha, A. E. Arfsten, S. T. Edwards, A. Hutchaleelaha, S. J. Hollennbach, J. L. Lambing, R. M. Scarborough and B. Y. Zhu, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 1229–1234.
7 E. V. Pimerova and E. V. Voronina, *Pharm. Chem. J.*, 2001, **35**, 18–21.
8 A. A. Bekhit, H. M. A. Ashour, Y. S. A. Ghany, A. E. Bekhit and A. Baraka, *Eur. J. Med. Chem.*, 2008, **43**, 456–463.
9 M. P. Donohue, D. A. Marchuk and H. A. Rockman, *J. Am. Coll. Cardiol.*, 2006, **48**, 1289–1298.
10 W. L. John, R. M. Patera, M. J. Plummer, B. P. Halling and D. A. Yuhas, *Pestic. Sci.*, 1994, **42**, 29–34.

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Published on 26 February 2013 on http://pubs.rsc.org | doi:10.1039/C3RA23124K

11 K. Hirai, A. Uchida and R. Ohno, in *Herbicide Classes in Development*, ed. P. Boger, K. Hirai and K. Wakabyashi, Springer-Verlag, Heidelberg, 2002, pp. 279–289.
12 M. J. Genin, C. Biles, B. J. Keiser, S. M. Poppe, S. M. Swaney, W. G. Tarpley, Y. Yagi and D. L. Romero, *J. Med. Chem.*, 2000, **43**, 1034–1038.
13 X. Li, X. Lu, M. Xing, X. H. Yang, T. T. Zhao, H. B. Gong and H. L. Zhu, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 3589–3593.
14 R. Ando, H. Ikegami, M. Sakiyama, S. Ooike, M. Hayashi, Y. Fujino, D. Abe, H. Nakamura, T. Mishina, H. Kato and Y. Iwase, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 4709–4711.
15 V. J. Cee, L. B. Schenkel and B. L. Hodous, *J. Med. Chem.*, 2010, **53**, 6368–6377.
16 A. H. Kishore, B. M. Vedamurthy and K. Mantelingu, *J. Med. Chem.*, 2008, **51**, 792–797.
17 T. E. Rawson, M. Rüth, El. Blackwood and D. Burdick, *J. Med. Chem.*, 2008, **51**, 4465–4475.
18 M. S. Coumar, C. Y. Chu and C. W. Lin, *J. Med. Chem.*, 2010, **53**, 4980–4988.
19 J. A. Markwalder, M. R. Arnone, P. A. Benfield, M. Boisclair, C. R. Burton, C. Chang, S. S. Cox, P. M. Czerniak, C. L. Dean, D. Doleniak, R. Grafstrom, B. A. Harrison, R. F. Kaltenbach, D. A. Nugiel, K. A. Rossi, S. R. Sherk, L. M. Sisk, P. Stouten, G. L. Trainor, P. Worland and S. P. Seitz, *J. Med. Chem.*, 2004, **47**, 5894–5911.
20 S. Schenone, O. Bruno, F. Bondavalli, A. Ranise, L. Mosti, G. Menozzi, P. Fossa, S. Donnini, A. Santoro, M. Ziche, F. Manetti and M. Botta, *Eur. J. Med. Chem.*, 2004, **39**, 939–946.
21 S. Gupta, L. M. Rodrigues, A. P. Esteves, A. M. Oliveira-Campos, M. S. Nascimento, N. Nazareth, H. Cidade, M. P. Neves, E. Fernandes, M. Pinto, N. M. Cerqueira and N. Bras, *Eur. J. Med. Chem.*, 2008, **43**, 771–780.
22 M. M. Ghorab, F. A. Ragab, S. I. Alqasoumi, A. M. Alafeefy and S. A. Aboulmagd, *Eur. J. Med. Chem.*, 2010, **45**, 171–178.
23 K. J. Curran, J. C. Verheijen, J. Kaplan, D. J. Richard, L. Toral-Barza, I. Hollander, J. Lucas, S. Ayral-Kaloustian, K. Yu and A. Zask, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 1440–1444.
24 G. Menozzi, P. Fossa, E. Cichero, A. Spallarossa, A. Ranise and L. Mosti, *Eur. J. Med. Chem.*, 2008, **43**, 2627–2638.
25 R. Silvestri, M. G. Cascio, G. La Regina, F. Piscitelli, A. Lavecchia, A. Brizzi, S. Pasquini, M. Botta, E. Novellino, V. Di Marzo and F. Corelli, *J. Med. Chem.*, 2008, **51**, 1560–1576.
26 X. L. Ding, H. Y. Zhang, L. Qi, B. X. Zhao, S. Lian, H. Shui and J. Y. Miao, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 5325–5328.
27 A. Andreani, M. Granaiola, A. Leoni, R. Morigi and M. Rambaldi, *Eur. J. Med. Chem.*, 2001, **36**, 743–746.
28 S. G. Kini, A. R. Bhat, B. Bryant, J. S. Williamson and F. E. Dayan, *Eur. J. Med. Chem.*, 2009, **44**, 492–500.
29 M. A. Ali, G. J. Samy, E. Manogaran, V. Sellappan, M. Z. Hassan, M. J. Ahsan, S. Pandian and M. ShaharYar, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 7000–7002.
30 M. J. Ahsan, G. J. Samy, K. R. Dutt, U. K. Agrawal, B. Shankar, S. Vyas, R. Kaur and G. Yadav, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 4451–4453.
31 M. J. Ahsan, J. G. Samy, H. Khalilullah, M. A. Bakht and M. Z. Hassan, *Eur. J. Med. Chem.*, 2011, **46**, 5694–5697.
32 R. Villar, I. Encio, M. Migliaccio, M. J. Gil and V. M. Merino, *Bioorg. Med. Chem.*, 2004, **12**, 963–969.
33 M. M. Alonso, A. Asumendi, J. Villar, M. J. Gil, V. M. Merino, I. Encio and J. Migliaccio, *Oncogene*, 2003, **22**, 3759.
34 M. M. Alonso, I. Encio, V. M. Merino, M. Gil and M. Migliaccio, *Br. J. Cancer*, 2001, **85**, 140021.
35 A. A. Sagardoy, M. J. Gil, R. Villar, M. J. Viñas, I. Encío and V. M. Merino, *Bioorg. Med. Chem.*, 2010, **18**, 5701–5707.
36 S. Kumaresan and S. R. Ramadas, *Sulfur Lett.*, 1984, **2**, 31–36.
37 (*a*) G. Doria, A. M. Isetta, M. Ferrari and D. Trizio, *Eur. Pat. Appl*, 1988, **88**, 300115; (*b*) G. Doria, A. M. Isetta, M. Ferrari and D. Trizio, *Chem. Abstr*, 1989, **111**, 57711.
38 A. H. Shamroukh, M. E. A. Zaki, E. M. H. Morsy, F. M. Abdel-Motti and F. M. E. Abdel-Megeid, *Arch. Pharm. Chem. Life Sci*, 2007, **340**, 345–351.
39 D. Janovska, K. Kubikova and L. Kokoska, *J. Food Sci.*, 2003, **21**, 107–110.
40 J. Bishnu, L. Sunil, S. Anuja and J. Science, *Eng. Technol*, 2009, **5**, 143–150.
41 A. Martin, M. Camacho, F. Portaels and J. C. Palomino, *Antimicrob. Agents Chemother.*, 2003, **47**, 3616–3619.
42 A. Martin and J. C. Palomino, *Resazurin Microtiter Assay (REMA): Resazurin Microtitre assay (REMA) Colorimetric redox indicator (CRI). Drug susceptibility testing for Mycobacterium tuberculosis*, Institute of Tropical Medicine, Belgium, procedure manual version 03, 2009.
43 T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55–63.
44 A. Monks, D. Scudiero, P. Skehan and R. Shoemaker, *J. Natl. Cancer Inst.*, 1991, **83**, 757–766.

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