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Synthesis, antimycobacterial and antitumor activities of new (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl *N*,*N*-disubstituted dithiocarbamate/*O*-alkyldithiocarbonate derivatives

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Abstract—Reaction of 2-chloromethylsaccharin with substituted potassium dithiocarbamates and substituted potassium dithiocarbonates furnished (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl *N*,*N*-disubstituted dithiocarbamates (**4**–**15**) and (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl *O*-alkyldithiocarbonates (**16**–**20**). The new derivatives were evaluated for in vitro antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv. Compounds **4**–**13**, **15**, and **16**–**20** described herein showed moderate to good inhibitory activity. In particular, seven analogs **4**, **5**, **6**, **13**, and **7**, **8**, and **12** exhibited excellent MIC values of 1.56 and 0.78 μg/mL, respectively. Compounds **4**, **5**, **10**, **12**, **13**, and **16** were selected and screened for antitumor activity. Among the tested compounds, **4** and **5** were found to be cytotoxic, especially against leukemia cell lines CCRF-CEM, HL-60(TB), RPMI-8226, and SR with $\log_{10}GI_{50}$ values lower than −6.69, and against non-small cell lung cancer NCI-H522 cell line with $\log_{10}GI_{50}$ values lower than −6.31. Compound **10** was cytotoxic against leukemia cell line HL-60(TB), whereas **16** displayed favorable cytotoxicity against ovarian cancer cell line OVCAR-3 with $\log_{10}GI_{50}$ values of −6.31 and −7.45, respectively.

1. Introduction

Mycobacterium tuberculosis is the main causative agent of a chronic and fatal condition in humans known as tuberculosis (TB). It is estimated that one third of the world's population is TB-infected, with ∼8 million new cases annually, and of these, 3.1 million die annually. TB is currently the leading killer of youths, women, and AIDS patients in the world.[1] The age of anti-TB chemotherapy began with the discovery of streptomycin in the early 1940s.[2] Although many active anti-TB agents have since been developed, a disturbing co-occurrence with the use of these drugs as single agents has been the development of drug resistance. While the development of this resistance can be forestalled through the use of combination regimens,[3] it is clear that drug resistance will continue to be a problem, so there is an urgent need for new anti-TB agents.

Cancer is another fatal condition which is continuing to be one of the largest causes of death in both men and women, claiming over 6 million lives each year in the world. In the last decade, basic cancer research has produced remarkable advances in understanding of cancer biology and cancer genetics.[4] To date, many anticancer drugs have been developed and applied by physicians. However, the resistance to anticancer drugs and side effects were discovered. Therefore, the research and development of new and safe drugs have become necessary by the pharmaceutical industry.[5]

Considerable interest has been focused on dithiocarbamates which have been shown to possess a broad spectrum of biological activities such as fungicidal[6–10] and antibacterial[7,11–14] effects. The antibacterial effect of dithiocarbamates was reported to arise by reaction with HS-groups of physiologically important enzymes by transfering the alkyl group of the dithioester to the HS-function of the enzyme.[11]

Dithiocarbamates are known also to be active as anticancer agents.[11,15–18] Since, brassinin[19] (Fig. 1), a crucial plant defense first isolated from cabbage, had cancer preventive activity, structural modification on this compound led to the synthesis of sulforamate[20] (Fig. 1) and a series of dithiocarbamates, some of which were found to have in vitro and in vivo antitumor

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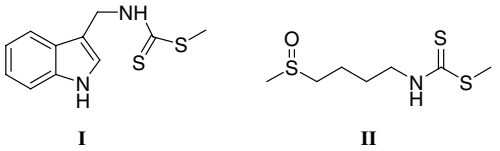


I II

Figure 1. Structures of brassinin (**I**) and sulforamate (**II**).

activity.[16] Investigations on various types of compounds possessing aminoalkylation ability showed that substituted aminomethyl *N*,*N*-dialkyldithiocarbamates have cytostatic features[17,18] presumably via a similar mechanism proposed for antibacterial action.

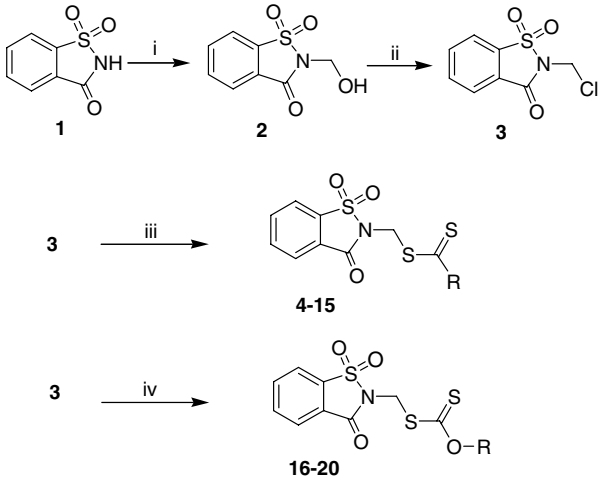
Furthermore, dithiocarbonate derivatives have been demonstrated to possess antifungal[21,22] and antibacterial[22,23] properties, and inhibitory action against brassinin detoxification by phytopathogenic fungus *Leptosphaeria maculans* (*Phoma lingam*).[24]

In view of above data, we have synthesized and evaluated seventeen new dithiocarbamate and dithiocarbonate derivatives possessing different alkyl/cycloalkyl groups, in order to obtain new and more potent antituberculosis and antitumor compounds which can improve the current tuberculosis and cancer treatments.

2. Results and discussion

2.1. Chemistry

Compounds **4**–**20** were synthesized as depicted in Scheme 1. Thus, *N*-chloromethylsaccharin (**3**) was synthesized from *N*-hydroxymethylsaccharin (**2**) formed by the reaction of saccharin (**1**) and formaldehyde. *N*-Chloromethylsaccharin (**3**) was reacted with potassium *N*,*N*-disubstituted dithiocarbamates and potassium *O*-alkyl dithiocarbonates to afford seventeen new (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl *N*,*N*-disubstituted dithiocarbamates (**4**–**15**) or (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl *O*-alkyldithiocarbonates (**16**–**20**).



Scheme 1. Reagents and conditions: (i) 37% HCHO, EtOH, 0 °C, 16 h; (ii) ether, $SOCl_2$, 0 °C, 24 h; (iii) RCS_2K, EtOH, reflux, 1 h; (iv) $ROCS_2K$, EtOH, reflux, 1 h.

The structures of the new compounds were established by elemental analysis, IR, ^{1}H NMR, HSQC, ^{13}C NMR (proton decoupled, APT, DEPT-135), and electron impact (EI) mass spectrometry.

The IR spectra of dithiocarbamates (**4**–**15**) supported the expected structures and showed absorption bands in the 1724–1743 and 1182–1248 cm^{-1} regions resulting from the C=O and C=S functions.[25,26] Further verification was obtained from the HSQC spectra of **8** and **14**, which explicitly showed the ^{1}H–^{13}C connections and allowed definite assignment of the ^{1}H and ^{13}C resonances. Thus, signals between δ 5.18 and 5.91 ppm in the ^{1}H NMR spectra of **4**–**15** were readily associated with the N–CH_2–S protons.[27] $C_{5,6,7}$–H protons of the benzoisothiazole moiety observed together as multiplets in the 200 MHz ^{1}H NMR spectra of **4**–**15** were separated and assigned starting from the proton having the lowest δ value as C_6–H < C_5–H < C_7–H on the basis of HSQC data. C=O signals were observed at δ 159.95 (**8**), δ 158.98 (**13**, APT), and δ 150.90 (**14**) ppm, and dithiocarbamate C=S was observed at δ 190.30 (**8** and **14**) and at δ 192.76 (**13**, APT) ppm.[28,29]

The C=O and C=S functions of the dithiocarbonates **16**–**20** gave rise to IR absorptions in the 1737–1745 and 1240–1256 cm^{-1} regions.[30] ^{13}C NMR spectra of **16** (proton decoupled and DEPT-135) and **18** (APT) supported the IR findings and displayed signals at δ 158.81 and 158.79 ppm, and δ 210.13 and 209.21 ppm attributed to the C=O and C=S functions.[31]

N–CH_2–S protons of **16**–**20** absorbed at δ 5.47–5.52 ppm as in compounds **4**–**15**.

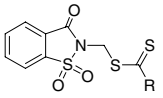
EI mass spectra of **4**–**20** (with the exception of **13** and **15**) displayed molecular ions (M^+) which confirmed their molecular weights. Further spectral details are presented in Section 4.

2.2. Biological activity

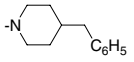
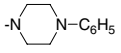
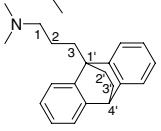
In vitro antimycobacterial activity evaluation of the synthesized derivatives against *M. Tuberculosis* H37Rv (ATCC 27294) was initially carried out using the microplate alamar blue assay (MABA) at a concentration of 6.25 μg/mL at the tuberculosis antimicrobial acquisition and coordinating facility (TAACF). Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system.[32] Primary antituberculosis activity results of dithiocarbamate (**4**–**13** and **15**) and dithiocarbonate (**16**–**20**) analogs are presented in Tables 1, 2.

Compounds demonstrating at least 90% inhibition in the primary screen were retested against *M. tuberculosis* H37Rv to determine the actual minimum inhibitory concentration (MIC) in the MABA. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. The MIC values of compounds **4**–**8**, **10**, **12**, **13**, and **16**–**19** are listed in Table 3.

Table 1. Primary in vitro antimycobacterial activity evaluation of **4**–**15** against *M. Tuberculosis* H37Rv at 6.25 μg/mL



4-15

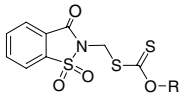
Compound	R	Assay	GI[a] (%)
4	$-N(C_2H_5)_2$	Alamar	100
5		Alamar	99
6		Alamar	99
7		Alamar	98
8		Alamar	100
9		Bactec	5
10		Alamar	99
11		Bactec	0
12		Alamar	99
13		Alamar	100
14[b]		—	—
15		Alamar	6

[a] Growth inhibition of virulent H37Rv strain of *M. Tuberculosis*.
[b] Not tested.

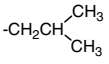
Among the dithiocarbamate analogs, **7**, **8**, and **12** displayed exceptional antituberculosis activity showing 98%, 100%, and 99% inhibition, respectively, at a minimum inhibitory concentration (MIC) of 0.78 μg/mL. Compounds **4**, **5**, **6**, and **13** also exhibited excellent activity showing 100%, 99%, 99%, and 100% inhibition, respectively, at a MIC value of 1.56 μg/mL. On the other hand, compound **10** demonstrated excellent inhibitory activity showing 99% inhibition at a MIC value of 6.25 μg/mL (Table 3).

Among the dithiocarbonate analogs, **18** exhibited very pronounced inhibitory activity (100%) with a MIC value of 3.13 μg/mL. Analogs **16**, **17**, and **19** produced 96%, 99% and 99% inhibition, respectively, at a MIC of 6.25 μg/mL (Table 3).

Table 2. Primary in vitro antimycobacterial activity evaluation of **16**–**20** against *M. Tuberculosis* H37Rv at 6.25 μg/mL



16-20

Compound	R	Assay	GI[a] (%)
16	$-CH_2CH_3$	Alamar	96
17	$-CH_2CH_2CH_3$	Alamar	99
18		Bactec	100
19	$-CH_2CH_2CH_2CH_3$	Alamar	99
20		Alamar	78

[a] Growth Inhibition of virulent H37Rv strain of *M. Tuberculosis*.

Table 3. The MIC and growth inhibition (GI) values of **4**–**8**, **10**, **12**, **13**, and **16**–**19** against *M. Tuberculosis* H37Rv

Compound	MIC[a] (μg/mL)	GI (%)
4	1.56	100
5	1.56	99
6	1.56	99
7	0.78	98
8	0.78	100
10	6.25	99
12	0.78	99
13	1.56	100
16	6.25	96
17	6.25	99
18	3.13	100
19	6.25	99

[a] MIC of Rifampin: 0.125 μg/mL versus *M. Tuberculosis* H37Rv (97% inhibition).

Although the structure–antituberculosis activity relationship in these structures was not straightforward, it may be speculated that dithiocarbamates were more active than dithiocarbonates. Among dithiocarbamates highest activity resided in compounds bearing 3-methyl piperidine (**7**), 4-methyl piperidine (**8**), and pyrrolidine (**12**) residues. Ring opening (**4**), demethylation (**5**), 2-methylsubstitution (**6**) or introduction of a second heteroatom (**10** and **13**) decreased activity. Substitution with benzyl (**9**), phenyl (**11**), and bulky groups (**15**) was not tolerated and thus these structural modifications resulted in inactive derivatives (Tables 1–3).

Compounds **4**, **5**, **10**, **12**, **13**, and **16** chosen by the National Cancer Institute were screened for antitumor activity. Primary anticancer assay was performed in accordance with the protocol of the Drug Evaluation Branch of the National Cancer Institute (Bethesda).[33–35]

Compounds were tested over a 5-log concentration range against each of the cell lines for their ability to inhibit the growth of, or to kill the cells. Three response parameters, the GI_{50} value (negative $\log_{10}$ of the concentration required to inhibit the growth of that cell line by

50% relative to untreated cells), TGI (the negative $\log_{10}$ minimum concentration that caused total growth inhibition), and LC_{50} (the negative $\log_{10}$ concentration needed to kill 50% of the cells), were calculated for each cell line (Tables 4, 5).

Negative values indicated the most sensitive cell lines. Compounds having $\log_{10}GI_{50}$ values −4 and <−4 were declared to be active (Table 4).

As can be seen from Table 4 the $\log_{10}GI_{50}$ values of the tested compounds were generally <−4, and compounds **4**, **5**, **10**, and **16** had the highest potencies.

Diethyl substituted compound **4** demonstrated the most marked effect on leukemia cancer cell lines CCRF-CEM, HL-60(TB), RPMI-8226, and SR ($\log_{10}GI_{50}$ value < −8.00), and non-small cell lung cancer cell line NCI-H522 ($\log_{10}GI_{50}$ value −7.57).

Piperidine containing compound **5** exhibited high cytotoxicity on leukemia cell lines CCRF-CEM, HL-60(TB), RPMI-8226, and SR, and non-small cell lung cancer cell line NCI-H522. The $\log_{10}GI_{50}$ values of compound **5** were −7.58, −7.48, −6.69, −6.99, and −6.31, respectively.

4-Methylpiperazine substituted compound **10** also exhibited high cytotoxicity on leukemia cell line HL-60(TB) with a $\log_{10}GI_{50}$ value of −6.31.

Among the dithiocarbonate analogs, **16** showed the most favorable cytotoxicity on ovarian cancer cell line (OVCAR-3) with a $\log_{10}GI_{50}$ value of −7.45.

The $\log_{10}LC_{50}$ values were generally >−4 with few exceptions (Table 4). From the above data it may be concluded that modification of the diethylamino residue of the most potent entry (**4**) by ring formation (**5** and **12**) or heteroatom substitution (**10** and **13**) decreased activity in dithiocarbamate derivatives.

3. Conclusion

We have herein described an efficient protocol for the synthesis of new saccharin derivatives with dithiocarbamate and dithiocarbonate side chains.

The in vitro antituberculosis screening results evidenced that many of the compounds from the series have emerged as potent antitubercular agents.

We have also shown that this class of compounds are especially active against leukemia, non-small cell lung cancer, and ovarian cancer cell lines.

In conclusion, we can assert that promising results obtained for the new dithiocarbamate and dithiocarbonate derivatives presented in this paper make them possible candidates for the treatment of tuberculosis and cancer, and encourage us to advance in the synthesis and evaluation of new derivatives.

4. Experimental

Melting points were determined in open capillary tubes with a Buchi 530 melting point apparatus and are uncorrected. IR (KBr) spectra were recorded using a Perkin-Elmer 1600 FTIR spectrophotometer and the values are expressed as υ_{max} cm^{-1}. ^{1}H-NMR, HSQC, and ^{13}C NMR (proton decoupled, APT, DEPT-135) spectra were recorded on Bruker AC 200/DPX 400 MHz and Varian^{UNITY} INOVA 500 MHz spectrometers. Mass spectra were recorded on a VG Zab Spec (EI, 70 eV) mass spectrometer. Elemental analyses were performed on a Carlo Erba Model 1106 elemental analyzer. All chemicals were purchased from E. Merck (Darmstadt, Germany).

4.1. *N*,*N*-Disubstituted dithiocarbamates (a)

An appropriate secondary amine (10 mmol) was added to an ethanolic solution of KOH (10 mmol/100 mL). The mixture was cooled in an ice bath and CS_2 (100 mmol) was added dropwise with stirring. Further agitation of the reaction mixture thus obtained for 1 h at room temperature, evaporation of the solvent under reduced pressure, and consequent addition of dry ether until precipitation reached completion and filtration afforded **a** which was recrystallized from ethanol or used without further purification.

4.2. *O*-Alkyldithiocarbonates (b)

KOH (10 mmol) was added to an appropriate alcohol (100 mL). The procedure described for **a** was followed beginning from addition of CS_2 with cooling to furnish **b** which was recrystallized from ethanol or used without further purification.

4.3. 2-(Hydroxymethyl)- 1,2-benzisothiazol-3(2*H*)-on 1,1-dioxide (2)

To a solution of compound **1** (100 mmol) in 100 mL of ethanol, 70 mL of 37% formaldehyde was added and the reaction mixture was kept at 0 °C for 16 h. The crude product thus obtained was recrystallized from ethanol to afford **2**.

4.4. 2-(Chloromethyl)- 1,2-benzisothiazol-3(2*H*)-on 1,1-dioxide (3)

To a solution of compound **2** (20 mmol) in 20 mL ether, 40 mL of thionyl chloride was added and kept in an ice bath for 24 h. Thionyl chloride was evaporated in vacuo and the residue was recrystallized from acetone to afford **3**.

4.5. General method for the synthesis of (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl *N*,*N*-disubstituted dithiocarbamates (4–15)

The ethanolic solution of **3** (5 mmol) and **a** (5 mmol) were refluxed for 1 h. After evaporation of the solvent in vacuo, the residue was washed with water and purified by recrystallization from ethanol.

Table 4. In vitro tumor cell growth inhibition of **4**, **5**, **10**, **12**, **13**, and **16**

Panel/cell line	**4**		**5**		**10**		**12**		**13**		**16**	
	$\log_{10}GI_{50}$	$\log_{10}TGI$	$\log_{10}GI_{50}$	$\log_{10}TGI$	$\log_{10}GI_{50}$	$\log_{10}TGI$	$\log_{10}GI_{50}$	$\log_{10}TGI$	$\log_{10}GI_{50}$	$\log_{10}TGI$	$\log_{10}GI_{50}$	$\log_{10}TGI$
Leukemia												
CCRF-CEM	<**−8.00**	−7.80	**−7.58**		−5.65	−5.00	−5.73	>−4.00	−5.10	−4.44	−4.79	−4.00
HL-60(TB)	<**−8.00**		**−7.48**	>−4.00	**−6.31**	−4.69			−5.32	−4.30		
K-562	−4.62	>−4.00	>−4.00	>−4.00	−4.84	−4.02	−5.79	>−4.00	>−4.00	>−4.00	−4.45	>−4.00
MOLT-4		−4.32		>−4.00	−5.29	−4.59	−5.80	>−4.00	−4.72	>−4.00	−4.49	>−4.00
RPMI-8226	<**−8.00**	<−8.00	**−6.69**	−6.22	−5.65	−5.16	−5.96	−4.89	−4.80	−4.29	−4.89	−4.42
SR	<**−8.00**	−4.90	**−6.99**	>−4.00	−5.39	−4.87			−4.76	−4.33		
Non-small cell lung cancer												
A549/ATCC	−4.55	>−4.00	>−4.00	>−4.00	−4.55	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.62	−4.23
EKVX	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.71	−4.44
HOP-62	−4.38	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.61	−4.18
NCI-H226	−4.15	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.53	−4.09
NCI-H23	−4.58	−4.08	>−4.00	>−4.00	−4.46	>−4.00	−4.21	>−4.00	>−4.00	>−4.00	−4.90	−4.56
NCI-H322M	−4.23	>−4.00	>−4.00	>−4.00	−4.02	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.60	−4.23
NCI-H460	−5.81	>−4.00		>−4.00	−4.75	−4.02	−4.28	>−4.00	−4.15	>−4.00	−4.34	>−4.00
NCI-H522	**−7.57**	−4.69	**−6.31**	>−4.00	−5.14	−4.47	−4.42	>−4.00	−4.61	>−4.00	−4.95	−4.56
Colon cancer												
COLO 205	−4.72	>−4.00	>−4.00	>−4.00	−5.46	−4.67	>−4.00	>−4.00	−4.20	>−4.00	−5.53	−4.95
HCC-2998	−4.57	−4.22	>−4.00	>−4.00	−4.51	−4.07	−4.55	>−4.00	>−4.00	>−4.00	−4.79	−4.53
HCT-116		−4.30		>−4.00	−4.89	−4.35	−4.20	>−4.00	>−4.00	>−4.00	−4.87	−4.58
HCT-15	−4.53	>−4.00	>−4.00	>−4.00	−4.67	−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.72	−4.44
HT29	−4.73	−4.25	−5.07	>−4.00	−5.37	−4.74	>−4.00	>−4.00	−4.58	>−4.00	−4.85	−4.34
KM12	−4.56	>−4.00	>−4.00	>−4.00	−4.60	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.46	>−4.00
SW-620	−4.47	>−4.00	>−4.00	>−4.00	−5.03	−4.13	>−4.00	>−4.00	−4.16	>−4.00	−5.30	−4.71
CNS cancer												
SF-268	−4.54	>−4.00	>−4.00	>−4.00	−4.43	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.53	>−4.00
SF-295	−4.35	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.75	−4.23
SF-539	−4.39	>−4.00	>−4.00	>−4.00	−4.43	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.61	−4.23
SNB-19	−4.15	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.54	−4.05
SNB-75	−4.59	−4.08	>−4.00	>−4.00	−4.61	−4.04	−4.64	>−4.00	−4.18	>−4.00	−5.33	−4.53
U251	−4.40	>−4.00	>−4.00	>−4.00	−4.58	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.73	−4.43
Melanoma												
LOX IMVI	−4.77	−4.35	>−4.00	>−4.00	−4.94	−4.43	>−4.00	>−4.00	>−4.00	>−4.00	−4.77	−4.50
MALME-3M	−4.63	−4.02	>−4.00	>−4.00	−4.64	−4.02	>−4.00	>−4.00	>−4.00	>−4.00	−5.71	−5.06
M14	−4.62	−4.17	>−4.00	>−4.00	−5.10	−4.64	>−4.00	>−4.00	−4.51	−4.05	−4.79	−4.48

SK-MEL-2	−4.05	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.35	>−4.00
SK-MEL-28	−4.67	−4.14	>−4.00	>−4.00	−4.84	−4.43	>−4.00	>−4.00	−4.21	>−4.00	−5.47	−4.82
SK-MEL-5	−4.81	>−4.00	−4.26	>−4.00	−4.79	−4.41	−5.12	>−4.00	−4.24	>−4.00	−4.94	−4.61
UACC-257	−4.42	>−4.00	>−4.00	>−4.00	−4.19	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.73	−4.26
UACC-62	−4.56	>−4.00	>−4.00	>−4.00	−4.72	−4.27	−4.24	>−4.00	>−4.00	>−4.00	−4.70	−4.42
Ovarian cancer												
IGROV1	−4.35	>−4.00	>−4.00	>−4.00	−4.35	>−4.00	−4.36	>−4.00	>−4.00	>−4.00	−4.73	−4.28
OVCAR-3	−4.46	>−4.00	>−4.00	>−4.00	−4.66	−4.2	−5.19	>−4.00	>−4.00	>−4.00	**−7.45**	−4.76
OVCAR-4	−4.56	>−4.00	>−4.00	>−4.00	−4.63	−4.14	−4.57	>−4.00	>−4.00	>−4.00	−5.78	−5.41
OVCAR-5	−4.33	>−4.00	>−4.00	>−4.00	−4.52	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.84	−4.52
OVCAR-8	−4.48	>−4.00	>−4.00	>−4.00	−4.33	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.68	>−4.00
SK-OV-3	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.49	>−4.00
Renal cancer												
786-O	−4.50	>−4.00	>−4.00	>−4.00	−4.92	−4.37	>−4.00	>−4.00	>−4.00	>−4.00	−4.68	−4.32
A498	−4.19	>−4.00	>−4.00	>−4.00	−4.03	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.70	−4.38
ACHN	−4.25	>−4.00	>−4.00	>−4.00	−4.38	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.77	−4.52
CAKI-1	−4.30	>−4.00	>−4.00	>−4.00	−4.28	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.89	−4.56
RXF 393	−4.58	−4.19	>−4.00	>−4.00	−4.40	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−5.16	−4.67
SN12C	−4.43	>−4.00	>−4.00	>−4.00	−4.28	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.68	−4.33
TK-10	−4.56	>−4.00	>−4.00	>−4.00	−4.36	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.53	>−4.00
UO-31	−4.40	>−4.00	>−4.00	>−4.00	−4.02	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.84	−4.53
Prostate cancer												
PC-3	−4.53	>−4.00		>−4.00	−4.97	−4.41	−5.22	>−4.00	−4.42	>−4.00	−4.48	>−4.00
DU-145	−4.35	>−4.00	>−4.00	>−4.00	−4.58	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.78	−4.47
Breast cancer												
MCF7	−4.60	>−4.00	>−4.00	>−4.00	−5.08	>−4.00	>−4.00	>−4.00	−4.31	>−4.00	−4.36	>−4.00
NCI/ADR-RES	−4.71	−4.38	>−4.00	>−4.00	−4.47	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.85	−4.29
MDA-MB 231 / ATCC	−4.19	>−4.00	>−4.00	>−4.00	−4.45	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.71	−4.39
HS 578T	>−4.00	>−4.00	>−4.00	>−4.00	−4.23	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.53	−4.08
MDA-MB 435	−4.34	>−4.00	>−4.00	>−4.00	−4.55	−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−5.19	−4.61
BT-549	−4.14	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.00	>−4.00	>−4.00	>−4.00	−4.80	−4.42
T-47D	−4.59	>−4.00	>−4.00	>−4.00	−4.96	>−4.00	−4.28	>−4.00	>−4.00	>−4.00	−4.62	−4.09
MG MID	−4.77	−4.21	−4.3	−4.04	−4.63	−4.17	−4.26	−4.02	−4.14	−4.02	−4.84	−4.37
Delta	3.23	3.79	3.28	2.18	1.68	0.99	1.7	0.86	1.18	0.42	2.61	1.04
Range	4.0	4.0	3.58	2.22	2.31	1.16	1.96	0.89	1.32	0.44	3.11	1.41

Table 5. The $\log_{10}LC_{50}$ values of **4, 5, 10, 12, 13**, and **16**

Panel/cell line	**4** $\log_{10}LC_{50}$	**5** $\log_{10}LC_{50}$	**10** $\log_{10}LC_{50}$	**12** $\log_{10}LC_{50}$	**13** $\log_{10}LC_{50}$	**16** $\log_{10}LC_{50}$
Leukemia						
CCRF-CEM	−4.04	>−4.00	>−4.00	>−4.00	>−4.00	−4.02
HL-60(TB)	>−4.00	>−4.00	−4.02		>−4.00	
K-562	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
MOLT-4	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
RPMI-8226	>−4.00	>−4.00	−4.09	>−4.00	>−4.00	>−4.00
SR	−4.11	>−4.00	−4.22		>−4.00	
Non-small cell lung cancer						
A549/ATCC	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
EKVX	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.17
HOP-62	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
NCI-H2 26	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
NCI-H2 3	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.21
NCI-H3 22M	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
NCI-H4 60	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
NCI-H5 22	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.17
Colon cancer						
COLO 205	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.28
HCC-2998	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.26
HCT-116	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.29
HCT-15	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.17
HT29	>−4.00	>−4.00	−4.23	>−4.00	>−4.00	>−4.00
KM12	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SW-620	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.18
CNS cancer						
SF-268	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SF-295	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SF-539	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SNB-19	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SNB-75	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
U251	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.13
Melanoma						
LOX IMVI	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.23
MALME-3M	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.23
M14	>−4.00	>−4.00	−4.21	>−4.00	>−4.00	−4.17
SK-MEL-2	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SK-MEL-28	>−4.00	>−4.00	−4.01	>−4.00	>−4.00	−4.14
SK-MEL-5	>−4.00	>−4.00	−4.03	>−4.00	>−4.00	−4.28
UACC-257	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
UACC-62	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.14
Ovarian cancer						
IGROV1	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
OVCAR-3	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.16
OVCAR-4	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−5.05
OVCAR-5	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.21
OVCAR-8	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SK-OV-3	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
Renal cancer						
786-O	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
A498	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.05
ACHN	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.26
CAKI-1	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.23
RXF 393	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.27
SN12C	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
TK-10	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
UO-31	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.22
Prostate cancer						
PC-3	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
DU-145	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.15

Table 5 (*continued*)

Panel/cell line	**4** $\log_{10}LC_{50}$	**5** $\log_{10}LC_{50}$	**10** $\log_{10}LC_{50}$	**12** $\log_{10}LC_{50}$	**13** $\log_{10}LC_{50}$	**16** $\log_{10}LC_{50}$
Breast cancer						
MCF7	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
NCI/ADR-RES	−4.04	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
MDA-MB 231 / ATCC	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.06
HS 578T	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
MDA-MB 435	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.13
BT-549	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.04
T-47D	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
MG MID	−4.0	−4.0	−4.01	−4.0	−4.0	−4.11
Delta	0.11	0	0.22	0	0	0.94
Range	0.11	0	0.23	0	0	1.05

4.6. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl *N*,*N*-diethyldithiocarbamate (4)

Yellow flakes (41%): mp 100–102 °C; IR(KBr): υ 1731 (C=O), 1238 (C=S); ^{1}H NMR ($CDCl_3$/200 MHz): δ 1.28 (t, 6H, J = 7.10 Hz, 2× CH_3), 3.71 (q, 2H, J = 6.89 Hz, N–CH_2), 4.03 (q, 2H, J = 6.80 Hz, N–CH_2), 5.84 (s, 2H, N–CH_2–S), 7.78–7.94 (m, 3H, bzi.[†] $C_{5,6,7}$–H), 8.05–8.09 (m, 1H, bzi. C_4–H); EIMS: *m*/*z* 344(M^+). Anal. Calcd for $C_{13}H_{16}N_2O_3S_3$ (344.46): C, 45.33; H, 4.68; N, 8.13; S, 27.93. Found: C, 45.59; H, 4.53; N, 8.02; S, 27.83.

4.7. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl piperidin-1-carbodithioate (5)

Shining white flakes (41%): mp 136–138 °C; IR(KBr): υ 1725 (C=O), 1239 (C=S); ^{1}H NMR ($CDCl_3$/200 MHz): δ 1.69 (s, 6H, pip. $C_{3,4,5}$–H), 3.87 (br s, 2H, pip. $C_{2,6}$–$H_{ax.}$), 4.27 (br s, 2H, pip. $C_{2,6}$–H_{equiv}), 5.86 (s, 2H, N–CH_2–S), 7.81–7.90 (m, 3H, bzi. $C_{5,6,7}$–H), 8.05–8.09 (m, 1H, bzi. C_4–H); EIMS: *m*/*z* 356 (M^+). Anal. Calcd for $C_{14}H_{16}N_2O_3S_3$ (356.48): C, 47.17; H, 4.52; N, 7.86; S, 26.99. Found: C, 47.55; H, 4.85; N, 7.70; S, 27.30.

4.8. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl 2-methylpiperidin-1-carbodithioate (6)

Light brown powder (45%): mp 108–113 °C; IR(KBr): υ 1736 (C=O), 1245 (C=S); ^{1}H NMR ($CDCl_3$/200 MHz): δ 1.27 (d, 3H, J = 6.98 Hz, pip. CH_3), 1.52–1.79 (m, 6H, pip. $C_{3,4,5}$–H), 3.17 (t, 1H, J = 12.06 Hz, pip. C_6–$H_{ax.}$), 4.41 (q, 1H, J = 7.10 Hz, pip. C_2–H_{equiv}), 5.51 (q, 1H, J = 7.90 Hz, pip. C_6–H_{equiv}), 5.85 (s, 2H, N–CH_2–S), 7.76–7.94 (m, 3H, bzi. $C_{5,6,7}$–H), 8.02–8.11 (m, 1H, bzi. C_4–H); EIMS: *m*/*z* 370 (M^+). Anal. Calcd for $C_{15}H_{18}N_2O_3S_3$ (370.51): C, 48.62; H, 4.90; N, 7.56; S, 25.96. Found: C, 47.38; H, 5.49; N, 7.23; S, 25.50.

4.9. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl 3-methylpiperidin-1-carbodithioate (7)

White powder (29%): mp 94–98 °C; IR(KBr): υ 1743 (C=O), 1248 (C=S); ^{1}H NMR ($CDCl_3$/200 MHz): δ 0.93 (s, 3H, pip. CH_3), 1.48–2.01 (m, 5H, pip. $C_{3,4,5}$–H), 2.85 (q, 1H, J = 13.28 Hz, pip. C_2–$H_{ax.}$), 3.17 (t, 1H, J = 12.37 Hz, pip. C_6–$H_{ax.}$), 4.38 (q, 1H, J = 14.03 Hz, pip. C_2–H_{equiv}), 5.32 (d, 1H, J = 11.85 Hz, pip. C_6–H_{equiv}), 5.86 (s, 2H, N–CH_2–S), 7.79–7.95 (m, 3H, bzi. $C_{5,6,7}$–H), 8.02–8.18 (m, 1H, bzi. C_4–H); EIMS: *m*/*z* 370 (M^+). Anal. Calcd for $C_{15}H_{18}N_2O_3S_3$ (370.51): C, 48.62; H, 4.90; N, 7.56; S, 25.96. Found: C, 48.99; H, 5.30; N, 7.45; S, 27.37.

4.10. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl 4-methylpiperidin-1-carbodithioate (8)

White flakes (16%): mp 145–150 °C; IR(KBr): υ 1720 (C=O), 1223 (C=S); ^{1}H NMR ($CDCl_3$/200 MHz): δ 0.95 (d, 3H, J = 6.00 Hz, pip. CH_3), 1.28–1.45 (m, 1H, pip. C_4–H), 1.58–1.84 (m, 4H, pip. $C_{3,5}$–H), 3.14 (q, 2H, J = 12.21 Hz, pip. $C_{2,6}$–$H_{ax.}$), 4.15 (d, 1H, J = 12.59 Hz, pip. C_2–H_{equiv}), 5.48 (d, 1H, J = 11.8 Hz, pip. C_6–H_{equiv}), 5.85 (d, 2H, J = 3.66 Hz, N–CH_2–S), 7.79–7.91 (m, 3H, bzi. $C_{5,6,7}$–H), 8.05–8.09 (m, 1H, bzi. C_4–H); ^{13}C NMR (HSQC, DMSO-d_6/125 MHz): δ 21.80 (CH_3), 30.90 (pip. C_4), 34.00 (pip. C_3), 34.90 (pip. C_5), 51.20 (pip. C_2), 52.80 (pip. C_6), 45.40 (N–CH_2–S), 122.10 (bzi. C_4), 126.10 (bzi. C_7), 126.60 (bzi. C_{3a}), 136.00 (bzi. C_6), 137.00 (bzi. C_5), 137.70 (bzi. C_{7a}), 159.95 (C=O), 190.30 (C=S); EIMS: *m*/*z* 370 (M^+). Anal. Calcd for $C_{15}H_{18}N_2O_3S_3$ (370.51): C, 48.62; H, 4.90; N, 7.56; S, 25.96. Found: C, 48.24; H, 4.58; N, 7.57; S, 25.07.

4.11. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl 4-benzylpiperidin-1-carbodithioate (9)

Light yellow powder (10%): mp 129–133 °C; IR(KBr): υ 1740 (C=O), 1244 (C=S); ^{1}H NMR ($CDCl_3$/200 MHz): δ 1.36–1.57 (m, 4H, pip. $C_{3,5}$–H), 1.79 (d, 1H, J = 14.09 Hz, pip. C_4–H), 2.58 (d, 2H, J = 6.91 Hz, benzyl CH_2), 3.25 (t, 4H, J = 11.45 Hz, pip. $C_{2,6}$–H), 5.18 (s, 2H, N–CH_2–S), 7.03–7.39 (m, 9H, Ar–H); EIMS: *m*/*z* 446 (M^+). Anal. Calcd for $C_{21}H_{22}N_2O_3S_3$ (446.61): C, 56.48; H, 4.97; N, 6.27; S, 21.54. Found: C, 56.34; H, 5.17; N, 6.01; S, 21.11.

4.12. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl 4-methylpiperazin-1-carbodithioate (10)

White flakes (16%): mp 145–150 °C; IR(KBr): υ 1727 (C=O), 1223 (C=S); ^{1}H NMR ($CDCl_3$/200 MHz): δ

[†] bzi: benzisothiazole.

1.85–1.91 (m, 2H, pip. C_3–H), 2.14 (s, 2H, C_5–H), 2.32–2.48 (m, 3H, N–CH_3), 3.95 (br s, 4H, pip. $C_{2,6}$–H), 5.61 (s, 2H, N–CH_2–S), 7.67–7.77 (m, 3H, bzi. $C_{5,6,7}$–H), 7.86–7.89 (m, 1H, bzi. C_4–H); EIMS: *m/z* 371 (M^+). Anal. Calcd for $C_{14}H_{17}N_3O_3S_3$ (371.50): C, 45.26; H, 4.61; N, 11.31; S, 25.89. Found: C, 45.51; H, 4.62; N, 11.16; S, 25.67.

4.13. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl 4-phenylpiperazin-1-carbodithioate (11)

Yellow powder (62%): mp 172–177 °C; IR(KBr): υ 1733 (C=O), 1243 (C=S); ^{1}H NMR ($CDCl_3$/200 MHz): δ 3.15 (t, 4H, *J* = 5.15 Hz, pip. $C_{3,5}$–H), 4.13 (br s, 4H, pip. $C_{2,6}$–H), 5.70 (s, 2H, N–CH_2–S), 6.69–6.77 (m, 3H, phenyl $C_{3,4,5}$–H), 7.06–7.14 (m, 2H, phenyl $C_{2,6}$–H), 7.71–7.81 (m, 3H, bzi. $C_{5,6,7}$–H), 7.91–7.95 (m, 1H, bzi. C_4–H); EIMS: *m/z* 433 (M^+). Anal. Calcd for $C_{19}H_{19}N_3O_3S_3$ (433.57): C, 52.63; H, 4.42; N, 9.69; S, 22.19. Found: C, 53.01; H, 4.32; N, 9.57; S, 22.15.

4.14. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl pyrrolidin-1-carbodithioate (12)

White needles (47%); mp 138–139 °C; IR(KBr): υ 1724 (C=O), 1238 (C=S); ^{1}H NMR ($CDCl_3$/200 MHz): δ 1.94–2.09 (m, 4H, pyr. $C_{3,4}$–H), 3.64, 3.95 (2t, 4H, *J* = 6.59 Hz, pyr. $C_{2,5}$–H), 5.87 (s, 2H, N–CH_2–S), 7.82–7.93 (m, 3H, bzi. $C_{5,6,7}$–H), 8.05–8.09 (m, 1H, bzi. C_4–H); EIMS: *m/z* 342 (M^+). Anal. Calcd for $C_{13}H_{14}N_2O_3S_3$ (342.46): C, 45.59; H, 4.12; N, 8.18; S, 28.09. Found: C, 45.90; H, 4.54; N, 8.07; S, 28.11.

4.15. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl morpholin-1-carbodithioate (13)

White powder (32%): mp 170–181 °C; IR(KBr): υ 1732 (C=O), 1222 (C=S); ^{1}H NMR ($CDCl_3$/200 MHz): δ 3.75 (s, 4H, morph. $C_{3,5}$–H), 3.91, 4.34 (2br s, 4H, morph. $C_{2,6}$–H), 5.87 (s, 2H, N–CH_2–S), 7.80–7.97 (m, 3H, bzi. $C_{5,6,7}$–H), 8.03–8.10 (m, 1H, bzi. C_4–H); ^{13}C NMR (APT, DMSO-d_6/125 MHz): δ 52.40 (morph. C_3), 55.00 (morph. C_5), 66.26 (morph. C_2 and C_6), 44.98 (N–CH_2–S), 122.45 (bzi. C_4), 126.07 (bzi. C_7), 126.42 (bzi. C_{3a}), 136.14 (bzi. C_6), 137.00 (bzi. C_5), 137.68 (bzi. C_{7a}), 158.98 (C=O), 192.76 (C=S). Anal. Calcd for $C_{13}H_{14}N_2O_4S_3$ (358.46): C, 43.56; H, 3.94; N, 7.81; S, 26.84. Found: C, 43.94; H, 4.00; N, 7.68; S, 26.78.

4.16. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl 2,6-dimethylmorpholin-1-carbodithioate (14)

Light green crystals (35%): mp 118–120 °C; IR(KBr): υ 1732 (C=O), 1236 (C=S); ^{1}H NMR ($CDCl_3$/200 MHz): δ 1.21 (s, 6H, 2× CH_3), 2.82 (s, 2H, morph. $C_{3,5}$–$H_{ax.}$), 3.63 (s, 2H, morph. $C_{2,6}$–H), 4.4 (br s, 1H, morph. C_3–H_{equiv}), 5.40 (br s, 1H, morph. C_5–H_{equiv}), 5.85 (s, 2H, N–CH_2–S), 7.77–7.92 (m, 3H, bzi. $C_{5,6,7}$–H), 8.00–8.07 (m, 1H, bzi. C_4–H); ^{13}C NMR (HSQC, DMSO-d_6/125 MHz): δ 19.75 (morph. C_2–CH_3), 19.85 (morph. C_6–CH_3), 55.18 (morph. C_3), 55.40 (morph. C_5), 70.30 (morph. C_2 and C_6), 45.00 (N–CH_2–S), 122.25 (bzi. C_4), 126.00 (bzi. C_7), 126.24 (bzi. C_{3a}), 136.18 (bzi. C_6), 137.00 (bzi. C_5), 137.60 (bzi. C_{7a}), 150.90 (C=O), 190.30 (C=S). Anal. Calcd for $C_{15}H_{18}N_2O_4S_3$ (386.51): C, 46.61; H, 4.69; N, 7.25; S, 24.89. Found: C, 46.93; H, 4.36; N, 7.06; S, 24.80.

4.17. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl *N*-methyl-*N*-[3-(dibenzo[*b*,*e*]-bicyclo[2.2.2]octadienyl)propyl]dithiocarbamate (15)

White powder (10%): mp 90–125 °C; IR(KBr): υ 1734 (C=O), 1182 (C=S); ^{1}H NMR ($CDCl_3$/200 MHz): δ 1.56 (s, 3 H, N–CH_3), 1.78–1.82 (m, 2H, C_2–H_2), 2.17–2.27 (m, 2H, $C_{2'}$–H_2), 2.42–2.53 (m, 2H, C_3–H_2), 3.42 (s, 1H, $C_{3'}$–H), 3.63 (s, 1H, $C_{3'}$–H), 4.02 (t, 1H, *J* = 7.70 Hz, $C_{4'}$–H), 4.25–4.38 (m, 2H, C_1–H_2), 5.85, 5.91 (2s, 2H, N–CH_2–S), 7.01–7.28 (m, 8H, Ar–H), 7.78–7.95 (m, 3H, bzi. $C_{5,6,7}$–H), 8.05-8.09 (m, 1H, bzi. C_4–H); Anal. Calcd for $C_{29}H_{28}N_2O_3S_3 \cdot 2.5\,H_2O$ (593.77): C, 58.65; H, 5.51; N, 4.71; S, 16.19. Found: C, 58.34; H, 5.10; N, 4.53; S, 16.18.

4.18. General method for the synthesis of (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl *O*-alkyl dithiocarbonates (16–20)

The ethanolic solution of **3** (5 mmol) and **b** (5 mmol) were refluxed for 1 h. After evaporation of the solvent in vacuo, products were washed with water and purified by recrystallization from ethanol.

4.19. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl *O*-ethyl dithiocarbonate (16)

White powder (37%): mp 60–64 °C; IR(KBr): υ 1743 (C=O), 1256 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 1.44 (t, 3H, *J* = 7.01 Hz, ethyl CH_3), 4.69 (q, 2H, *J* = 6.99Hz, O–CH_2), 5.50 (s, 2H, N–CH_2–S), 7.99–8.14 (m, 3H, bzi. $C_{5,6,7}$–H), 8.32 (d, 1H, *J* = 7.32 Hz, bzi. C_4–H); ^{13}C NMR (proton decoupled, DEPT-135 / DMSO-d_6/125 MHz): δ 14.14 (ethyl CH_3), 42.43 (N–CH_2–S), 71.89 (ethyl O–CH_2), 122.44 (bzi. C_4), 126.11 (bzi. C_7), 126.39 (bzi. C_{3a}), 136.16 (bzi. C_6), 137.02 (bzi. C_5), 137.57 (bzi. C_{7a}), 158.81 (C=O), 210.13 (C=S); EIMS: *m/z* 317 (M^+). Anal. Calcd for $C_{11}H_{11}NO_4S_3$ (317.41): C, 41.62; H, 3.49; N, 4.41; S, 30.31. Found: C, 41.69; H, 3.13; N, 4.36; S, 30.73.

4.20. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl *O*-propyl dithiocarbonate (17)

Light yellow flakes (40%): mp 45–50 °C; IR(KBr): υ 1745 (C=O), 1246 (C=S); ^{1}H NMR (DMSO-d_6/400MHz): δ 0.98 (t, 3H, *J* = 7.31 Hz, propyl CH_3), 1.84–1.89 (m, 2H, propyl–CH_2–), 4.61 (t, 2H, *J* = 6.58 Hz, propyl O–CH_2), 5.50 (s, 2H, N–CH_2–S), 7.99–8.14 (m, 3H, bzi. $C_{5,6,7}$–H), 8.32 (d, 1H, *J* = 7.32 Hz, bzi. C_4–H); EIMS: *m/z* 331 (M^+). Anal. Calcd for $C_{12}H_{13}NO_4S_3$ (331.43): C, 43.49; H, 3.95; N, 4.23; S, 29.02. Found: C, 43.50; H, 3.92; N, 4.19; S, 28.75.

4.21. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl *O*-isopropyl dithiocarbonate (18)

White powder (46%): mp 58–61 °C; IR(KBr): υ 1739 (C=O), 1256 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 1.43 (d, 6H, J = 6.34 Hz, 2× CH_3), 5.67–5.74 (m, 1H, O–CH), 5.47 (s, 2H, N–CH_2–S), 7.99–8.14 (m, 3H, bzi. $C_{5,6,7}$–H), 8.32 (d, 1H, J = 7.32 Hz, bzi. C_4–H); ^{13}C NMR (APT, DMSO-d_6/125 MHz): δ 21.48(2× CH_3), 42.10 (N–CH_2–S), 80.40 (O–CH), 122.42 (bzi. C_4), 126.11 (bzi. C_7), 126.40 (bzi. C_{3a}), 136.17 (bzi. C_6), 137.02 (bzi. C_5), 137.58 (bzi. C_{7a}), 158.79 (C=O), 209.21 (C=S); EIMS: m/z 331 (M^+). Anal. Calcd for $C_{12}H_{13}NO_4S_3$ (331.43): C, 43.49; H, 3.95; N, 4.23; S, 29.02. Found: C, 43.64; H, 3.77; N, 4.19; S, 29.38.

4.22. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl *O*-butyl dithiocarbonate (19)

Light yellow needles (42%): mp 52–54 °C; IR(KBr): υ 1737 (C=O), 1246 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 0.92 (t, 3H, J = 7.31 Hz, butyl CH_3), 1.39–1.48 (m, 2H, butyl C_3–H_2), 1.79–1.86 (m, 2H, butyl C_2–H_2), 4.65 (t, 2H, J = 6.58 Hz, O–CH_2), 5.49 (s, 2H, N–CH_2–S), 7.99–8.14 (m, 3H, bzi. $C_{5,6,7}$–H), 8.33 (d, 1H, J = 6.82 Hz, bzi. C_4–H); EIMS: m/z 345 (M^+). Anal. Calcd for $C_{13}H_{15}NO_4S_3$ (345.46): C, 45.20; H, 4.38; N, 4.05; S, 27.85. Found: C, 45.49; H, 3.98; N, 4.07; S, 27.55.

4.23. (1,1-Dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methyl *O*-isobutyl dithiocarbonate (20)

Ivory powder (44%): mp 71–74 °C; IR(KBr): υ 1745 (C=O), 1240 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 1.00 (d, 6H, J = 6.34 Hz, 2× CH_3), 2.17–2.24 (m, 1H, CH), 4.45 (d, 2H, J = 6.83 Hz, O–CH_2), 5.52 (s, 2H, N–CH_2–S), 7.99–8.14 (m, 3H, bzi. $C_{5,6,7}$–H), 8.33 (d, 1H, J = 7.28 Hz, bzi. C_4–H); EIMS: m/z 345 (M^+). Anal. Calcd for $C_{13}H_{15}NO_4S_3$ (345.46): C, 45.20; H, 4.38; N, 4.05; S, 27.85. Found: C, 45.22; H, 4.36; N, 4.01; S, 27.78.

4.24. In vitro evaluation of antituberculosis activity

Primary screening was conducted at 6.25 μg/mL against *M. tuberculosis* H37Rv in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA). Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system. Compounds showing ⩾90 inhibition in the primary screen were considered active and then re-tested at lower concentrations against *M. tuberculosis* H37Rv in order to determine the actual minimum inhibitory concentration (MIC). The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% with respect to the controls.

4.25. Microplate alamar blue susceptibility assay (MABA)

Antimicrobial susceptibility testing was performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument, Meriden, CN) in order to minimize background fluorescence. Outer perimeter wells were filled with sterile water to prevent dehydration in experimental wells. Initial drug dilutions were prepared in either dimethylsulfoxide or distilled deionized water, and subsequent twofold dilutions were performed in 0.1 mL of 7H9GC (no Tween 80) in the microplates. BACTEC 12B-passaged inocula were initially diluted 1:2 in 7H9GC, and 0.1 mL was added to wells. Subsequent determination of bacterial titer yielded 1×10^6 CFU/mL in plate wells for H37Rv. Frozen inocula were initially diluted 1:20 in BACTEC 12B medium followed by a 1:50 dilution in 7H9GC. Addition of 1/10 mL to wells resulted in a final bacterial titer of 2.0×10^5 CFU/mL for H37Rv. Wells containing drug only were used to detect autofluorescence of compounds. Addition control wells consisted of bacteria only (B) and medium only (M). Plates were incubated at 37 °C. Starting at day 4 of incubation, 20 μl of 10× Alamar Blue solution (Alamar Biosciences/Accumed, Westlake, OH) and 12.5 μl of 20% Tween 80 were added to one B well and one M well, and plates were reincubated at 37 °C. Wells were observed at 12 and 24 h for a color change from blue to pink and for a reading of ⩾50,000 fluorescence units (FU). Fluorescence was measured in a Cytofluor II microplate fluorometer (PerSeptive Biosystems, Framingham, MA.) in bottom-reading mode with excitation at 530 nm and emission at 590 nm. If the B wells became pink by 24 h, reagent was added to the entire plate. If the well remained blue or ⩽50,000 FU was measured, additional M and B wells were tested daily until a color change occurred, at which time reagents were added to all remaining wells. Plates were then incubated at 37 °C, and results were recorded at 24 h post-reagent addition. Visual MICs were defined as the lowest concentration of drug that had prevented a color change. For fluorometric MICs, a background subtraction was performed on all wells with a mean of triplicate M wells. Percent inhibition was defined as 1-(test well FU/mean FU of triplicate B wells) × 100. The lowest drug concentration effecting an inhibition of ⩾90% was considered the MIC.

4.26. BACTEC radiometric method of susceptibility testing

Inocula for susceptibility testing were either from a positive BACTEC isolation vial with a growth index (GI) of 500 or more, or a suspension of organisms isolated earlier on a conventional medium. The culture was well mixed with a syringe and 0.1 mL of a positive BACTEC culture was added to each of the vials containing the test compounds (6.25 μg/mL). The Standard vials contained rifampin (RMP) (0.25 μg/mL). A control vial was inoculated with a 1:100 dilution of the culture.

Each vial was tested immediately on a BACTEC instrument to provide CO_2 in the headspace. The vials were incubated at 37 °C and tested daily with a BACTEC instrument. When the GI in the control read at least 30, the increase in GI (Δ GI) from the previous day in the control was compared with that in the drug vial. The following formula was used to interpret the results:

Δ GI control $>\Delta$ GI drug = susceptible

Δ GI control $<\Delta$ GI drug = resistant

If a clear susceptibility pattern (the difference of Δ GI of control and the drug bottle) was not seen at the time the control GI was 30 the vials were read for 1 or 2 additional days to establish a definite pattern of Δ GI differences.

4.27. Methodology of the in vitro cancer screen

The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96-well microtiter plates in 100 μL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs were solubilized in dimethylsulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/mL gentamicin. Additional four, 10-fold or 1/2 log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 μl of these different drug dilutions were added to the appropriate microtiter wells already containing 100 μl of medium, resulting in the required final drug concentrations. Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 μl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air-dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 μl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as:

$[(Ti - Tz)/(C - Tz)] \times 100$ for concentrations for which $Ti >/= Tz$

$[(Ti - Tz)/Tz] \times 100$ for concentrations for which $Ti < Tz$.

Three dose response parameters were calculated for each experimental agent. Growth inhibition of 50% (GI50) was calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from $Ti = Tz$. The LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from $[(Ti - Tz)/Tz] \times 100 = -50$. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested.

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