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Synthesis and In Vitro Antitumoral Activity of New *N*-Phenyl-3-pyrrolecarbothioamides

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Abstract—A new series of *N*-phenylpyrrolecarbothioamides were obtained from base catalyzed intramolecular cyclization of 3-amino-3-(alkyl or arylamino)propenethioamides. Pyrrole derivatives were evaluated for their in vitro anticancer activity toward cell lines of nine different types of human cancer. Some of newly prepared compounds demonstrated inhibitory effects on the growth of a wide range of cancer cell lines generally at 10^{-6} M level and in some case at 10^{-8} M concentrations.

Introduction

Cancer represents a serious human health problem despite much progress in understanding the biology and pharmacology. The main problem is that cancer is not one disease, but a group of diseases affecting different organs and system of the body. There are cogent reasons why cancer is difficult to cure, one is that the differences between normal and neoplastic human cells are mostly quantitative, another is that by their very nature, the cancer cells have eluded or overcome the immune surveillance system of the body. The traditional therapeutic strategies for treatment of cancer are surgery, radiotherapy, immunotherapy and chemotherapy. Today, about 50% of the patients diagnosed with cancer are cured through one of these methods or by combination of them. Chemotherapy is, at present, the only one effective therapy for some types of disseminated cancers because anticancer drugs travel through the circulatory system. At present, at least ten different neoplasms can be cured by chemotherapy in the majority of patients.[1] Many drugs exert their action by interfering with the cellular functions by alkylation of nucleic acids and proteins, by DNA intercalation with groove binding, by inhibition of topoisomerase II, by inhibition of microtubule assembly and by stabilization of polymerization of microtubules or by prevention of the biosynthesis or utilization of normal cellular metabolites. Most antineoplastic drugs are highly toxic to the patient and must be administered with extreme caution. The toxicity usually involves rapidly proliferating tissues such us bone marrow and the intestinal epithelium. However, individual drugs produce distinctive toxic effects on the heart, lungs, kidneys, and other organs.

Although the development of cancer chemotherapy the search of new lead structures, particularly those interacting with novel biological targets is necessary. Thus we began a random search for cytotoxic molecules in order to provide novel structural leads. Pyrrole based chemotherapeutic agents have a long history and include anti-inflammatory drugs such as Clopirac, Tolmetine, Viminol, antihelminthic drugs such as Pyrivinium, antimycotic drugs such us pyrrolnitrin, antibiotic virustatic drugs such as Stallimycin. Furthermore the pyrrole moiety has been incorporated in several non-nucleoside reverse transcriptase inhibitors[2–8] and antiproliferative agents[9,10] as well as the DNA minor groove binders Distamycin A[11,12] and Tallimustine[13,14] are pyrrolecarbamoyl oligomers. In recent years it has been reported the in vitro anticancer activity of diazopyrroles and triazenopyrroles.[15–17] These last may be considered isosters of Dacarbazine that is the most active drug available for treatment of malignant melanomas and Hodgkin tumours resistant to MOPP therapy. A number of agents of these classes are under intensive development by research groups throughout the world. On the other hand the presence of sulfur containing functionalities, like carbothioamide, is known to enhance biological activity of bioactive molecules. Prompted by the above observations we under-

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took a study on the influence of introduction of carbothioamide moiety on pyrrole nucleus. Thus in this paper we report on the synthesis and antitumoral activity of a series of *N*-phenylpyrrole-3-carbothioamides bearing various substituents such as aliphatic, aromatic or heteroaromatic secondary amine on 2-position and acetyl or (substituted)benzoyl moieties on 5-position.

Results and Discussion

Chemistry

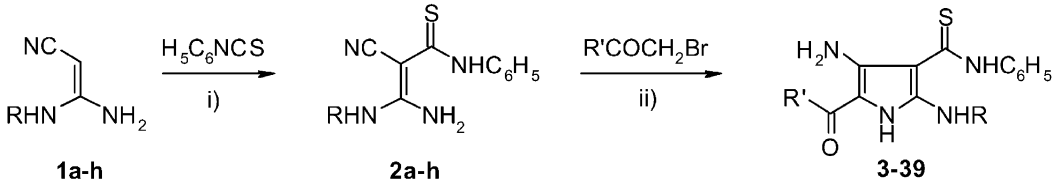
The synthetic approach to obtain pyrrole derivatives **3–39** followed the reactions shown in Scheme 1. The required 3-amino-3-(alkyl or arylamino)propenethioamides **2** were prepared by treatment of 3-amino-3-(alkyl or arylamino)propenenitriles **1** with phenyl isothiocyanate using the method described in our previous paper.[18] The reaction was found to be highly regioselective since none of the corresponding products of addition on 3-amino group was detected in the crude product. Pyrrolecarbothioamides **3–39** (Table 1) were obtained in good to excellent yield by coupling thioamides **2a–h** with α-bromoketones under basic conditions.[19] The formation of pyrrole derivatives can be interpreted as arising from initial alkylation of the more nucleophilic primary amino group of propenethioamides. The formed *N*-alkylated intermediate undergoes subsequent intramolecular cyclization through selective nucleophilic attack of the active methylene to cyano group.

All the newly synthesized compounds gave corrected analytical data. The IR spectra of pyrroles showed no absorption bands of CN thus confirming the assigned structure. The ^{1}H NMR spectra show five deuterium oxide exchangeable protons, the one that lowerfield resonates was easily assigned to pyrrolic NH, even if in some cases this signal is so broad that it does not permit its assignment.

Pharmacology

Evaluation of anticancer activity on pyrrolecarbothioamides **3–39** was performed at National Cancer Institute (NCI). First all pyrroles have been evaluated in primary anticancer assay at 10^{-4} M concentration against NCI-H460 (Lung), MCF7 (Breast) and SF-268 (CNS) cell lines (Table 2).

For NCI criteria, compounds, which reduce the growth of any one of the cell lines to approximately 32% or less, are passed on for evaluation in the full panel of cell lines over a 5-log dose range. Pyrroles **3**, **9**, **16–18**, **26–28**, **30–32**, **34–39**, that meet these criteria, were evaluated for their anticancer activity following the known in vitro disease-oriented antitumor screening program,



1a-h **2a-h** **3-39**

1, 2	R	1, 2	R
a	*n*-Pr	**e**	4-MeOC$_6$H$_4$
b	*i*-Pr	**f**	4-ClC$_6$H$_4$
c	CH$_2$C$_6$H$_5$	**g**	5-MePyridin-2-yl
d	4-MeC$_6$H$_4$	**h**	4-MePyridin-2-yl

Scheme 1. Synthesis of pyrrolecarbothioamides **3–39**. Reagents and conditions: (i) MeCN, rt; (ii) $CHCl_3$, TEA, rt.

Table 1. Pyrrolecarbothioamides **3–39**

Compd	R	R′	Compd	R	R′	Compd	R	R′
3	4-MeC$_6$H$_4$	Me	**16**	4-ClC$_6$H$_4$	4-MeC$_6$H$_4$	**29**	4-MePyridin-2-yl	3,4-Cl$_2$C$_6$H$_3$
4	4-MeC$_6$H$_4$	C$_6$H$_5$	**17**	4-ClC$_6$H$_4$	4-MeOC$_6$H$_4$	**30**	CH$_2$C$_6$H$_5$	Me
5	4-MeC$_6$H$_4$	4-MeC$_6$H$_4$	**18**	4-ClC$_6$H$_4$	4-ClC$_6$H$_4$	**31**	CH$_2$C$_6$H$_5$	C$_6$H$_5$
6	4-MeC$_6$H$_4$	4-MeOC$_6$H$_4$	**19**	4-ClC$_6$H$_4$	3,4-Cl$_2$C$_6$H$_3$	**32**	CH$_2$C$_6$H$_5$	4-MeC$_6$H$_4$
7	4-MeC$_6$H$_4$	4-ClC$_6$H$_4$	**20**	5-MePyridin-2-yl	C$_6$H$_5$	**33**	CH$_2$C$_6$H$_5$	4-MeOC$_6$H$_4$
8	4-MeC$_6$H$_4$	3,4-Cl$_2$C$_6$H$_3$	**21**	5-MePyridin-2-yl	4-MeC$_6$H$_4$	**34**	CH$_2$C$_6$H$_5$	4-ClC$_6$H$_4$
9	4-MeOC$_6$H$_4$	Me	**22**	5-MePyridin-2-yl	4-MeOC$_6$H$_4$	**35**	CH$_2$C$_6$H$_5$	3,4-Cl$_2$C$_6$H$_3$
10	4-MeOC$_6$H$_4$	C$_6$H$_5$	**23**	5-MePyridin-2-yl	4-ClC$_6$H$_4$	**36**	*n*-Pr	Me
11	4-MeOC$_6$H$_4$	4-MeC$_6$H$_4$	**24**	5-MePyridin-2-yl	3,4-Cl$_2$C$_6$H$_3$	**37**	*n*-Pr	C$_6$H$_5$
12	4-MeOC$_6$H$_4$	4-MeOC$_6$H$_4$	**25**	4-MePyridin-2-yl	C$_6$H$_5$	**38**	*i*-Pr	Me
13	4-MeOC$_6$H$_4$	4-ClC$_6$H$_4$	**26**	4-MePyridin-2-yl	4-MeC$_6$H$_4$	**39**	*i*-Pr	C$_6$H$_5$
14	4-MeOC$_6$H$_4$	3,4-Cl$_2$C$_6$H$_3$	**27**	4-MePyridin-2-yl	4-MeOC$_6$H$_4$			
15	4-ClC$_6$H$_4$	C$_6$H$_5$	**28**	4-MePyridin-2-yl	4-ClC$_6$H$_4$			

Table 2. Antiproliferative activity of pyrrolecarbothioamides **3–39** at 10^{-4} M concentration expressed in growth percentage

Compd	Cell lines			Compd	Cell lines			Compd	Cell lines		
	MCF7	NCI–H460	SF-268		MCF7	NCI–H460	SF-268		MCF7	NCI–H460	SF-268
3	1	1	1	**16**	100	1	30	**29**	70	63	57
4	99	90	101	**17**	48	23	46	**30**	1	1	1
5	99	83	90	**18**	76	22	49	**31**	2	1	2
6	100	79	99	**19**	74	99	125	**32**	4	3	3
7	89	58	74	**20**	83	96	99	**33**	99	63	83
8	58	96	117	**21**	95	101	122	**34**	2	1	2
9	2	1	3	**22**	90	90	93	**35**	2	1	2
10	99	90	98	**23**	72	33	72	**36**	14	26	31
11	99	90	103	**24**	81	80	70	**37**	3	2	4
12	86	69	60	**25**	95	100	97	**38**	9	13	11
13	95	75	83	**26**	3	11	55	**39**	3	2	3
14	78	98	114	**27**	35	8	38				
15	104	91	108	**28**	39	15	64				

which is based upon use of multiple panel of 60 human tumor cell lines.[20,21] Each compound is tested at minimum of five concentrations at 10-fold dilution against every cell line in the panel. A 48 h continuous drug exposure protocol is used and a sulforhodamine B (SRB) protein assay is used to estimate cell viability or growth.[22] The anticancer activity of each compound is deduced from dose–response curves and is presented in three different tables according to the data provided by NCI.[23] The response parameters GI_{50}, TGI and LC_{50} (Tables 3–5) refer to the drug concentration that produced 50% inhibition, total growth inhibition and 50% cytotoxicity, respectively, and are expressed in 10^{-6} molar concentrations.

From the analysis of GI_{50} data reported in Table 3 we can evince that, except for compound **31** and **37**, all the other pyrrolecarbothioamides maintained a good level of cytostatic activity at 10^{-5} M and in some cases at 10^{-8} M concentration. Compounds **34** displayed inhibitory activity at 10^{-6} M concentration on all 60 cell lines with particular selectivity against HCC-2998 colon cancer cell line (GI_{50} $3.71{\cdot}10^{-7}$ M, TGI $1.98{\cdot}10^{-6}$ M and LC_{50} $4.60{\cdot}10^{-6}$ M). Although pyrroles **16**, **18** and **32** have demonstrated less activity respect to **34**, they inhibited the growth of the same cell lines. Compound **30** selectively exhibited high potency against lung cancer NCI-H522 cell line (GI_{50} $3.83{\cdot}10^{-8}$ M), melanoma SK-MEL-2 cell line (GI_{50} $2.58{\cdot}10^{-7}$ M), IGROV-1 cell line of ovarian cancer (GI_{50} $3.17{\cdot}10^{-7}$ M), UO31 cell line of renal cancer (GI_{50} $4.52{\cdot}10^{-7}$ M) and SF-539 of CNS cancer (GI_{50} $1.88{\cdot}10^{-6}$ M). Furthermore compound **30** inhibited totally the growth of NCI-H522 and SK-MEL-2 cell lines at $1.66{\cdot}10^{-7}$ M and $1.07{\cdot}10^{-6}$ M concentration and showed LC_{50} values of $5.96{\cdot}10^{-7}$ M and $5.50{\cdot}10^{-6}$ M respectively. IGROV-1 and UO-31 cell lines are also susceptible to compound **36** (GI_{50} $5.07{\cdot}10^{-7}$ and $7.81{\cdot}10^{-7}$ M respectively). Pyrrole **38** is particularly selective on RPMI-8226 cell line of leukemia (GI_{50} $5.9{\cdot}10^{-8}$ M) but it is not cytotoxic on this cell line.

The activity appears to be related to some structural requirements, to the presence of a 2-benzylamino moiety (**30–35**) and to acyl substituents. In fact the presence of a 4-chlorobenzoyl on 5-position (**34**) favorably affects the activity. The introduction in the same position of other acyl groups produces variable effects: a benzoyl or a 4-methoxybenzoyl (**31** and **33**) is detrimental while a 4-methylbenzoyl, a 3,4-dichlorobenzoyl or an acetyl are positive. When benzylamino group is replaced by a propylamino moiety as in **36** the activity is maintained. The substitution of acetyl of **36** with a benzoyl leds to the completely inactive compound **37**. Similarly, the introduction of an isopropylamino function at 2-position have a helpful effect on the activity, while substitution of acetyl group with a benzoyl produces negative effect, as matter of fact pyrrole **38** is more active than **39**. Among 2-(arylamino)pyrroles **3–19** a 4-chlorine atom on phenylamino moiety is effective in inducing strong antitumoral activity, whereas 4-methyl or 4-methoxy groups led to clear reduction in inhibitory activity. Significant antiproliferative activity is shown by the 5-acetyl derivatives **3** and **9** only. Generally, a drop in activity is observed when a pyridylamino group is introduced at 2-position, being moderately active only 4-methylpyridylamino derivatives **27** and **28**.

Experimental

Chemistry. Melting points were determined on a Stuart Scientific Melting point SMP1 and are uncorrected. Proton NMR spectra were recorded on a Varian Unity 300 spectrometer. The chemical shift are reported in part per million (δ, ppm) downfield from tetramethylsilane (TMS), which was used as internal standard. Infrared spectra were obtained with a Bruker Vector 22 spectrophotometer. Elemental analyses were carried out with a Carlo Erba model 1106 Elemental Analyzer and the values found were within 0.4% of theoretical values. The 3-amino-3-(1-propylamino)-2-[(phenylamino)thioxamethyl]propenenitrile **2a**, 3-amino-3-(2-propylamino)-2-[(phenylamino)thioxamethyl] propenenitrile **2b**, 3-amino-3-benzylamino-2-[(phenylamino) thioxamethyl]propenenitrile **2c**, 3-amino-3-[(4-methylphenyl)amino]-2-[(phenylamino) thioxamethyl] propene-nitrile **2d**, 3-amino-3-[(4-methoxyphenyl)-amino]-2-[(phenylamino)thioxamethyl]-2-propenenitrile **2e**, pyrrolecarbothioamides **3**, **4**, **9**, **10**, **30**, **31**, **36**, **38**, **39** were prepared by previously described procedures.[18,19]

General procedure for the synthesis of propenethioamides 2f–h. The appropriate amine (10 mmol) was added to a solution of 3-amino-3-ethoxypropenenitrile (1.10 g, 10

Table 3. GI_{50} values of pyrrolecarbothioamides

Panel/cell line	Compound														
	3	**9**	**16**	**17**	**18**	**26**	**27**	**28**	**30**	**32**	**34**	**35**	**36**	**38**	**39**
Leukemia															
CCRF-CEM	21.0	>100	8.65	>100	6.27	>100	n.t.	25.8	28.1	5.16	2.44	n.t.	46.4	25.5	>100
HL-60(TB)	15.8	45.1	44.4	>100	49.5	>100	24.3	23.8	5.33	16.3	3.92	19.1	37.7	51.7	>100
K-562	9.31	45.9	7.38	>100	3.29	>100	25.1	18.3	24.9	2.13	2.09	3.32	43.3	33.4	60.8
MOLT-4	30.2	>100	12.1	>100	9.43	>100	23.5	10.9	36.7	2.83	2.58	3.31	69.2	37.2	80.9
RPMI-8226	n.t.	n.t.	5.04	n.t.	14.5	>100	13.3	11.0	n.t.	2.15	1.79	2.65	n.t.	0.059	n.t.
SR	22.3	33.7	2.05	>100	1.92	>100	15.2	12.6	28.7	2.43	2.13	3.10	17.2	23.7	43.4
Non small cell lung cancer															
A549/ATCC	14.9	41.9	2.27	>100	3.21	>100	23.4	16.0	27.1	9.21	2.18	5.15	33.4	32.3	90.5
EKVX	24.3	99.8	5.49	>100	4.89	>100	22.3	14.5	27.1	13.6	7.21	14.6	35.2	37.0	>100
HOP-62	20.8	>100	4.41	>100	5.65	>100	24.7	20.5	19.9	15.5	3.67	17.3	42.0	38.1	>100
HOP-92	13.0	>100	12.9	>100	5.21	>100	n.t.	17.2	14.9	6.58	2.84	n.t.	34.0	17.8	>100
NCI-H226	14.4	36.2	13.9	46.9	3.64	>100	17.1	17.6	18.2	11.5	7.91	11.3	21.7	21.1	41.3
NCI-H23	17.8	65.0	3.14	>100	3.29	>100	21.9	19.3	38.8	13.3	2.39	18.2	41.0	35.9	>100
NCI-H322M	18.1	65.4	7.95	>100	14.4	>100	12.0	12.8	30.7	2.96	2.03	5.15	53.4	51.4	50.2
NCI-H460	15.7	48.3	4.20	>100	6.58	>100	6.04	5.82	22.8	5.63	1.77	5.41	33.9	31.8	57.0
NCI-H522	15.6	36.7	9.69	>100	4.83	>100	13.8	12.8	0.0383	2.21	1.81	4.83	4.15	16.6	63.8
Colon cancer															
COLO 205	23.9	21.9	1.44	>100	4.13	>100	19.4	16.0	28.4	3.88	11.5	1.78	31.5	27.9	28.1
HCC-2998	18.6	28.2	2.29	>100	2.74	>100	17.1	14.7	20.5	1.29	0.371	1.74	>100	28.2	21.8
HCT-116	16.7	38.6	3.72	60.7	3.70	>100	20.7	14.2	18.4	4.34	1.83	3.48	34.8	24.1	36.2
HCT-15	18.3	35.6	10.7	>100	4.01	>100	21.1	16.1	19.1	2.14	1.79	2.51	31.8	26.8	32.8
HT29	25.8	35.3	4.43	24.8	4.99	>100	17.6	14.9	31.3	3.23	1.94	3.23	20.0	38.8	27.8
KM12	15.9	37.5	5.25	>100	7.60	>100	13.3	10.0	17.9	2.28	1.67	3.17	33.9	29.6	31.7
SW-620	28.4	>100	11.5	>100	6.42	>100	25.3	25.9	32.1	2.38	1.88	3.22	>100	54.1	47.0
CNS cancer															
SF-268	14.5	29.6	5.79	>100	4.22	>100	21.9	21.2	21.5	12.9	3.03	14.1	23.3	25.3	67.8
SF-295	17.8	61.6	2.13	>100	2.38	>100	17.9	14.5	25.6	5.05	1.83	3.47	43.3	38.1	>100
SF-539	29.1	73.2	5.30	>100	5.41	>100	18.3	12.7	1.88	2.86	4.63	2.68	10.8	>100	>100
SNB-19	16.8	82.8	5.61	>100	4.35	>100	18.8	23.4	34.3	14.7	4.08	18.5	38.4	27.8	>100
SNB-75	19.9	>100	2.94	43.9	3.00	>100	20.5	18.1	>100	4.85	1.71	10.0	37.0	>100	69.3
U251	14.3	43.8	3.01	n.t.	2.97	>100	15.3	15.1	17.3	4.04	1.72	3.97	31.7	n.t.	43.1
Melanoma															
LOX IMVI	16.4	32.1	3.91	>100	3.64	>100	18.7	14.8	17.4	2.01	1.80	2.21	23.3	19.1	23.2
MALME-3M	17.1	50.6	4.17	>100	8.33	>100	14.6	13.8	19.7	n.t.	n.t.	3.13	28.0	25.7	19.3
M14	17.1	26.8	12.9	n.t.	11.9	>100	20.5	16.4	18.9	1.96	1.96	3.57	19.4	19.8	23.2
SK-MEL-2	15.5	32.9	14.5	>100	11.7	>100	10.1	8.59	0.258	10.8	1.91	12.4	92.1	17.8	23.8
SK-MEL-28	19.5	36.2	4.21	22.8	4.27	>100	17.0	18.6	24.2	3.64	1.77	4.63	24.7	32.5	17.4
SK-MEL-5	15.5	37.2	n.t.	>100	n.t.	>100	n.t.	n.t.	17.1	n.t.	n.t.	n.t.	22.7	18.3	28.9
UACC-257	18.5	52.2	18.7	>100	11.2	>100	15.9	15.6	24.2	5.76	1.68	13.8	30.1	24.0	22.8
UACC-62	16.2	25.9	14.4	>100	13.4	>100	12.9	10.6	16.6	11.8	8.15	14.3	17.9	23.5	16.9
Ovarian cancer															
IGROV1	16.8	78.1	4.41	>100	4.15	>100	12.2	9.02	0.317	7.34	2.37	11.8	0.507	26.4	>100
OVCAR-3	15.2	>100	11.3	>100	3.19	>100	41.6	25.7	26.7	11.8	6.65	17.6	42.3	32.1	>100
OVCAR-4	17.5	36.5	1.97	23.8	2.08	>100	10.9	6.01	26.4	2.73	1.66	3.81	23.1	30.4	72.0
OVCAR-5	32.9	>100	11.0	>100	11.8	>100	26.1	21.2	24.3	14.0	3.00	13.6	68.8	61.8	>100
OVCAR-8	18.1	60.8	4.68	>100	3.12	>100	17.2	12.2	18.6	12.1	1.77	14.6	27.7	28.4	84.7
SK-OV-3	34.3	>100	14.2	>100	6.52	>100	19.5	13.8	>100	2.77	20.1	17.2	45.5	65.9	>100
Renal cancer															
786-0	17.7	42.4	2.73	>100	2.89	>100	21.0	19.2	17.3	5.90	1.72	11.2	26.3	24.3	36.5
A498	13.8	>100	13.6	>100	12.7	>100	15.9	19.1	17.9	17.0	9.99	15.3	27.4	18.9	77.6
ACHN	18.8	77.6	3.27	>100	2.21	>100	30.4	21.8	29.0	11.4	2.28	12.2	40.8	33.2	63.1
CAKI-1	16.9	87.3	8.67	>100	6.05	>100	36.6	27.9	31.0	14.3	5.20	15.3	46.8	32.9	>100
RXF 393	13.3	16.6	4.95	>100	2.70	>100	16.9	15.9	18.9	5.79	1.80	10.4	20.4	18.4	42.8
SN12C	18.8	86.7	13.1	>100	7.70	>100	18.2	15.7	22.5	14.5	4.89	14.9	32.8	27.9	>100
TK-10	17.3	68.9	5.36	>100	5.39	>100	20.0	17.2	27.5	11.2	2.10	17.8	39.1	40.8	57.2
UO-31	13.2	51.6	9.15	>100	3.73	>100	21.8	18.0	0.452	5.33	1.58	6.70	0.781	20.5	77.2
Prostate cancer															
PC-3	n.t.	n.t.	6.84	n.t.	10.6	>100	21.2	20.8	39.6	11.9	5.31	13.8	>100	n.t.	n.t.
DU-145	16.3	>100	8.05	>100	5.41	>100	36.7	22.1	28.8	13.8	3.45	15.3	54.7	26.8	>100
Breast cancer															
MCF7	15.7	23.6	12.5	70.5	4.41	87.5	20.4	9.16	19.3	2.88	1.59	2.47	19.2	19.4	40.4
NCI/ADR-RES	26.8	>100	4.37	>100	2.53	>100	3.49	2.57	30.6	2.58	7.83	2.08	28.7	68.6	>100
MDA-MB-231/ATCC	16.5	52.0	10.1	>100	4.55	>100	20.0	16.6	15.1	6.55	1.90	4.73	36.6	22.3	28.5
HS 578T	20.0	73.9	n.t.	>100	n.t.	>100	n.t.	n.t.	24.0	n.t.	n.t.	n.t.	39.3	36.0	86.0
MDA-MB-435	15.0	49.4	6.68	>100	7.14	>100	19.4	15.7	18.2	3.28	1.74	5.09	21.0	21.7	30.7
MDA-N	16.0	76.3	6.73	>100	6.75	>100	15.3	15.2	18.7	3.51	1.73	3.64	41.4	22.1	40.4
BT-549	15.6	77.9	8.04	>100	2.93	>100	19.6	14.2	23.5	7.95	2.49	15.8	24.1	30.5	>100
T-47D	12.8	56.7	15.5	>100	4.12	>100	51.1	19.7	22.1	10.3	3.76	16.5	47.1	21.4	69.8

Table 4. TGI values of pyrrolecarbothioamides

Panel/cell line	Compound													
	3	**9**	**16**	**17**	**18**	**27**	**28**	**30**	**32**	**34**	**35**	**36**	**38**	**39**
Leukemia														
CCRF-CEM	62.1	>100	>100	>100	>100	n.t.	>100	>100	28.9	5.54	n.t.	>100	72.9	>100
HL-60(TB)	54.1	>100	>100	>100	>100	64.0	64.6	35.3	33.5	14.0	38.4	>100	>100	>100
K-562	72.2	>100	24.0	>100	17.8	>100	52.4	76.0	5.11	4.04	>100	>100	>100	>100
MOLT-4	>100	>100	32.6	>100	42.6	>100	48.5	>100	6.38	6.49	>100	>100	>100	>100
RPMI-8226	n.t.	>100	18.0	>100	56.3	37.3	31.3	n.t.	5.00	4.34	6.03	>100	27.9	n.t.
SR	>100	>100	10.7	>100	7.41	>100	39.0	>100	5.40	4.49	>100	>100	76.8	>100
Non small cell lung cancer														
A549/ATCC	30.7	>100	5.29	>100	13.9	>100	53.0	93.9	22.1	4.87	23.3	>100	94.3	>100
EKVX	96.7	>100	19.0	>100	19.5	>100	44.6	>100	26.5	20.1	29.6	>100	>100	>100
HOP-62	42.1	>100	15.3	>100	20.5	72.2	47.3	40.0	29.2	12.9	34.7	>100	>100	>100
HOP-92	35.9	>100	28.0	>100	22.2	n.t.	35.3	59.3	18.9	9.02	n.t.	>100	92.4	>100
NCI-H226	32.4	>100	43.4	>100	19.4	47.3	45.6	40.2	32.3	27.2	31.4	75.0	60.4	>100
NCI-H23	42.8	>100	9.10	>100	11.9	72.6	46.5	>100	27.1	5.95	41.6	>100	>100	>100
NCI-H322M	40.7	>100	27.7	>100	66.2	38.4	51.7	>100	11.4	5.28	18.1	>100	>100	>100
NCI-H460	31.9	>100	15.4	>100	20.8	31.5	27.3	56.5	18.8	3.49	20.7	>100	>100	>100
NCI-H522	32.5	>100	23.6	>100	20.3	29.9	27.1	0.166	4.96	3.38	17.7	>100	31.7	>100
Colon cancer														
COLO 205	>100	48.1	2.84	>100	16.5	41.5	29.9	>100	18.0	26.2	3.79	>100	>100	97.5
HCC-2998	56.5	>100	5.31	>100	7.70	37.4	29.7	40.6	2.65	1.98	3.48	>100	75.5	46.2
HCT-116	35.4	>100	11.0	>100	13.4	50.6	30.0	34.1	15.2	3.31	12.6	>100	64.4	>100
HCT-15	46.1	>100	28.5	>100	16.9	55.9	34.1	39.9	4.33	3.36	6.27	>100	>100	>100
HT29	>100	>100	16.8	>100	18.0	39.4	28.6	>100	10.5	3.70	11.0	53.2	>100	91.3
KM12	34.8	>100	18.8	>100	31.5	39.2	32.4	36.9	6.34	3.24	10.5	>100	>100	>100
SW-620	96.9	>100	37.7	>100	51.9	71.0	69.6	>100	5.19	3.63	10.1	>100	>100	>100
CNS cancer														
SF-268	30.2	>100	23.3	>100	17.8	68.2	59.8	55.3	28.8	8.44	31.5	86.8	65.9	>100
SF-295	39.7	>100	5.28	>100	5.87	49.5	36.8	66.0	17.2	3.32	15.0	>100	>100	>100
SF-539	>100	>100	17.7	>100	18.4	35.8	26.6	32.1	5.63	13.8	6.47	65.4	>100	>100
SNB-19	42.9	>100	21.9	>100	21.8	56.0	64.1	>100	29.1	15.1	37.7	>100	91.5	>100
SNB-75	50.3	>100	7.14	>100	6.60	50.9	39.7	>100	16.6	3.33	24.1	>100	>100	>100
U251	29.9	>100	9.02	>100	9.73	36.4	33.2	32.7	15.6	3.13	15.7	>100	n.t.	>100
Melanoma														
LOX IMVI	33.3	>100	14.3	>100	16.6	42.2	28.3	32.8	3.97	3.19	4.53	63.4	37.8	80.4
MALME-3M	31.9	>100	19.8	>100	36.1	30.8	29.6	38.6	n.t.	n.t.	11.7	83.9	89.2	43.8
M14	36.4	69.3	35.8	>100	34.1	42.5	31.9	33.7	3.97	3.54	12.6	36.8	39.7	48.5
SK-MEL-2	31.9	>100	31.7	>100	25.8	24.2	21.5	1.07	22.8	3.58	25.9	>100	33.0	52.1
SK-MEL-28	45.3	>100	14.5	87.0	14.4	34.0	35.9	52.0	9.97	3.22	18.9	68.3	87.7	35.1
SK-MEL-5	31.0	>100	n.t.	>100	n.t.	n.t.	n.t.	34.9	n.t.	n.t.	n.t.	52.3	42.2	74.1
UACC-257	36.5	>100	43.7	>100	31.8	32.6	32.7	49.2	18.4	3.26	30.6	>100	65.8	46.7
UACC-62	31.7	95.7	28.1	>100	27.1	26.7	25.3	32.5	25.2	21.0	29.2	41.6	53.8	35.2
Ovarian cancer														
IGROV1	35.7	>100	15.0	>100	17.3	42.0	30.9	14.1	19.9	6.43	26.5	>100	>100	>100
OVCAR-3	35.5	>100	56.8	>100	12.9	>100	93.0	97.9	25.2	20.2	38.5	>100	>100	>100
OVCAR-4	54.9	>100	4.32	93.6	5.14	36.5	29.4	79.9	12.5	4.15	15.8	74.5	>100	>100
OVCAR-5	>100	>100	40.9	>100	27.3	74.1	55.3	55.1	27.0	9.17	28.3	>100	>100	>100
OVCAR-8	39.4	>100	18.1	>100	11.0	52.4	34.5	39.2	26.1	3.37	30.2	>100	94.9	>100
SK-OV-3	>100	>100	27.3	>100	19.3	47.0	35.0	>100	8.04	49.7	32.2	>100	>100	>100
Renal cancer														
786-0	34.3	>100	6.40	>100	9.42	49.7	34.8	32.5	18.7	3.14	27.9	75.6	61.8	>100
A498	28.8	>100	27.9	>100	28.9	33.1	36.9	53.7	31.6	23.0	31.5	>100	82.8	>100
ACHN	36.2	>100	10.7	>100	5.08	97.4	55.8	85.2	23.5	4.71	28.1	>100	>100	>100
CAKI-1	35.6	>100	30.6	>100	23.2	>100	>100	98.2	27.5	18.6	32.2	>100	>100	>100
RXF 393	27.9	77.7	17.4	>100	7.45	38.4	32.5	35.6	20.7	4.16	27.3	65.3	50.3	62.9
SN12C	35.0	>100	27.4	>100	22.5	39.2	31.0	50.8	28.8	17.0	29.2	>100	75.1	>100
TK-10	35.5	>100	16.8	>100	16.5	61.5	69.2	63.8	23.3	4.25	33.4	>100	>100	>100
UO-31	28.4	>100	23.0	>100	15.1	57.4	37.2	6.34	18.2	3.02	21.6	>100	77.7	>100
Prostate cancer														
PC-3	n.t.	n.t.	23.3	n.t.	41.2	>100	>100	>100	26.1	18.4	30.5	>100	n.t.	>100
DU-145	34.2	>100	24.8	>100	24.1	>100	63.7	>100	27.0	13.1	32.4	>100	>100	>100
Breast cancer														
MCF7	30.6	68.6	33.5	>100	21.6	75.2	31.5	37.2	13.2	3.08	12.3	45.4	42.2	>100
NCI/ADR-RES	89.4	>100	17.1	>100	13.3	24.3	11.1	>100	8.51	24.2	4.72	>100	>100	>100
MDA-MB-231/ATCC	35.7	>100	24.2	>100	18.1	44.5	31.6	34.1	19.6	3.91	17.6	>100	66.3	>100
HS 578T	52.3	>100	n.t.	>100	n.t.	n.t.	n.t.	58.9	n.t.	n.t.	n.t.	>100	>100	>100
MDA-MB-435	30.9	>100	19.7	>100	20.0	59.6	35.3	33.9	10.2	3.14	18.3	55.9	49.0	99.1
MDA-N	37.2	>100	20.6	>100	21.5	56.2	47.9	39.1	12.3	3.32	14.5	>100	61.3	>100
BT-549	31.6	>100	20.5	>100	8.02	42.5	30.5	64.7	20.3	7.06	30.5	68.4	86.4	>100
T-47D	37.2	>100	>100	>100	>100	>100	>100	63.3	33.5	65.1	81.2	>100	82.6	>100

Table 5. LC_{50} values of pyrrolecarbothioamides

Panel/cell line	Compound											
	3	**16**	**18**	**27**	**28**	**30**	**32**	**34**	**35**	**36**	**38**	**39**
Leukemia												
CCRF-CEM	>100	>100	>100	n.t.	>100	>100	>100	21.7	n.t.	>100	>100	>100
HL-60(TB)	>100	>100	>100	>100	>100	>100	69.1	50.5	77.5	>100	>100	>100
K-562	>100	71.9	98.0	>100	>100	>100	33.7	7.82	>100	>100	>100	>100
MOLT-4	>100	87.5	>100	>100	>100	>100	31.0	31.1	>100	>100	>100	>100
RPMI-8226	n.t.	46.2	>100	>100	89.3	n.t.	25.0	12.5	38.2	>100	>100	n.t.
SR	>100	44.2	62.3	>100	>100	>100	25.0	9.45	>100	>100	>100	>100
Non small cell lung cancer												
A549/ATCC	63.3	>100	>100	>100	>100	>100	50.4	14.7	94.9	>100	>100	>100
EKVX	>100	51.7	59.4	>100	>100	>100	52.0	46.4	60.0	>100	>100	>100
HOP-62	85.2	51.6	63.1	>100	>100	80.4	55.0	42.8	69.6	>100	>100	>100
HOP-92	99.0	60.8	66.3	>100	72.4	>100	45.8	31.3	n.t.	>100	>100	>100
NCI-H226	73.0	>100	92.3	>100	>100	89.0	90.4	82.5	87.4	>100	>100	>100
NCI-H23	>100	33.3	41.5	>100	>100	>100	54.9	22.1	95.0	>100	>100	>100
NCI-H322M	91.5	87.3	>100	>100	>100	>100	33.9	17.8	46.4	>100	>100	>100
NCI-H460	64.5	45.3	53.3	>100	>100	>100	47.7	6.89	63.2	>100	>100	>100
NCI-H522	67.9	56.5	64.6	64.8	57.3	0.596	13.3	6.31	49.0	>100	60.4	>100
Colon cancer												
COLO 205	>100	5.59	50.0	88.8	56.0	>100	47.1	59.3	8.08	>100	>100	>100
HCC-2998	>100	16.6	43.6	81.7	59.9	80.4	5.41	4.60	6.94	>100	>100	97.6
HCT-116	75.0	34.5	43.0	>100	63.3	63.1	39.0	5.98	65.2	>100	>100	>100
HCT-15	>100	75.9	70.0	>100	72.4	83.3	8.78	6.28	36.4	>100	>100	>100
HT29	>100	51.9	53.8	88.5	54.8	>100	33.2	7.07	52.7	>100	>100	>100
KM12	76.1	52.1	>100	>100	>100	76.2	24.1	6.29	50.1	>100	>100	>100
SW-620	>100	>100	>100	>100	>100	>100	16.3	7.01	78.4	>100	>100	>100
CNS cancer												
SF-268	62.8	79.6	76.3	>100	>100	>100	64.0	38.0	70.5	>100	>100	>100
SF-295	88.2	18.1	26.9	>100	93.6	>100	42.9	6.02	49.2	>100	>100	>100
SF-539	>100	43.5	46.2	70.0	55.7	>100	14.0	37.4	27.9	>100	>100	>100
SNB-19	>100	72.2	>100	>100	>100	>100	57.7	40.7	76.9	>100	>100	>100
SNB-75	>100	27.0	21.3	>100	87.2	>100	41.6	6.45	58.0	>100	>100	>100
U251	62.3	52.3	69.9	86.7	73.2	61.9	41.7	5.68	55.2	>100	>100	>100
Melanoma												
LOX IMVI	67.5	47.2	78.9	95.0	54.1	61.9	7.84	5.65	9.29	>100	74.6	>100
MALME-3M	59.7	74.5	>100	65.1	63.3	75.8	n.t.	n.t.	45.2	>100	>100	99.3
M14	77.1	99.6	97.4	88.1	62.0	60.0	8.05	6.37	51.1	69.9	79.5	>100
SK-MEL-2	65.9	69.4	56.8	57.9	49.5	5.50	48.4	6.69	54.2	>100	60.9	>100
SK-MEL-28	>100	41.9	42.1	67.9	69.1	>100	32.3	5.88	48.4	>100	>100	70.9
SK-MEL-5	61.8	n.t.	n.t.	n.t.	n.t.	71.1	n.t.	n.t.	n.t.	>100	97.0	>100
UACC-257	72.3	>100	90.3	67.1	68.2	>100	44.4	6.36	67.6	>100	>100	95.5
UACC-62	61.9	54.8	55.0	55.4	60.3	63.4	54.1	47.3	59.6	96.6	>100	73.4
Ovarian cancer												
IGROV1	75.9	41.0	53.8	>100	99.7	>100	45.0	23.9	59.6	>100	>100	>100
OVCAR-3	83.1	>100	77.6	>100	>100	>100	53.9	47.0	84.5	>100	>100	>100
OVCAR-4	>100	9.45	22.9	>100	>100	>100	35.9	10.9	45.1	>100	>100	>100
OVCAR-5	>100	>100	63.1	>100	>100	>100	52.1	30.6	58.9	>100	>100	>100
OVCAR-8	85.9	56.5	53.9	>100	97.6	82.7	56.5	6.41	62.2	>100	>100	>100
SK-OV-3	>100	52.6	44.6	>100	88.8	>100	28.3	>100	60.3	>100	>100	>100
Renal cancer												
786-0	66.5	24.6	32.4	>100	63.2	60.8	44.6	5.74	69.4	>100	>100	>100
A498	60.0	57.3	65.6	68.8	71.3	>100	58.6	52.7	65.2	>100	>100	>100
ACHN	69.7	33.0	14.6	>100	>100	>100	48.5	9.74	64.6	>100	>100	>100
CAKI-1	74.9	>100	72.0	>100	>100	>100	52.9	47.6	67.9	>100	>100	>100
RXF 393	58.3	49.9	33.2	87.3	66.3	66.9	58.7	9.63	71.6	>100	>100	>100
SN12C	65.1	57.2	56.7	84.6	61.1	>100	57.3	41.7	57.3	>100	>100	>100
TK-10	72.9	42.1	43.1	>100	>100	>100	48.5	8.61	62.7	>100	>100	>100
UO-31	61.0	54.9	45.5	>100	76.6	>100	42.7	5.75	55.0	>100	>100	>100
Prostate cancer												
PC-3	n.t.	67.4	>100	>100	>100	>100	57.2	47.5	67.6	>100	n.t.	>100
DU-145	72.1	67.8	88.1	>100	>100	>100	52.8	36.5	68.6	>100	>100	>100
Breast cancer												
MCF7	59.7	89.8	>100	>100	>100	71.8	40.4	5.98	84.8	>100	91.5	>100
NCI/ADR-RES	>100	48.1	67.7	>100	>100	>100	46.1	65.7	13.7	>100	>100	>100
MDA-MB-231/ATCC	77.3	58.0	55.0	99.1	60.2	77.1	48.7	8.08	52.2	>100	>100	>100
HS 578T	>100	n.t.	n.t.	n.t.	n.t.	>100	n.t.	n.t.	n.t.	>100	>100	>100
MDA-MB-435	63.6	46.9	46.3	>100	79.2	63.3	32.3	5.67	51.1	>100	>100	>100
MDA-N	86.9	50.8	54.5	>100	>100	81.7	39.1	6.38	57.5	>100	>100	>100
BT-549	64.1	46.5	29.0	91.9	65.7	>100	45.1	25.2	58.5	>100	>100	>100
T-47D	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

mmol) in anhydrous acetonitrile (20 mL). The resulting solution was kept at room temperature for 4 days and then phenyl isothiocyanate (1.35 g, 10 mmol) was added. The resulting mixture was stirred at room temperature for 5 h and the formed precipitate was separated by filtration and recrystallized from a suitable solvent.

3-Amino-3-[(4-chlorophenyl)amino]-2-[(phenylamino) thioxamethyl]-2-propenenitrile 2f. Yield 80%. Mp 203–204 °C (1-PrOH). ^{1}H NMR (DMSO-d_6) δ 7.11, 7.27, 7.43 (m, 9H, aryl), 8.56 (br s, 2H, NH_2), 9.64 (s, 1H, NH), 10.85 (s, 1H, NH). IR (Nujol) 3420, 3340, 3300, 3200, 2180, 1610, 1590, 1570 cm^{-1}. Anal. calcd for $C_{16}H_{13}ClN_4S$: C, 58.44; H, 3.98; N, 17.04. Found: C, 58.50; H, 3.96; N, 17.00.

3-Amino-3-[(5-methylpyridin-2-yl)amino]-2-[(phenylamino) thioxamethyl]-2-propenenitrile 2g. Yield 60%. Mp 171–173 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 2.19 (s, 3H, CH_3), 7.16, 7.32, 7.62, 8.09 (m, 10H, aryl, pyridyl and NH_2), 10.76 (s, 1H, NH), 13.71 (s, 1H, NH). IR (Nujol) 3390, 3190, 3120, 3030, 2180, 1605 cm^{-1}. Anal. calcd for $C_{16}H_{15}N_5S$: C, 62.11; H, 4.89; N, 22.64. Found: C, 62.16; H, 4.90; N, 22.67.

3-Amino-3-[(4-methylpyridin-2-yl)amino]-2-[(phenylamino) thioxamethyl]-2-propenenitrile 2 h. Yield 78%. Mp 153–154 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 2.26 (s, 3H, CH_3), 6.91, 7.03, 7.17, 7.34, 7.65, 8.14 (m, 10H, aryl, pyridyl and NH_2), 10.76 (s, 1H, NH), 13.90 (s, 1H, NH). IR (Nujol) 3390, 3190, 3120, 3030, 2180, 1605 cm^{-1}. Anal. calcd for $C_{16}H_{15}N_5S$: C, 62.11; H, 4.89; N, 22.64. Found: C, 62.06; H, 4.91; N, 22.60.

General procedure for the synthesis of pyrrole-carbothiomides 3–39

A solution of propenethioamides (2.5 mmol), the appropriate bromoketone (2.5 mmol) and triethylamine (0.35 mL, 2.5 mmol) in anhydrous chloroform (50 mL) was stirred at room temperature for 24 h. After removal of the solvent, the residue was washed with water, filtered and recrystallized from the appropriate solvent to give target pyrroles.

***N*-Phenyl-4-amino-2-(4-methylphenylamino)-5-(4-methylbenzoyl)-pyrrole-3-carbothioamide 5.** Yield 98%. Mp 206–208 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 2.26 (s, 3H, CH_3), 3.74 (s, 3H, CH_3), 6.80–7.53 (m, 15H, aryl and NH_2), 8.12 (s, 2H, NH), 11.18 (brs, 1H, NH). IR (Nujol) 3470, 3410, 3310, 3200, 1640, 1595 cm^{-1}. Anal. calcd for $C_{26}H_{24}N_4OS$: C, 70.88; H, 5.49; N, 12.72. Found: C, 70.85; H, 5.47; N, 12.75.

***N*-Phenyl-4-amino-2-(4-methylphenylamino)-5-(4-methoxybenzoyl)-pyrrole-3-carbothioamide 6.** Yield 91%. Mp 195–197 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 2.24 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 6.89-7.51 (m, 15H, aryl and NH_2), 8.10 (s, 2H, NH) 10.90 (s, 1H, NH). IR (Nujol) 3420, 3370, 3250, 3200, 3120, 1620, 1560 cm^{-1}. Anal. calcd for $C_{26}H_{24}N_4O_2S$: C, 68.40; H, 5.30; N, 12.27. Found: C, 68.46; H, 5.29; N, 12.31.

***N*-Phenyl-4-amino-2-(4-methylphenylamino)-5-(4-chlorobenzoyl)-pyrrole-3-carbothioamide 7.** Yield 92%. Mp 214-215 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 2.25 (s, 3H, CH_3), 6.79–7.97 (m, 15H, aryl and NH_2), 8.24 (s, 2H, NH), 10.90 (s, 1H, NH) . IR (Nujol) 3430, 3350, 3240, 3130, 1580, 1560 cm^{-1}. Anal. calcd for $C_{25}H_{21}ClN_4OS$: C, 65.14; H, 4.59; N, 12.15. Found: C, 65.08; H, 4.60; N, 12.19.

***N*-Phenyl-4-amino-2-(4-methylphenylamino)-5-(3,4-dichlorobenzoyl)-pyrrole-3-carbothioamide 8.** Yield 95%. Mp 228–230 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 2.25 (s, 3H, CH_3), 6.95–7.63 (m, 14H, aryl and NH_2), 8.32 (s, 2H, NH), 10.91 (s, 1H, NH) . IR (Nujol) 3420, 3300, 3270, 3180, 1635, 1600 cm^{-1}. Anal. calcd for $C_{25}H_{20}Cl_2N_4OS$: C, 60.61; H, 4.07; N, 11.31. Found: C, 60.55; H, 4.08; N, 11.35.

***N*-Phenyl-4-amino-2-(4-methoxyphenylamino)-5-(4-methylbenzoyl)-pyrrole-3-carbothioamide 11.** Yield 88%. Mp 196–198 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 2.28 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 6.96–7.41 (m, 15H, aryl and NH_2), 8.17 (s, 2H, NH) 10.97 (s, 1H, NH). IR (Nujol) 3450, 3390, 3260, 3120, 1620, 1585 cm^{-1}. Anal. calcd for $C_{26}H_{24}N_4O_2S$: C, 68.40; H, 5.30; N, 12.27. Found: C, 68.46; H, 5.28; N, 12.22.

***N*-Phenyl-4-amino-2-(4-methoxyphenylamino)-5-(4-methoxybenzoyl)-pyrrole-3-carbothioamide 12.** Yield 98%. Mp 196–198 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 3.71 (s, 3H, CH_3), 3.73 (s, 3H, CH_3), 6.76–7.92 (m, 15H, aryl and NH_2), 8.15 (s, 2H, NH), 11.01 (s, 1H, NH). IR (Nujol) 3450, 3380, 3250, 3120, 1625, 1585 cm^{-1}. Anal. calcd for $C_{26}H_{24}N_4O_3S$: C, 66.08; H, 5.12; N, 11.86. Found: C, 66.13; H, 5.10; N, 11.81.

***N*-Phenyl-4-amino-2-(4-methoxyphenylamino)-5-(4-chlorobenzoyl)-pyrrole-3-carbothioamide 13.** Yield 98%. Mp 219–220 °C (MeCN). ^{1}H NMR (DMSO-d_6) d 3.71 (s, 3H, CH_3), 6.92-7.50 (m, 15H, aryl and NH_2), 8.27 (s, 2H, NH). IR (Nujol) 3440, 3370, 3230, 3110, 1615, 1585 cm^{-1}. Anal. calcd for $C_{25}H_{21}ClN_4O_2S$: C, 62.95; H, 4.44; N, 11.75. Found: C, 62.89; H, 4.46; N, 11.80.

***N*-Phenyl-4-amino-2-(4-methoxyphenylamino)-5-(3,4-dichlorobenzoyl)-pyrrole-3-carbothioamide 14.** Yield 98%. Mp 214–215 °C (1-PrOH). ^{1}H NMR (DMSO-d_6) δ 3.71 (s, 3H, CH_3), 6.93-7.61 (m, 14H, aryl and NH_2), 8.33 (s, 2H, NH), 10.93 (s, 1H, NH). IR (Nujol) 3410, 3380, 3240, 3140, 1625, 1590 cm^{-1}. Anal. calcd for $C_{25}H_{20}Cl_2N_4O_2S$: C, 58.71; H, 3.94; N, 10.96. Found: C, 58.66; H, 3.96; N, 10.92.

***N*-Phenyl-4-amino-2-(4-chlorophenylamino)-5-benzoyl-pyrrole-3-carbothioamide 15.** Yield 98%. Mp 174–175 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 6.46–7.54 (m, 16H, aryl and NH_2), 8.13 (s, 2H, NH), 10.69 (s, 1H, NH). IR (Nujol) 3450, 3400, 3240, 1610, 1580 cm^{-1}. Anal. calcd for $C_{24}H_{19}ClN_4OS$: C, 64.49; H, 4.28; N, 12.54. Found: C, 64.43; H, 4.25; N, 12.57.

***N*-Phenyl-4-amino-2-(4-chlorophenylamino)-5-(4-methylbenzoyl)-pyrrole-3-carbothioamide 16.** Yield 95%. Mp 190–192 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H, CH_3), 6.87–7.45 (m, 15H, aryl and NH_2), 8.09 (s, 2H, NH) 10.67 (s, 1H, NH). IR (Nujol) 3460, 3410, 3360, 3330, 1600 cm^{-1}. Anal. calcd for $C_{25}H_{21}ClN_4OS$: C, 65.14; H, 4.59; N, 12.15. Found: C, 65.20; H, 4.60; N, 12.10.

***N*-Phenyl-4-amino-2-(4-chlorophenylamino)-5-(4-methoxybenzoyl)-pyrrole-3-carbothioamide 17.** Yield 98%. Mp 222–224 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 3H, CH_3), 6.91–7.54 (m, 15H, aryl and NH_2), 8.04 (s, 2H, NH), 10.61 (s, 1H, NH). IR (Nujol) 3450, 3390, 3290, 3220, 3200, 1630, 1595 cm^{-1}. Anal. calcd for $C_{25}H_{21}ClN_4O_2S$: C, 62.95; H, 4.44; N, 11.75. Found: C, 63.00; H, 4.45; N, 11.71.

***N*-Phenyl-4-amino-2-(4-chlorophenylamino)-5-(4-chlorobenzoyl)-pyrrole-3-carbothioamide 18.** Yield 98%. Mp 234–235 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 6.99–7.55 (m, 15H, aryl and NH_2), 8.17 (s, 2H, NH). IR (Nujol) 3460, 3390, 3240, 3120, 3050, 1630, 1580 cm^{-1}. Anal. calcd for $C_{24}H_{18}Cl_2N_4OS$: C, 59.88; H, 3.77; N, 11.64. Found: C, 59.82; H, 3.79; N, 11.70.

***N*-Phenyl-4-amino-2-(4-chlorophenylamino)-5-(3,4-dichlorobenzoyl)-pyrrole-3-carbothioamide 19.** Yield 98%. Mp 225–227 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 7.14–7.69 (m, 14H, aryl and NH_2), 8.27 (s, 2H, NH_2), 10.68 (s, 1H, NH). IR (Nujol) 3410, 3240, 3150, 1630, 1590 cm^{-1}. Anal. calcd for $C_{24}H_{17}Cl_3N_4OS$: C, 55.88; H, 3.32; N, 10.86. Found: C, 55.93; H, 3.30; N, 10.92.

***N*-Phenyl-4-amino-2-[(5-methylpyridin-2-yl)amino]-5-benzoyl-pyrrole-3-carbothioamide 20.** Yield 94%. Mp 153–154 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 2.72 (s, 3H, CH_3), 6.87–7.73 (m, 15H, aryl and NH_2), 8.38 (s, 2H, NH), 10.03 (s, 1H, NH), 13.82 (s, 1H, NH). IR (Nujol) 3350, 3060, 1625, 1590 cm^{-1}. Anal. calcd for $C_{24}H_{21}N_5OS$: C, 67.42; H, 4.95; N, 16.38. Found: C, 67.48; H, 4.97; N, 16.35.

***N*-Phenyl-4-amino-2-[(5-methylpyridin-2-yl)amino]-5-(4-methylbenzoyl)-pyrrole-3-carbothioamide 21.** Yield 91%. Mp 159–160 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 2.16 (s, 3H, CH_3), 2.18 (s, 3H, CH_3), 6.60-7.48 (m, 14H, aryl and NH_2), 8.11 (s, 2H, NH). IR (Nujol) 3400, 1615, 1580 cm^{-1}. Anal. calcd for $C_{25}H_{23}N_5OS$: C, 68.00; H, 5.25; N, 15.86. Found: C, 68.08; H, 5.22; N, 15.80.

***N*-Phenyl-4-Amino-2-[(5-methylpyridin-2-yl)amino]-5-(4-methoxybenzoyl)-pyrrole-3-carbothioamide 22.** Yield 95%. Mp 148–150 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 2.17 (s, 3H, CH_3), 3.62 (s, 3H, CH_3), 6.59–7.40 (m, 14H, aryl and NH_2), 8.10 (s, 2H, NH). IR (Nujol) 3120, 3040, 1610, 1580 cm^{-1}. Anal. calcd for $C_{25}H_{23}N_5O_2S$: C, 65.63; H, 5.07; N, 15.31. Found: C, 65.68; H, 5.05; N, 15.30.

***N*-Phenyl-4-amino-2-[(5-methylpyridin-2-yl)amino]-5-(4-chlorobenzoyl)-pyrrole-3-carbothioamide 23.** Yield 98%. Mp 152–154 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 2.18 (s, 3H, CH_3), 6.60–7.51 (m, 14H, aryl and NH_2), 8.10 (s, 2H, NH). IR (Nujol) 3150, 1625, 1600 cm^{-1}. Anal. calcd for $C_{24}H_{20}ClN_5OS$: C, 62.40; H, 4.36; N, 15.16. Found: C, 62.34; H, 4.35; N, 15.20.

***N*-Phenyl-4-amino-2-[(5-methylpyridin-2-yl)amino]-5-(3,4-dichlorobenzoyl)-pyrrole-3-carbothioamide 24.** Yield 98%. Mp 174–175 °C (MeCN). ^{1}H NMR (DMSO–d_6) δ 2.18 (s, 3H, CH_3), 6.54–7.72 (m, 13H, aryl and NH_2), 8.12 (s, 2H, NH). IR (Nujol) 3140, 1620, 1600, 1580 cm^{-1}. Anal. calcd for $C_{24}H_{19}Cl_2N_5OS$: C, 58.07; H, 3.86; N, 14.11. Found: C, 58.01; H, 3.85; N, 14.15.

***N*-Phenyl-4-amino-2-[(4-methylpyridin-2-yl)amino]-5-benzoyl-pyrrole-3-carbothioamide 25.** Yield 94%. Mp 148–150 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 2.17 (s, 3H, CH_3), 6.54–7.48 (m, 15H, aryl and NH_2), 8.13 (s, 2H, NH). IR (Nujol) 3030, 1620, 1595 cm^{-1}. Anal. calcd for $C_{24}H_{21}N_5OS$: C, 67.42; H, 4.95; N, 16.38. Found: C, 67.36; H, 4.94; N, 16.41.

***N*-Phenyl-4-Amino-2-[(4-methylpyridin-2-yl)amino]-5-(4-methylbenzoyl)-pyrrole-3-carbothioamide 26.** Yield 91%. Mp 119–120 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 2.16 (s, 6H, CH_3), 6.53–7.36 (m, 14H, aryl and NH_2), 8.12 (s, 2H, NH). IR (Nujol) 3210, 1600, 1580 cm^{-1}. Anal. calcd for $C_{25}H_{23}N_5OS$: C, 68.00; H, 5.25; N, 15.86. Found: C, 67.94; H, 5.24; N, 15.90.

***N*-Phenyl-4-amino-2-[(4-methylpyridin-2-yl)amino]-5-(4-methoxybenzoyl)-pyrrole-3-carbothioamide 27.** Yield 93%. Mp 140–142 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 2.16 (s, 3H, CH_3), 3.41 (s, 3H, CH_3), 6.53–7.36 (m, 14H, aryl and NH_2), 8.13 (s, 2H, NH). IR (Nujol) 3150, 3040, 1625, 1590 cm^{-1}. Anal. calcd for $C_{25}H_{23}N_5O_2S$: C, 65.63; H, 5.07; N, 15.31. Found: C, 65.58; H, 5.08; N, 15.26.

***N*-Phenyl-4-amino-2-[(4-methylpyridin-2-yl)amino]-5-(4-chlorobenzoyl)-pyrrole-3-carbothioamide 28.** Yield 94%. Mp 138–140 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 2.17 (s, 3H, CH_3), 6.54–7.51 (m, 14H, aryl and NH_2), 8.13 (s, 2H, NH). IR (Nujol) 3150, 1595, 1580 cm^{-1}. Anal. calcd for $C_{24}H_{20}ClN_5OS$: C, 62.40; H, 4.36; N, 15.16. Found: C, 62.44; H, 4.34; N, 15.24.

***N*-Phenyl-4-amino-2-[(4-methylpyridin-2-yl)amino]-5-(3,4-dichlorobenzoyl)-pyrrole-3-carbothioamide 29.** Yield 98%. Mp 130–132 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 2.18 (s, 3H, CH_3), 6.55–7.68 (m, 13H, aryl and NH_2), 8.21 (s, 2H, NH). IR (Nujol) 3060, 1630, 1605, 1590 cm^{-1}. Anal. calcd for $C_{24}H_{19}Cl_2N_5OS$: C, 58.07; H, 3.86; N, 14.11. Found: C, 58.12; H, 3.87; N, 14.06.

***N*-Phenyl-4-amino-2-benzylamino-5-(4-methylbenzoyl)-pyrrole-3-carbothioamide 32.** Yield 93%. Mp 192–193 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 2.23 (s, 3H, CH_3), 4.56 (s, 2H, CH_2), 6.84–7.35 (m, 16H, aryl and NH_2), 7.77 (s, 2H, NH), 8.08 (s, 1H, NH), 11.24 (s, 1H, NH) . IR (Nujol) 3420, 3330, 3150, 1600 cm^{-1}. Anal. calcd for $C_{26}H_{24}N_4OS$: C, 70.88; H, 5.49; N, 12.72. Found: C, 70.93; H, 5.50; N, 12.69.

***N*-Phenyl-4-amino-2-benzylamino-5-(4-methoxylbenzoyl)-pyrrole-3-carbothioamide 33.** Yield 76%. Mp 171–172 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 3.68 (s, 3H, CH_3), 4.55 (s, 2H, CH_2), 6.86-7.41 (m, 16H, aryl and NH_2), 7.92 (s, 1H, NH), 8.05 (s, 1H, NH), 11.23 (s, 1H, NH) . IR (Nujol) 3410, 3310, 3150, 1600 cm^{-1}. Anal. calcd for $C_{26}H_{24}N_4O_2S$: C, 68.40; H, 5.30; N, 12.27. Found: C, 68.34; H, 5.32; N, 12.23.

***N*-Phenyl-4-amino-2-benzylamino-5-(4-chlorobenzoyl)-pyrrole-3-carbothioamide 34.** Yield 85%. Mp 155–156 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 4.56 (s, 2H, CH_2), 6.82–7.42 (m, 16H, aryl and NH_2), 7.95 (s, 1H, NH), 8.17 (s, 1H, NH), 11.18 (s, 1H, NH) . IR (Nujol) 3420, 3360, 3230, 1660, 1620 cm^{-1}. Anal. calcd for $C_{25}H_{21}ClN_4OS$: C, 65.14; H, 4.59; N, 12.15. Found: C, 65.20; H, 4.61; N, 12.09.

***N*-Phenyl-4-amino-2-benzylamino-5-(3,4-dichlorobenzoyl)-pyrrole-3-carbothioamide 35.** Yield 77%. Mp 174–175 °C (MeCN). ^{1}H NMR (DMSO-d_6) δ 4.56 (s, 2H, CH_2), 7.15–7.57 (m, 15H, aryl and NH_2), 7.98 (s, 1H, NH), 8.22 (s, 1H, NH), 11.20 (s, 1H, NH). IR (Nujol) 3440, 3420, 3310, 3250, 3180, 1640, 1610, 1590 cm^{-1}. Anal. calcd for $C_{25}H_{20}Cl_2N_4OS$: C, 60.61; H, 4.07; N, 11.31. Found: C, 60.66; H, 4.06; N, 11.27.

Primary anticancer assay

The compounds were tested by NCI in an in vitro three-cell line, one dose primary anticancer assay as a primary cancer screen. The three-cell line panel consists of the MCF7 (breast), NCI-H460 (lung) and SF-268 (CNS). Each cell line is inoculated and preincubated on a microtriter plate. Test agents are then added at single 10^{-4} M concentration and the culture incubated for 48 h. End-point determinations are made with alamar blue.[24] Results for each test agent are reported as the percent of growth of the treated cells when compared to untreated control cells. Compounds, which reduce the growth of any one of the cell lines to approximately 32% or less, are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range.

Determination of GI_{50}, TGI and LC_{50} values

A total of 60 human tumor cell lines, derived from nine cancer types (leukemia, lung, colon, brain, melanoma, ovarian, renal, prostate and breast) formed the basis of this test. The tumor cells were cultured in RPMI1640 medium supplemented with 5% foetal calf serum and 2 mM L-glutamine. The tumor cells are inoculated over a series of standard 96-well microtrite plates in 100 μL of medium.[20,21] Density of inoculum depends on the type of tumor cell and from its growth characteristics.[25] These cells are then preincubated on the microtrite plate for 24 h before adding the compounds. These were tested in DMSO solution at five different concentration (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M). After an incubation of the chemical agent for 48 h with the tumor cell lines a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. The cytotoxic effects are evaluated and the assay results and dose–response parameters were calculated as previously described.[23]

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