Synthesis and biological evaluation of new 5-methyl-*N*-(3-oxo-1-thia-4-azaspiro[4.5]-dec-4-yl)-3-phenyl-1*H*-indole-2-carboxamide derivatives

Özlen Güzel*, Nalan Terzioğlu, Gültaze Çapan, and Aydın Salman

Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry 34116 Beyazıt, Istanbul, Turkey
E-mail: ozlen_guzel@yahoo.com

Abstract

A series of new 5-methyl-N-(3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-3-phenyl-1*H*-indole-2-carboxamide derivatives (**2** and **3**) was synthesized and characterized by IR, ^{1}H-NMR, HSQC, HMBC, ^{13}C-NMR (APT), APCI mass spectral data and elemental analysis. All synthesized compounds were evaluated for *in vitro* antituberculosis activity against *M.tuberculosis* H37Rv. Compound **3c** was found to provide the highest (90%) inhibition of mycobacterial growth in the primary screen conducted at 6.25 μg/mL.

Compounds **2a**, **2b**, **3a**, **3b** and **3c** chosen as prototypes were evaluated against the full panel of 55 human tumour cell lines in the National Cancer Institute's *in vitro* primary cytotoxicity assay. **2b** showed the most favourable cytotoxic effects on ovarium cancer cell line IGROV1 ($\log_{10}$ GI_{50} value –7.31) and renal cancer cell line UO-31 ($\log_{10}$ GI_{50} value –7.56), whereas **3a** showed favorable cytotoxicity on renal cancer cell line RXF-393 ($\log_{10}$ GI_{50} value –6.38).

Keywords: Indole, spirothiazolidinones, antituberculosis activity, anticancer activity

Introduction

Tuberculosis (TB), an infection of *Mycobacterium tuberculosis*, still remains the leading cause of worldwide death among infectious diseases. One-third of the population is infected with *M. Tuberculosis* and the World Health Organization (WHO) estimates that within the next 20 years about 30 million people will be infected with the bacillus[1]. Active disease following new infection, as well as reactivation of latent tuberculosis, is particularly prevalent in individuals with compromised immune systems, such as those that are HIV positive. Duration of the treatment of cases is very prolonged especially when caused by resistant bacteria.

Although advances in cancer detection and treatment over the past 10 years have led an increase in the expected survival period, the majority of patients diagnosed with the disease relapse after cytoreductive surgery and standard adjuvant chemotherapy. While much research is being done to establish active salvage regimens, successful treatment depends upon effective adjuvant therapy. Intrinsic or acquired resistance of cancer cells to chemotherapeutics in several malignant tumors decrease the response rate and new therapeutic agents are needed.

Indole derivatives have been shown to possess antibacterial, antifungal and anti HIV activities[2-7]. There have also been several reports on the anticancer properties of compounds bearing this ring system[8,9]. A very recent report deals with the apoptosis inducing effect of 5-methyl-3-phenyl-indole-2-carboxylic acid benzylidene hydrazides[9].

On the other hand, 4-thiazolidinones and their spiroheterocyclic analogs have been reported to exhibit antibacterial, antifungal and antimycobacterial activity[10-17]. Furthermore 2-aryl-4-oxothiazolidin-3-yl amides have been reported to demonstrate antiproliferative activity in prostate cancer[18].

As a consequence of this research and with the aim of obtaining new and more potent antituberculosis and antitumor compounds which can improve the current chemotherapeutic antituberculosis and antitumor treatments, we have synthesized and evaluated a series of new 5-methyl-*N*-(3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-3-phenyl-1*H*-indole-2-carboxamide derivatives bearing various substituents on the spiroheterocyclic residue.

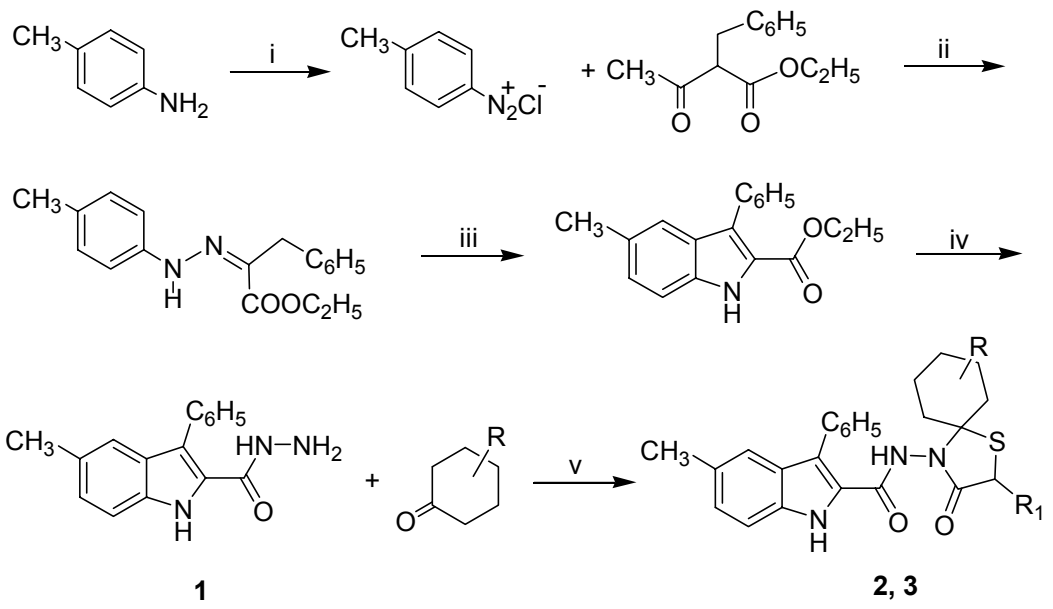
Results and Discussion

5-Methyl-3-phenyl-1*H*-indole-2-carbohydrazide (**1**), obtained via a multistep synthesis[19], an appropriate cyclic ketone and mercaptoacetic acid or α-mercaptopropionic acid were refluxed in dry benzene using a Dean-Stark water separator to produce 5-methyl-*N*-(3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-3-phenyl-1*H*-indole-2-carboxamide derivatives (**2** and **3)** in a one-pot reaction (Scheme 1).

The structures of the new compounds were established by IR, ^{1}H-NMR, HMBC, HSQC, ^{13}C-NMR (APT), atmospheric pressure chemical ionization [APCI(+)] mass spectrometry and elemental analysis (Table 1).

The IR spectra exhibited N-H and C=O bands in the 3241-3370 cm^{-1} and 1651-1678 cm^{-1} regions attributed to the common CONH functions of **2** and **3** [20]. Observation of new endocyclic C=O bands (1692-1713 cm^{-1}) characteristic for such structures besides C=O amide bands (1651-1678 cm^{-1}) in the IR spectra of **2** and **3** supported the aimed cyclization[12,13].

^{1}H-NMR spectra of **2** showed the spirodecane C_2-H_2 at δ 3.52-3.63 ppm and C_2-H of **3** at δ 3.74-3.92 ppm [13,21,22]. The remaining spirodecane protons resonated at about δ 0.81-2.10 ppm together with the alkyl substituents on the spiroheterocycle. Peaks associated with the indole subunit were observed in the expected regions[20].



Scheme 1. Reagents and conditions: i) $NaNO_2$, HCl, 0°C; ii) KOH, 0°C; iii) 37% HCl, reflux, 4h; iv) $H_2NNH_2.H_2O$, EtOH, reflux, 6h; v) $HSCH_2COOH$/$HSCH(CH_3)COOH$, dry C_6H_6, reflux, 6h.

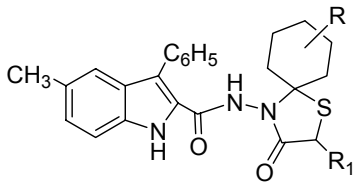
^{13}C-APT run on **2c** and 2D NMR experiments HSQC and HMBC run on **3c** allowed explicit assignments for the proton and carbon chemical shifts. The spectra substantiated the expected conversion and revealed the typical spirodecane C_2, C_3 (lactam C=O) and C_5 resonances at δ 28.57-37.45, 168.05-170.71 and 71.40-73.00 ppm, respectively[13,22]. Cross peaks observed between 2-CH_3 and C_2-H protons and spirodecane C_3 (lactam C=O) in the HMBC spectrum of **3c** enabled definite assignment of the lactam C=O (δ 170.71 ppm) and CONH (δ 162.57 ppm) carbons.

Abundant $(M+H)^+$ ions observed in the APCI mass spectra of **2d** and **3c** (**2d**: m/z 496; **3c**: m/z 448) confirmed their molecular weights. The molecules fragmented via loss of m/z 74 or m/z 88 fragments or rupture of the exocyclic CO-NH bond which afforded the base peaks (**2d**: m/z 422; **3c**: m/z 234)[13,20]. A proposed mass fragmentation route of **2d** and **3c** recorded employing APCI in the positive ionization mode is depicted in Scheme 2.

Further spectral details are presented in the experimental section.

The *in vitro* antimycobacterial activity evaluation of **2** and **3** against *M. Tuberculosis* H37Rv (ATCC 27294) was initially carried out in BACTEC 12B medium using the microplate alamar blue assay (MABA) at a concentration of 6.25 μg/mL at the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF)[23] (Table 1).

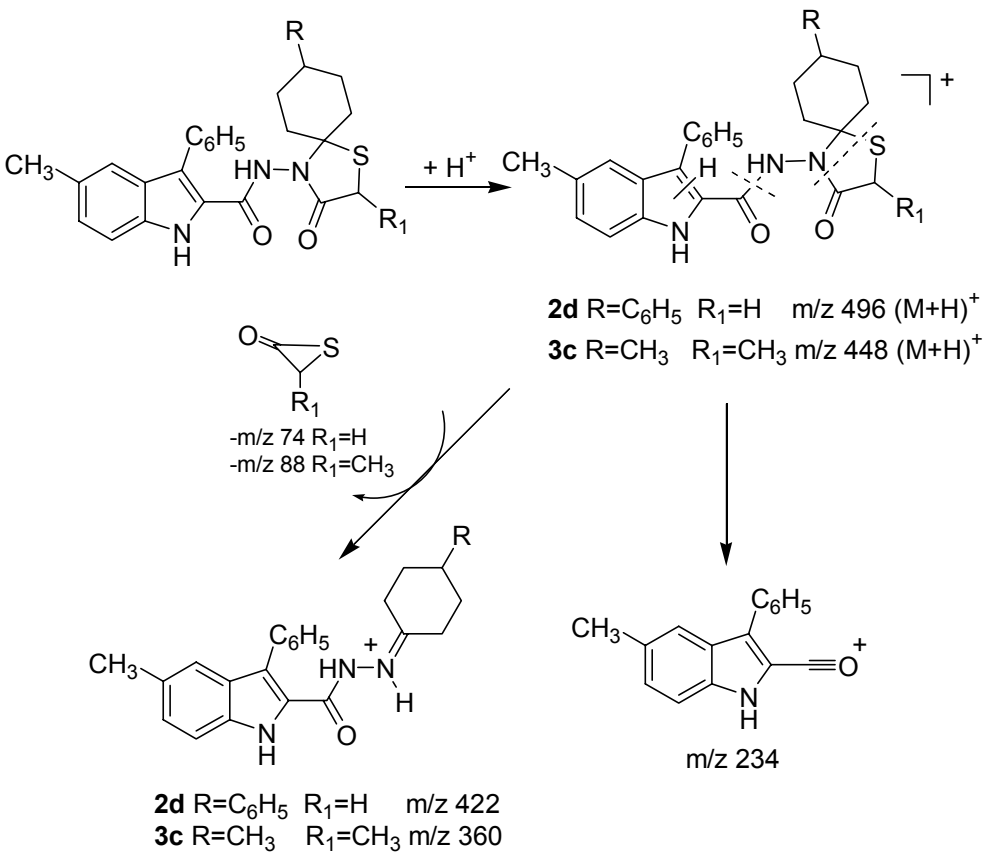
Table 1. Formulas, physical constants, elemental analysis and primary *in vitro* antimycobacterial activity evaluation of **2** and **3** against *M. Tuberculosis* H37Rv



2, 3

Comp.	R	R_1	Formula (M.W.)	Yield (%)	M.p. (°C)	Analysis (%) (calc./found)			GI (%)[a]
						C	H	N	
2a	6-CH_3	H	$C_{25}H_{27}N_3O_2S$	47	176-8	69.26	6.28	9.69	72
			(433.57)			68.73	6.32	9.54	
2b	7-CH_3	H	$C_{25}H_{27}N_3O_2S.H_2O$	25	156-8	66.49	6.49	9.31	63
			(451.58)			66.03	6.25	9.16	
2c	8-C_3H_7	H	$C_{27}H_{31}N_3O_2S$	71	196-8	70.25	6.77	9.10	85
			(461.62)			70.12	6.81	8.98	
2d	8-C_6H_5	H	$C_{30}H_{29}N_3O_2S$	57	108-10	72.70	5.90	8.48	56
			(495.64)			72.33	5.89	8.16	
3a	6-CH_3	CH_3	$C_{26}H_{29}N_3O_2S$	43	156-8	69.77	6.53	9.39	63
			(447.60)			70.30	6.69	9.40	
3b	7-CH_3	CH_3	$C_{26}H_{29}N_3O_2S$	14	197-8	69.77	6.53	9.39	71
			(447.60)			69.45	6.49	9.84	
3c	8-CH_3	CH_3	$C_{26}H_{29}N_3O_2S$	46	172-4	69.77	6.53	9.39	90
			(447.60)			70.04	6.69	9.37	
3d	8-C_3H_7	CH_3	$C_{28}H_{33}N_3O_2S$	48	170-3	70.70	6.99	8.83	86
			(475.65)			70.18	7.22	9.14	
3e	8-C_6H_5	CH_3	$C_{31}H_{31}N_3O_2S.1.5H_2O$	79	132-5	69.37	6.38	7.82	69
			(536.66)			69.69	6.29	7.32	

[a]Growth Inhibition of virulent H37Rv strain of *M. Tuberculosis* at 6.25µg/ml. MIC of Rifampin: 0.125 µg/mL versus *M. Tuberculosis H37Rv* (97% inhibition).



Scheme 2. Proposed mass fragmentation pattern of **2d** and **3c** under APCI (+).

As can be seen in Table 1, **2** and **3** produced growth inhibitions ranging from 56% to 90%. The correlation between structure and antituberculosis activity in this series was not straightforward, however, it may be speculated that introduction of a methyl group at 2- position of the spirodecane system increased activity (except **3a**) and thus compounds **3** were more active than **2** and **3c**, the 8-C_6H_5 and 2-CH_3 substituted entry showed the highest inhibition (90%).

Compounds **2a, 2b, 3a, 3b, 3c** 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)[24-26]. For the compounds, the 50% growth inhibition (GI_{50}) and total growth inhibition (TGI) were obtained for each cell line. The $\log_{10} GI_{50}$ and $\log_{10}$ TGI defined as the mean of the $\log_{10}$'s of the invidual GI_{50} and TGI values were then determined. Negative values indicated the most sensitive cell lines. Compounds having values –4 and <-4 were declared to be active. As shown in Table 2. $\log_{10} GI_{50}$ values of compounds **2a**, **2b**, **3a**, **3b** and **3c** were smaller than –4 for all tested tumour cell lines.

Among the compounds tested, compound **2b** demonstrated the most marked effects in the National Cancer Institute's 55 human tumour cell line *in vitro* screen on ovarium cancer cell line IGROV1 ($\log_{10} GI_{50}$ value –7.31), renal cancer cell line UO-31 ($\log_{10} GI_{50}$ value –7.56), and CNS cancer cell line SNB-19 ($\log_{10} GI_{50}$ value –4.73). On the same cancer cell line, the $\log_{10} GI_{50}$

values of thioguanine used as an anticancer agent were –5.32, -5.98 and –4.10, respectively. When these data are examined, it is observed that **2b** is more active than thioguanine on ovarium cancer cell line IGROV1, renal cancer cell line UO-31 and CNS cancer cell line SNB-19. On the other hand compound **3a** exhibited high cytotoxicity on renal cancer cell line RXF 393 and CNS cancer cell line SNB-19. The $\log_{10}$ GI_{50} values of compound **3a** and thioguanine were –6.38, -4.57 and -5.83, -4.10 on the same cancer cell lines, respectively.

Table 2. Invitro tumor cell growth inhibition of **2a, 2b, 3a, 3b** and **3c**

Panel/cell line	**2a**		**2b**		**3a**		**3b**		**3c**		Thioguanine	
	$\log_{10}GI_{50}$	$\log_{10}TGI$	$\log_{10}GI_{50}$	$\log_{10}TGI$	$\log_{10}GI_{50}$	$\log_{10}TGI$	$\log_{10}GI_{50}$	$\log_{10}TGI$	$\log_{10}GI_{50}$	$\log_{10}TGI$	$\log_{10}GI_{50}$	$\log_{10}TGI$
Leukemia												
CCRF-CEM	-4.77	-4.41	-4.97	-4.50	-4.66	-4.14	-4.13	>-4.00	-4.69	-4.10	-6.81	-4.59
K-562	-4.97	-4.38	-4.90	-4.41	-4.73	-4.22	-4.46	>-4.00	-4.79	-4.21	-5.96	-4.75
MOLT-4	-5.03	-4.55	-5.06	-4.50	-4.91	-4.39	-4.71	>-4.00	-5.10	-4.49	-6.39	-4.15
RPMI-8226	-4.86	-4.45	-4.86	-4.50	-4.67	-4.19	-4.27	>-4.00	-4.94	-4.28	-6.57	-4.81
Non-small cell lung cancer												
A549/ATCC	-4.88	-4.55	-4.82	-4.52	-4.80	-4.48	-4.51	>-4.00	-4.85	-4.50	-5.45	-3.98
EKVX	-4.77	-4.47	-4.62	-4.23	-4.61	-4.21	>-4.00	>-4.00	-4.71	-4.25	-5.72	-3.91
HOP-62	-4.75	-4.47	-4.72	-4.45	-4.70	-4.42	>-4.00	>-4.00	-4.74	-4.42	-6.13	-4.54
HOP-92	-4.90	-4.56	-4.88	-4.55	-4.71	-4.41	>-4.00	>-4.00	-4.78	-4.44	-5.39	-4.01
NCI-H226	-4.76	-4.47	-4.74	-4.45	-4.76	-4.47	>-4.00	>-4.00	-4.76	-4.45	-5.97	-4.64
NCI-H23	-4.77	-4.48	-4.70	-4.44	-4.61	-4.27	>-4.00	>-4.00	-4.82	-4.50	-5.13	-3.65
NCI-H322M	-4.77	-4.45	-4.75	-4.42	-4.65	-4.29	>-4.00	>-4.00	-4.73	-4.37	-6.17	-4.70
NCI-H460	-4.74	-4.36	-4.74	-4.42	-4.66	-4.23	-4.08	>-4.00	-4.81	-4.36	-6.04	-5.20
NCI-H522	-4.88	-4.55	-4.92	-4.59	-5.62	-4.78	-4.11	>-4.00	-4.89	-4.51		
Colon cancer												
COLO 205	-4.69	-4.31	-4.76	-4.45	-4.74	-4.41	-4.03	>-4.00	-4.75	-4.39	-5.77	-5.05
HCC-2998	-4.79	-4.50	-4.84	-4.54	-4.71	-4.45	-4.12	>-4.00	-4.78	-4.50	-6.00	-4.74
HCT-116	-4.84	-4.47	-4.84	-4.51	-4.76	-4.45	-4.12	>-4.00	-4.82	-4.46	-6.27	-4.79
HCT-15	-4.75	-4.41	-4.74	-4.43	-4.74	-4.41	-4.06	>-4.00	-4.71	-4.27	-5.96	-4.29
HT29	-4.84	-4.43	-4.86	-4.53	-4.81	-4.41	-4.47	>-4.00	-4.88	-4.53	-5.94	-3.98
KM12	-4.83	-4.46	-4.79	-4.46	-4.73	-4.40	-4.18	>-4.00	-4.81	-4.45	-5.76	-4.50
SW-620	-4.62	-4.22	-4.59	-4.17	-4.70	-4.27	>-4.00	>-4.00	-4.50	-4.08	-5.81	-3.86
CNS cancer												
SF-268	-4.77	-4.43	-4.81	-4.49	-4.73	-4.36	-4.19	>-4.00	-4.83	-4.45	-5.94	-3.80
SF-295	-4.80	-4.50	-4.76	-4.48	-4.75	-4.45	-4.16	>-4.00	-4.85	-4.48	-5.99	-3.80
SF-539	-4.71	-4.42	-4.68	-4.40	-4.65	-4.35	>-4.00	>-4.00	-4.68	-4.34	-5.99	-4.16
SNB-19	**-4.72**	-4.37	**-4.73**	-4.44	**-4.57**	-4.11	>-4.00	>-4.00	**-4.64**	-4.20	-4.10	-3.61
SNB-75	-4.71	-4.42	-4.77	-4.46	-4.67	-4.38	-4.22	>-4.00	-4.61	-4.29	-5.84	-4.38

U251	-4.78	-4.49	-4.76	-4.47	-4.79	-4.48	-4.31	>-4.00	-4.80	-4.47	-5.31	-3.62
Melanoma												
LOX IMVI	-4.72	-4.45	-4.70	-4.43	-4.72	-4.45	>-4.00	>-4.00	-4.75	-4.46	-6.68	-5.04
MALME-3M	-4.76	-4.45	-4.74	-4.46	4.69	-4.42	-4.16	>-4.00	-4.77	-4.48	-5.87	-4.54
M14	-4.71	-4.42	-4.73	-4.45	4.70	-4.41	>-4.00	>-4.00	-4.74	-4.41	-6.23	-4.84
SK-MEL-2	-4.68	-4.41	-5.04	-4.64	4.92	-4.58	>-4.00	>-4.00	-4.75	-4.44	-6.03	-4.97
SK-MEL-28	-4.70	-4.42	-4.71	-4.42	-4.71	-4.41	>-4.00	>-4.00	-4.75	-4.44	-5.05	-3.81
SK-MEL-5	-4.82	-4.50	-4.76	-4.46	-4.80	-4.47	>-4.00	>-4.00	-4.74	-4.44	-5.45	-4.78
UACC-257	-4.78	-4.50	-4.72	-4.46	-4.69	-4.42	-4.18	>-4.00	-4.72	-4.35	-5.70	-4.01
UACC-62	-4.89	-4.57	-4.86	-4.53	-4.87	-4.55	-4.11	>-4.00	-4.93	-4.59	-6.30	-5.34
Ovarian cancer												
IGROV1			**-7.31**	-4.92			-4.23	>-4.00	-4.87	-4.44	-5.32	-3.84
OVCAR-3	-4.86	-4.52	-4.86	-4.52	-4.84	-4.50	-4.01	>-4.00	-4.93	-4.56	-6.15	-5.17
OVCAR-4	-4.78	-4.47	-4.73	-4.40	-4.80	-4.44	-4.30	>-4.00	-4.83	-4.46	-5.82	-4.00
OVCAR-5	-4.71	-4.44	-4.74	-4.46	-4.69	-4.40	>-4.00	>-4.00	-4.77	-4.46	-6.18	-4.32
OVCAR-8	-4.76	-4.45	-4.81	-4.48	-4.68	-4.36	-4.46	>-4.00	-4.67	-4.27	-6.16	-4.22
SK-OV-3	-4.67	-4.33	-4.67	-4.32	-4.42	>-4.00	>-4.00	>-4.00	-4.48	>-4.00	-6.26	-4.78
Renal cancer												
786-O	-4.76	-4.42	-4.71	-4.41	-4.65	-4.29	-4.23	>-4.00	-4.72	-4.28	-6.08	-3.97
A498	-4.76	-4.46	-4.76	-4.44	-4.82	-4.49	>-4.00	>-4.00	-4.76	-4.46	-5.17	-4.02
ACHN	-4.76	-4.48	-4.74	-4.44	-4.76	-4.44	>-4.00	>-4.00	-4.70	-4.35	-5.71	-3.95
CAKI-1	-4.79	-4.49	-4.81	-4.47	-4.77	-4.39	-4.02	>-4.00	-4.81	-4.42	-6.27	-4.80
SN12C	-4.75	-4.47	-4.78	-4.49	-4.80	-4.50	-4.63	>-4.00	-4.86	-4.52	-6.12	-5.15
TK-10	-4.76	-4.47	-4.76	-4.46	-4.72	-4.45	-4.05	>-4.00	-4.89	-4.55	-5.90	-3.76
UO-31	-4.81	-4.51	**-7.56**	-4.98	-4.77	-4.49	>-4.00	>-4.00	-4.85	-4.53	-5.98	-4.10
RXF 393			-5.23	-4.69	**-6.38**	-4.68	-4.77	>-4.00	-4.85	-4.53	-5.83	-4.49
Prostate cancer												
PC-3	-4.90	-4.52	-4.85	-4.53	-4.81	-4.42	-4.26	>-4.00	-4.86	-4.34	-5.75	-3.76
DU-145	-4.74	-4.36	-4.73	-4.37	-4.66	-4.29	>-4.00	>-4.00	-4.66	-4.27	-6.10	-3.87
Breast cancer												
MCF7	-4.83	-4.41	-4.81	-4.41	-4.64	>-4.00	-4.49	>-4.00	-4.76	>-4.00	-6.15	-4.37
MDA-MB 231/ATCC	-4.77	-4.46	-4.76	-4.47	-4.76	-4.46	>-4.00	>-4.00	-4.81	-4.50	-6.23	-4.44
HS 578T	-4.56	-4.02	-4.55	-4.17	-4.84	-4.36	-4.06	>-4.00	-4.38	>-4.00	-5.71	-3.78
MDA-MB 435	-4.75	-4.46	-4.75	-4.46	-4.79	-4.48	>-4.00	>-4.00	-4.78	-4.48	-5.13	-3.74
BT-549	-4.70	-4.43	-4.71	-4.44	-4.73	-4.46	-4.12	>-4.00	-4.71	-4.44	-6.07	-4.49
T-47D	-4.79	-4.40	-4.69	-4.26	-4.61	-4.10	>-4.00	>-4.00	-4.71	-4.07	-5.44	-3.79
MG MID	-4.78	-4.44	-4.88	-4.47	-4.77	-4.40	-4.15	-4.00	-4.77	-4.38	-5.90	-4.33
Delta	0.25	0.13	2.67	0.51	1.60	0.65	0.62	0.06	0.32	0.21		
Range	0.47	0.55	3.01	0.81	1.96	1.06	0.77	0.07	0.72	0.59		

In conclusion, these preliminary results are promising and some of these compounds especially **2b**, **3a** and **3c** may be potential candidates for new antituberculosis and anticancer lead molecules.

Experimental Section

General Procedures. Cyclic ketones, mercaptoacetic acid and 2-mercaptopropionic acid were commercially available. 5-methyl-3-phenyl-1*H*-indole-2-carbohyrazide (**1**) was synthesized as depicted in Scheme 1 according to ref. 19. The reactions were monitored by TLC on silica gel HF_{254} coated plates (E.Merck, Darmstadt, Germany). Melting points were measured 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 all values are expressed as υ_{max} cm^{-1}. ^{1}H-NMR, HSQC, HMBC and ^{13}C-NMR (APT) spectra were recorded on Bruker AC 200 MHz and Varian^{UNITY}INOVA 500 MHz spectrometers using DMSO-d_6. Mass spectra (LC/MS-APCI) were recorded on a Finnigan ^{TM} LCQ ^{TM} Mass Spectrometer in the positive ionization mode. Elemental analyses were performed on a Carlo Erba Model 1106 elemental analyzer.

Synthesis of 5-methyl-*N*-(3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-3-phenyl-1*H*-indole-2-carboxamide derivatives (2 and **3).** A mixture of 1 (0.005 mol), an appropriate cyclic ketone (0.005 mol) and mercaptoacetic acid or α-mercaptopropionic acid (0.02 mol) was refluxed in 20 ml dry benzene for 5-6 h using a Dean-Stark water separator. Excess benzene was evaporated *in vacuo*. The resulting residue was triturated with saturated $NaHCO_3$ solution until CO_2 evolution ceased and was allowed to stand overnight or in some cases refrigerated until solification. The solid thus obtained was washed with water, dried, and recrstallized from C_2H_5OH.

5-Methyl-*N*-(6-methyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (2a). IR(KBr): υ 3279 (NH/OH), 1704, 1667 (C=O); ^{1}H-NMR (200 MHz): δ 0.81 (s, 3H, 6-CH_3 sp.[1]), 1.03-1.10 (m, 4H, sp.), 1.34-1.81 (m, 5H, sp.), 2.37 (s, 3H, 5-CH_3 ind.[2]), 3.52 (s, 2H, C_2-H_2), 7.12 (d, J=8.35 Hz, 1H, C_6-H ind.), 7.32-7.56 (m, 7H, $C_{4,7}$-H ind. and Ar-H), 9.80 (s, 1H, CONH), 11.81 (s, 1H, NH ind.).

5-Methyl-*N*-(7-methyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (2b). IR(KBr): υ 3241 (NH/OH), 1711,1651 (C=O); ^{1}H-NMR (500 MHz): δ 0.89 (s, 3H, 7-CH_3 sp.), 1.30-1.80 (m, 9H, sp.), 2.38 (s, 3H, 5-CH_3 ind.), 3.58 (s, 2H, C_2-H_2), 7.12 (dd, J=8.3, 1.5 Hz, 1H, C_6-H ind.), 7.32 (s, 1H, C_4-H ind.), 7.36 (t, J=7.3 Hz, 1H, Ar-H), 7.41 (d, J=8.3 Hz, 1H, C_7-H ind.), 7.46 (t, J=7.6 Hz, 2H, Ar-H), 7.53 (dd, J=8.3, 1.4 Hz, 2H, Ar-H), 10.02 (s, 1H, CONH), 11.92 (s, 1H, NH ind.).

5-Methyl-*N*-(3-oxo-8-propyl-1-thia-4-azaspiro[4.5]dec-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (2c). IR (KBr): υ 3329, 3255 (NH/OH), 1692, 1674 (C=O); ^{1}H-NMR (200 MHz):

[1] sp: spirodecane
[2] ind: indole

δ 0.88 (t, J=7.03 Hz, 3H, 8-$CH_2CH_2CH_3$ sp.), 1.14 (s, 4H, 8-$CH_2CH_2CH_3$ sp.), 1.29 (s, 4H, sp.), 1.71 (br.s, 5H, sp.), 2.35 (s, 3H, 5-CH_3 ind.), 3.56 (s, 2H, C_2-H_2), 7.11 (d, J=8.39 Hz, 1H, C_6-H ind.), 7.31-7.54 (m, 7H, $C_{4,7}$-H ind. and Ar-H), 9.94 (s, 1H, CONH), 11.75 (s, 1H, NH ind.). ^{13}C-NMR (APT)(125 MHz): δ 14.81 (8-$CH_2CH_2CH_3$ sp.), 20.24 (8-$CH_2CH_2CH_3$ sp.), 21.94 (5-CH_3 ind.), 28.57 (C_2 sp.), 30.02 ($C_{7,9}$ sp.), 35.52 (C_8 sp.), 39.04 (8-$CH_2CH_2CH_3$ sp.), 37.12 ($C_{6,10}$ sp.), 73.00 (C_5 sp.), 112.85 (C_7 ind.), 119.07 (C_3 ind.), 119.92 (C_4 ind.), 126.80 (C_2 ind.), 127.21 (C_6 ind.), 127.45 (C_{3a} ind.), 128.40 (C_4 3-C_6H_5 ind.), 128.89 ($C_{3,5}$ 3-C_6H_5 ind.), 130.90 ($C_{2,6}$ 3-C_6H_5 ind.), 134.60 (C_1 3-C_6H_5 ind.), 129.77 (C_5 ind.), 134.88 (C_{7a} ind.), 162.62 (amide C=O), 168.05 (lactam C=O).

5-Methyl-*N*-(3-oxo-8-phenyl-1-thia-4-azaspiro[4.5]dec-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (2d). IR(KBr): υ 3284 (NH/OH), 1709, 1659 (C=O); ^{1}H-NMR (200 MHz): δ 1.67-2.10 (m, 9H, sp.), 2.38 (s, 3H, 5-CH_3 ind.), 3.63 (br.s, 2H, C_2-H_2), 7.11-7.60 (m, 13H, Ar-H), 10.11 (s, 1H, CONH), 11.89 (s, 1H, NH ind.); MS (APCI): m/z 496 $(M+H)^+$ (41%), 498 (6%), 497 (20%), 422 (100%), 234 (96%) .

5-Methyl-*N*-(2,6-dimethyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (3a). IR(KBr): υ 3370,3288 (NH/OH), 1702, 1678 (C=O); ^{1}H-NMR (200 MHz): δ 0.79 (br.s, 3H, 6-CH_3 sp.), 1.09-1.13 (m, 4H, sp.), 1.39 (d, J=6.89 Hz, 3H, 2-CH_3 sp.), 1.51-1.64 (m, 5H, sp.), 2.37 (s, 3H, 5-CH_3 ind.), 3.79-3.87 (2q, J=6.97 Hz, 1H, C_2-H), 7.12 (d, J=8.45 Hz, 1H, C_6-H ind.), 7.31-7.55 (m, 7H, $C_{4,7}$-H ind. and Ar-H), 9.82 (s, 1H, CONH), 11.84 (s, 1H, NH ind.).

5-Methyl-*N*-(2,7-dimethyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (3b). IR(KBr): υ 3250 (NH/OH), 1693, 1665 (C=O); ^{1}H-NMR (500 MHz): δ 0.89 (d, J=5.9 Hz, 3H, 7-CH_3 sp.), 1.30-1.80 (m, 12H, sp. and 2-CH_3 sp.), 2.38 (s, 3H, 5-CH_3 ind.), 3.87 (br.s, 1H, C_2-H), 7.13 (dd, J=8.5, 1.4 Hz, 1H, C_6-H ind.), 7.32 (s, 1H, C_4-H ind.), 7.36 (t, J=7.3 Hz, 1H, Ar-H), 7.41 (d, J=8.3 Hz, 1H, C_7-H ind.), 7.46 (t, J=7.6 Hz, 2H, Ar-H), 7.53-7.54 (m, 2H, Ar-H), 9.91 (s, 1H, CONH), 11.75 (s, 1H, NH ind.).

5-Methyl-*N*-(2,8-dimethyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (3c). IR(KBr): υ 3290 (NH/OH), 1712, 1654 (C=O); ^{1}H-NMR (500 MHz): δ 0.97 (d, J=6.83 Hz, 3H, 8-CH_3 sp.), 1.05-1.20 (m, 2H, $C_{7/9}$-H sp.), 1.31-1.35 (m, 1H, C_8-H sp.), 1.67 (d, 2H, J=13.18 Hz, $C_{7/9}$-H sp.), 1.41 (d, J=6.83 Hz, 3H, 2-CH_3 sp.), 1.73-1.85 (m, 2H, $C_{6/10}$-H sp.), 1.87-1.97 (m, 2H, $C_{6/10}$-H sp.), 2.37 (s, 3H, 5-CH_3 ind.), 3.85-3.88 (m, 1H, C_2-H), 7.12 (d, J=8.54 Hz, 1H, C_6-H ind.), 7.33-7.36 (m, 2H, C_4-H ind. and C_4-H 3-C_6H_5 ind.), 7.41 (m, 1H, C_7-H ind.), 7.45 (t, J=7.56 Hz, 2H, $C_{3,5}$-H 3-C_6H_5 ind.), 7.54 (d, J=7.56 Hz, 2H, $C_{2,6}$-H 3-C_6H_5 ind.), 9.89 (s, 1H, CONH), 11.73 (s, 1H, NH ind.). ^{13}C-NMR (125 MHz): δ 20.49 (2-CH_3 sp.), 21.93 (5-CH_3 ind.), 22.60 (8-CH_3 sp.), 31.07 (C_8 sp.), 31.97, 32.39 ($C_{7/9}$ sp.), 37.28, 38.15 ($C_{6/10}$ sp.), 37.45 (C_2 sp.), 71.40 (C_5 sp.), 112.81 (C_7 ind.), 119.12 (C_3 ind.), 120.00 (C_4 ind.), 126.65 (C_6 ind.), 126.80 (C_{3a} ind.), 127.27 (C_4 3-C_6H_5 ind.), 127.47 (C_2 ind.), 128.94 ($C_{3,5}$ 3-C_6H_5 ind.), 129.82 (C_5 ind.), 130.91 ($C_{2,6}$ 3-C_6H_5 ind.), 134.49 (C_1 3-C_6H_5 ind.), 134.55 (C_{7a} ind.), 162.57 (amide C=O), 170.71 (lactam C=O); MS (APCI): m/z 448 $(M+H)^+$ (20%), 449 (6%), 360 (72%), 234 (100%).

5-Methyl-*N*-(2-methyl-3-oxo-8-propyl-1-thia-4-azaspiro[4.5]dec-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (3d). IR(KBr): υ 3291 (NH/OH), 1694, 1658 (C=O); ^{1}H-NMR (200 MHz): δ 0.75 (br.s, 3H, 8-$CH_2CH_2CH_3$ sp.), 1.02-1.28 (m, 8H, sp. and 8-$CH_2CH_2CH_3$ sp.), 1.59 (br.s, 3H, 2-CH_3 sp.), 2.23 (br.s, 5H, sp.), 2.36 (s, 3H, 5-CH_3 ind.), 3.74 (br.s, 1H, C_2-H), 6.99-7.38 (m, 8H, Ar-H), 10.09 (s, 1H, CONH), 11.88 (s, 1H, NH ind.).

5-Methyl-*N*-(2-methyl-3-oxo-8-phenyl-1-thia-4-azaspiro[4.5]dec-4-yl)-3-phenyl-1*H*-indole-2-carboxamide (3e). IR(KBr): υ 3262 (NH/OH), 1713, 1672 (C=O); ^{1}H-NMR (200 MHz): δ 1.42 (d, J=6.89 Hz, 3H, 2-CH_3 sp.), 1.50-2.10 (m, 9H, sp.), 2.36 (s, 3H, 5-CH_3 ind.), 3.92 (q, J=6.89 Hz, 1H, C_2-H), 7.09-7.53 (m, 13H, Ar-H), 10.45 (s, 1H, CONH), 12.22 (s, 1H, NH ind.).

In vitro evaluation of antituberculosis activity [microplate alamar blue assay (MABA)]. Antimicrobial susceptibility testing was performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument, Meriden, Conn.) 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 dimethyl sulfoxide 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 $1x10^6$ 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 $2.0x10^5$ 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 10x 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)x100. The lowest drug concentration effecting an inhibition of ≥90% was considered as the MIC.

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 5,000 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 % CO2, 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 dimethyl sulfoxide 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 ½ 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 % CO2, 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 minutes 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 minutes 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 concentrations levels. Percentage growth inhibition was calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz

[(Ti-Tz)/Tz] x 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)] x 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] x 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.

Acknowledgements

Antimycobacterial activity was determined by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) through a research and development contract with the U.S. National Institute of Allergy and Infectious Diseases. Thanks are addressed to Dr.Joseph A. Maddry (TAACF) and his team for their collaboration.
We thank the Division of Cancer Research, National Cancer Institute, Bethesda, Maryland, for the anticancer activity screening.
This work was supported in part by The Research Fund of Istanbul University (Project Number 1267/050599).

References

1. Savini, L.; Chiasserini, L.; Gaeta, A.; Pellerano, C. *Bioorg.Med.Chem.* **2002,** *10*, 2193.
2. Mahboobi, S.; Eichhorn, E.; Popp, A.; Sellmer, A.; Elz, S.; Möllmann, U. *Eur. J. Med. Chem.* **2006,** *41*, 176.
3. Hoemann, M. Z.; Kumaravel, G.; Xie, R. L.; Rossi, R. F.; Meyer, S.; Sidhu, A.; Cuny, G. D.; Hauske, J. R. *Bioorg. Med. Chem. Lett.* **2000,** *10*, 2675.
4. Kutschy, P.; Suchỳ, M.; Andreani, A.; Dzurilla, M.; Kováčik, V.; Alföldi, J.; Rossi, M.; Gramatová, M. *Tetrahedron* **2002,** *58*, 9029.
5. Levy, L. M.; Cabrera, G. M.; Wright, J. E.; Seldes, A. M.; *Phytochemistry* **2000,** *54*, 941.
6. Sugiyama, H.; Yokokawa, F.; Aoyama, T.; Shioiri, T. *Tetrahedron Lett.* **2001,** *42*, 7277.
7. Font, M.; Monge, A.; Cuartero, A.; Elorriaga, A.; Martínez-Irujo, J. J.; Alberdi, E.; Santiago, E.; Prieto, I.; Lasarte, J. J.; Sarobe, P.; Borrás, F. *Eur. J. Med. Chem.* **1995,** *30*, 963.
8. Adreani, A.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Garaliene, V.; Farruggia, G.; Masotti, L. *Bioorg. Med. Chem.* **2004,** *12*, 1121.
9. Zhang, H.-Z.; Drewe, J.; Tseng, B.; Kasibhatla, S.; Cai, S. X. *Bioorg. Med. Chem.* **2004,** *12*, 3649.
10. Andres, C. J.; Bronson, J. J.; D'Andrea, S. V.; Deshpande, M. S.; Falk, P. J.; Grant-Young, K. A.; Harte, W. E.; Ho, H. T.; Misco, P. F.; Robertson, J. G.; Stock, D.; Sun, Y.; Walsh, A.W. *Bioorg. Med. Chem.Lett.* **2000,** *10*, 715.
11. Bonde, C. G.; Gaikwad, N. J. *Bioorg. Med. Chem.* **2004,** *12*, 2151.
12. Çapan, G.; Ulusoy, N.; Ergenç, N.; Kiraz, M. *Monatsh. Chem.* **1999,** *130*, 1399.
13. Ulusoy, N. *Arzneim-Forsch /Drug Res.* **2002,** *52(7)*, 565.
14. Babaoglu, K.; Page, M. A.; Jones, V. C.; McNeil, M. R.; Dong, C.; Naismith, J. H.; Lee, R. E. *Bioorg. Med. Chem.Lett.* **2003,** *13*, 3227.
15. Srivastava, T.; Gaikwad, A. K.; Haq, W.; Sinha, S.; Kati, S. B. *Arkivoc* **2005,** *ii*, 120.
16. Sharma, R.; Nagda, D. P.; Talesara, G. L. *Arkivoc* **2006,** *i*, 1.

17. Sayyed, M.; Mokle, S.; Bokhare, M.; Mankar, A.; Surwase, S.; Bhusare, S.; Vilohute, Y. *Arkivoc* **2006,** *ii*, 187.
18. Gududuru, V.; Hurh, E.; Dalton, J. T.; Miller, D. D. *Bioorg. Med. Chem. Lett.* **2004,** *14*, 5289.
19. Ergenç, N.; Salman, A.; Bankaoğlu, G. *Pharmazie* **1990,** *45*, 346.
20. Kaynak, F. B.; Öztürk, D.; Özbey. S.; Çapan, G. *J.Mol.Struct.* **2005,** *740*, 213.
21. Bicking, J. B.; Bock, M. G.; Cragoe, E. J.; Dipardo, R. M.; Gould, N. P.; Holltz, W. J.; Lee, T. J.; Robb, C. M.; Smith, R. L.; Springer, J. P.; Blaine, E. H. *J. Med. Chem*. **1983,** *26*, 342.
22. Karalı, N.; İlhan, E.; Gürsoy, A.; Kiraz, M.; *Farmaco* **1998,** *52*, 1.
23. Collins, L. A.; Franzblau, S. G. *Antimicrob. Agents Chemother* **1997,** *41*, 1004.
24. Alley, M. C.; Scudiero, D. A.; Monks, P. A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H. and Boyd, M. R. Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay. *Cancer Res.* **1988,** *48*, 589.
25. Grever, M. R.; Schepartz, S. A. and Chabner, B. A. The National Cancer Institute: Cancer Drug Discovery and Development Program. Seminars in Oncology, 1992; Vol. 19, No. 6, pp 622.
26. Boyd, M. R. and Paull, K. D. Some Practical Considerations and Applications of the National Cancer Institute In Vitro Anticancer Drug Discovery Screen. *Drug Development Research* **1995**, *34*, 91.