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Short communication

Synthesis and anticancer evaluation of some new hydrazone derivatives of 2,6-dimethylimidazo[2,1-*b*]-[1,3,4]thiadiazole-5-carbohydrazide

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Abstract

In this study, some novel 2,6-dimethyl-N′-substituted phenylmethylene-imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbohydrazides (**3a**–**3h**) were synthesized from 2,6-dimethylimidazo-[2,1-*b*][1,3,4]thiadiazole-5-carbohydrazide (**2**). The newly synthesized compounds (**3a**–**3h**) were evaluated in the National Cancer Institute's 3-cell line, one dose in vitro primary cytotoxicity assay. Compounds **3c** and **3h** which passed the criteria for activity in this assay (20–29% growth percentages) were scheduled automatically for evaluation against the full panel of 60 human tumor cell lines at a minimum of five concentrations at 10-fold dilutions. Sulforhodamine B (SRB) protein assay was used to estimate cell stability or growth. 2,6-Dimethyl-N′-(2-hydroxyphenylmethylidene)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbohydrazide (**3c**) showed the most favorable cytotoxicity. This compound demonstrated the most marked effects in the National Cancer Institute's 60 human tumor cell line in vitro screen on an ovarian cancer cell line (OVCAR $\log_{10}$ GI_{50} value −5.51).

Keywords: Imidazo[2,1-*b*][1,3,4]thiadiazoles; Hyrazide-hydrazones; Anticancer activity

1. Introduction

The search for anticancer drugs led to the discovery of several imidazo-fused heterocycles having anticancer activity [1–5]. An early report on 2-amino-1,3,4-thiadiazole derivatives deals with the activity of these compounds against several transplanted animal tumors [6]. Recently Gadad et al. [7] reported on the cytotoxic effects of imidazo[2,1-*b*][1,3,4]thiadiazoles (**I**). Andreani et al. [8] studied on some imidazo[2,1-*b*]thiazole guanyl hydrazones (**II**) which were active against various cancer cell lines. Much interest has also been focused on the chemistry and anticonvulsant, analgesic [9], antibacterial [10] and antisecretory [11] activities displayed by compounds incorporating this heterocyclic system. Since the imidazo[2,1-*b*][1,3,4]thiadiazole system is similar in part to Levamisole, a well-known immunomodulator [12], the possibility of reducing the harmful effects of the cytotoxic agents on the immune system also appears to be very challenging (Fig. 1).

In an attempt to achieve new compounds with possible anticancer properties, we designed and synthesized some novel 2,6-dimethyl-N′-substituted phenylmethylene-imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbohydrazide hydrazones (**3a**–**3h**) to evaluate their anticancer properties.

2. Chemistry

The synthetic pathway used in the preparation of the compounds is outlined in Fig. 2. Thus the reaction of 2-amino-5-methyl-1,3,4-thiadiazole and ethyl 2-chloroacetoacetate afforded ethyl-2,6-dimethylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carboxylate (**1**) which in turn was refluxed with hydrazine hydrate to obtain 2,6-dimethylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbohydrazide (**2**) [13].

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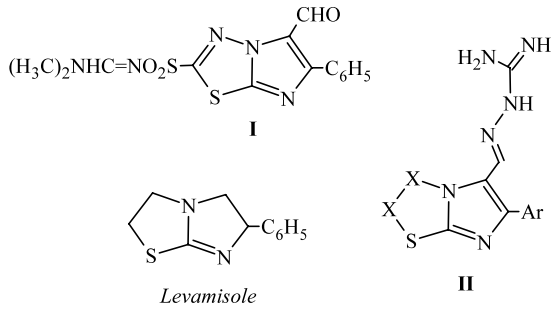

Fig. 1.

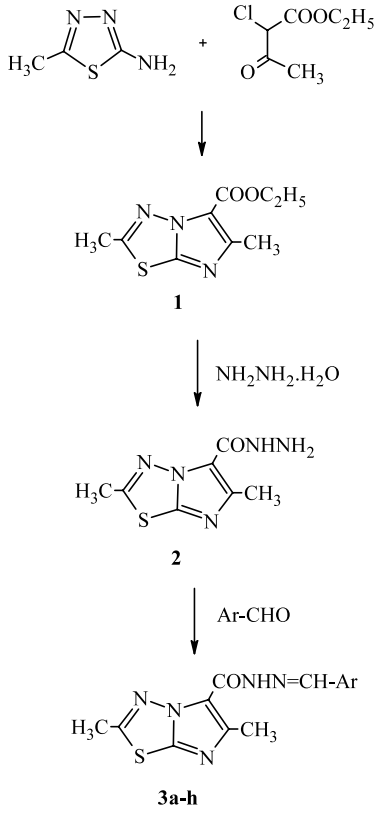

Fig. 2. Synthesis of **3a**–**3h**.

Condensation of **2** with appropriate aldehydes yielded the corresponding hydrazide hydrazones (**3a**–**3h**). All the synthesized compounds are listed in Table 1 and their structures are determined by elemental analyses and spectral data (IR, ^{1}H-NMR, EI-MS).

In the IR spectra, the NH and C=O bands were observed in the 3460–3446 cm^{-1} and 1695–1690 cm^{-1} regions, respectively. In the ^{1}H-NMR spectra of hydrazide hydrazones (**3a**–**3h**), the absence of the NH_2 absorptions of the hydrazide **2** ($\delta = 5.25$ ppm) and the presence of new resonances assigned to the –CH= proton of **3** provided evidence for hydrazone formation. These protons resonated in 8.84–9.21 ppm region as singlets. EI mass spectra of three representative examples (**3a**, **3f** and **3g**) provided molecular ions at *m*/*z* 299, 333 and 317 with different intensities confirming their molecular weights. The major fragmentation pathway in **3a**, **3f** and **3g** involved the cleavage of the N–N bond and migration of the CH proton to the nitrogen atom, giving the fragment at *m*/*z* 196 (Fig. 3). Additional spectral characteristics are presented in Section 4.

3. Anticancer evaluation and discussion

The 2,6-dimethyl-N′-substituted phenylmethylideneimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbohydrazides (**3a**–**3h**) were evaluated in the 3-cell line panel consisting of NCI-H460 (Lung), MCF7 (Breast), and SF-268 (CNS). Primary anticancer assay was performed in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda [14–16]. The compounds were added at a single concentration (10^{-4} M) and the culture was incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). Results for each compound were reported as the percent of growth of the treated cells when compared to the untreated control cells. The 2-hydroxy derivative **3c** and 4-nitro derivative **3h** which reduced the growth of the cell lines to 32% or less (negative numbers indicate cell kill) were passed on for evaluation in the full panel of 60 human tumor cell lines. Primary results are shown in Table 2. The cytotoxic and/or growth inhibitory effects of the compounds were tested in vitro against the full panel of 60 human tumor cell lines derived from nine neoplastic diseases at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. A 48-h continuous drug exposure protocol was followed and an SRB protein assay was used to estimate cell viability or growth. For each compound, the 50% growth inhibition (GI_{50}) and total growth inhibition (TGI) were obtained for all the cell lines. The $\log_{10} GI_{50}$ and $\log_{10}$ TGI were then determined, defined as the mean of the $\log_{10}$'s of the individual GI_{50} and TGI values. The lowest values are obtained with the most sensitive cell lines. Compounds having values -4 and less than -4 were declared to be active. The cell lines used in the NCI screen were Leukemia (L) lines CCRF-CEM, HL-60 (TB), K-562, MOLT-4, RPMI-8226, SR; non-small cell lung cancer (NSCLC) lines A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522; colon cancer (CL) lines COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620; central nervous system cancer (CNSC) lines SF-268, SF-295, SF-539, SNB-19, SNB-75, U251; melanoma (M) lines LOX IMVI, MALME-3M, M14, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62; ovarian cancer (OC) lines IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, SK-OV-3; renal cancer (RC) lines 786-O,

Table 1
Some characteristics of **3a**–**3h**

Compound	Ar	Yield (%)	Melting point (°C)	Formula (M_w)	Analysis calc./found		
					C	H	N
3a	C_6H_5	40	288–289	$C_{14}H_{13}N_5OS$ (299.34)	56.17, 55.78	4.37, 4.19	23.39, 23.49
3b	4-$CH_3C_6H_4$	51	298	$C_{15}H_{15}N_5OS$ (313.37)	57.48, 57.37	4.82, 4.73	22.35, 22.52
3c	2-HOC_6H_4	43	295–297	$C_{14}H_{13}N_5O_2S$ (315.35)	53.32, 53.09	4.15, 4.05	22.21, 22.28
3d	4-$CH_3OC_6H_4$	68	290–292	$C_{15}H_{15}N_5O_2S$ (329.37)	54.69, 54.47	4.59, 4.67	21.26, 21.91
3e	4-BrC_6H_4	87	270	$C_{14}H_{12}BrN_5OS\cdot\frac{1}{2}H_2O$ (387.26)	43.41, 42.97	3.38, 2.74	18.08, 18.29
3f	4-ClC_6H_4	63	300	$C_{14}H_{12}ClN_5OS\cdot\frac{1}{2}C_2H_5OH$ (356.82)	50.49, 51.06	4.23, 3.57	19.62, 19.82
3g	4-FC_6H_4	83	270	$C_{14}H_{12}FN_5OS$ (317.34)	52.98, 53.16	3.81, 3.91	22.07, 22.20
3h	4-$NO_2C_6H_4$	62	274–276	$C_{14}H_{12}N_6O_3S\cdot\frac{1}{2}H_2O$ (353.36)	47.58, 48.14	3.70, 3.23	23.78, 23.81

A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, UO-31; prostate cancer (PC) lines PC-3, DU-145 and breast cancer (BC) lines MCF7, NCI/ADR-RES, MDA-MB 231/ATCC, HS 578T, MDA-MB-435, MDA-N, BT-549, T-47D (Table 3).

The hydroxy derivative **3c** was highly active in the in vitro screen on an OC cell line (OVCAR-3, $\log_{10} GI_{50}$ value −5.51), a BC cell line (MCF7, $\log_{10} GI_{50}$ value −4.51) and an L cell line (HL-60(TB), $\log_{10} GI_{50}$ value −4.50). On the OC cell line (OVCAR-3), the $\log_{10} GI_{50}$ values of chlorambucil, 5-fluorouracil and melfalan used as anticancer agents are −3.96, −4.53 and −4.42, respectively. In addition, the nitro derivative **3h** demonstrated the most marked effects on two CNSC cell lines (SF-539, $\log_{10} GI_{50}$ value −4.50 and U251, $\log_{10} GI_{50}$ value −4.63), an OC cell line (SK-OV-3, $\log_{10} GI_{50}$ value −4.55) and a BC cell line (MDA-MD-231/ATCC, $\log_{10} GI_{50}$ value −4.62). The $\log_{10} GI_{50}$ values of chlorambucil, 5-fluorouracil and melfalan on an OC cell line (SK-OV-3) and a BC cell line (MDA-MD-231/ATCC) are −3.96, −3.90, −4.47 and −3.83, −3.49, −4.31, respectively. When these data are examined, it is observed that both the compounds (**3c** and **3h**) are much more active than chlorambucil, 5-fluorouracil and melfalan against an OC cell line, OVCAR-3 and SK-OV-3, respectively. In conclusion, these preliminary results are promising and some of these compounds may be potential candidates for new anticancer agents.

4. Experimental

4.1. Chemistry

Melting points were estimated with a Büchi 530 melting point apparatus in open capillaries and are uncorrected. Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer. IR spectra were

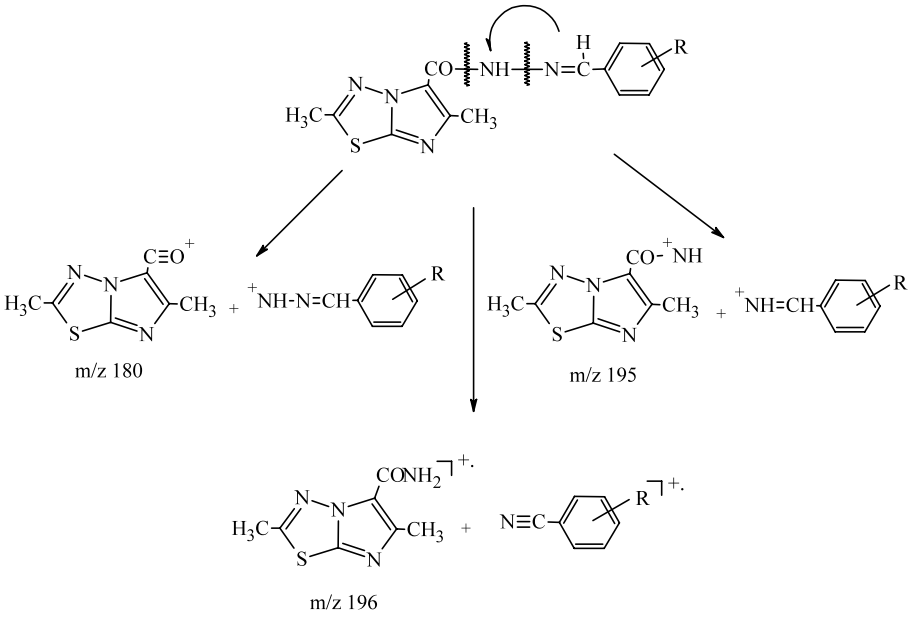

Fig. 3. The proposed fragmentation pattern of hydrazide hydrazones (**3a**–**3h**).

Table 2
Three-cell panel (growth percentages after inoculation with compounds **3a**–**3h**)

Compound (10^{-4} M)	NCI-H460 (lung)	MCF7 (breast)	SF-268 (CNS)
3a	94	64	98
3b	96	62	101
3c	29	25	54
3d	106	73	103
3e	86	63	49
3g	104	65	93
3h	85	72	20

Table 3
In vitro tumor cell growth inhibition of **3c** and **3h**

Panel/cell line	**3c**		**3h**	
	$\log_{10}$ GI_{50}	$\log_{10}$ TGI	Log_{10} GI_{50}	$\log_{10}$ TGI
Leukemia				
CCRF-CEM	−4.39	> −4.00	> −4.00	> −4.00
HL-60 (TB)	−4.50	> −4.00	> −4.00	> −4.00
MOLT-4	−4.28	> −4.00	−4.39	> −4.00
Non-small cell lung cancer				
HOP-62	> −4.00	> −4.00	−4.33	> −4.00
NCI-H460	−4.30	> −4.00	> −4.00	> −4.00
NCI-H522	> −4.00	> −4.00	−4.29	> −4.00
Colon cancer				
COLO 205	−4.23	> −4.00	> −4.00	> −4.00
HCT-116	−4.29	> −4.00	> −4.00	> −4.00
HCT-15	−4.38	> −4.00	> −4.00	> −4.00
HCC-2998	−4.26	> −4.00	> −4.00	> −4.00
CNS cancer				
SF-539	> −4.00	> −4.00	−4.50	> −4.00
SNB-19	> −4.00	> −4.00	−4.34	> −4.00
SNB-75	> −4.00	> −4.00	−4.30	> −4.00
U251	−4.42	> −4.00	−4.63	−4.27
Melanoma				
M14	−4.28	> −4.00	> −4.00	> −4.00
SK-MEL-5	−4.22	> -4.00	> −4.00	> −4.00
Ovarian cancer				
OVCAR-3	−5.51	−4.04	> −4.00	> −4.00
SK-OV-3	–	> −4.00	−4.55	−4.06
Renal cancer				
786-O	−4.32	> −4.00	> −4.00	> −4.00
A498	−4.21	> −4.00	> −4.00	> −4.00
ACHN	−4.25	> −4.00	−4.30	> −4.00
TK-10	> −4.00	> −4.00	−4.43	> −4.00
UO-31	> −4.00	> −4.00	−4.46	> −4.00
Breast cancer				
MCF7	−4.51	> −4.00	−4.04	> −4.00
NCI/ADR-RES	−4.21	> −4.00	> −4.00	> −4.00
MDA-MB 231/ATCC	> −4.00	> −4.00	−4.62	−4.31
T-47D	−4.21	> −4.00	> −4.00	> −4.00

recorded as KBr discs, using a Perkin–Elmer Model 1600 FT-IR spectrometer. ^{1}H-NMR spectra were obtained on a Bruker AC 200 (200 MHz), a Bruker DPX 400 (400 MHz) spectrophotometer using DMSO-d_6 (E. Merck, Darmstadt, Germany). EI-MS were determined on a VG Zab Spec (70 eV) mass spectrometer. Starting materials were purchased from E. Merck (Darmstadt, Germany).

*4.1.1. Synthesis of ethyl-2,6-dimethylimidazo[2,1-b]-[1,3,4]thiadiazole-5-carboxylate (**1**)*

0.1 mol of 2-amino-5-methyl-1,3,4-thiadiazole was refluxed with 0.1 mol of ethyl-2-chloroacetoacetate in 50 ml ethanol (70%) in the presence of 2 g pyridine for 5 h. The solid that separated was recrystallized with water; m.p., 120 °C [13].

*4.1.2. Synthesis of 2,6-dimethylimidazo[2,1-b]-[1,3,4]thiadiazole-5-carbohydrazide (**2**)*

0.01 mol of **1** was refluxed with 0.04 mol of the hydrazine hydrate (80%) in 20 ml ethanol for 2 h. The solid that separated was recrystallized with ethanol (60%); m.p. 310 °C (decomp.) [13].

2: IR [KBr, ν (cm^{-1})]: 3329, 3226 (N–H), 1699 (C=O). ^{1}H-NMR [200 MHz, δ, ppm, DMSO-d_6]: 2.35 (3H, s, 6-CH_3), 2.41 (3H, s, 2-CH_3), 5.25 (2H, s, NH_2), 13.10 (1H, s, NH).

*4.1.3. Synthesis of 2,6-dimethyl-N′-substituted phenylmethylidene-imidazo[2,1-b][1,3,4]thiadiazole-5-carbohydrazides (**3a**–**3h**): general procedure*

0.0025 mol of **2** was refluxed with 0.0025 mol of the appropriate aldehyde in 50 ml ethanol (60%) for 5 h. The solid that separated was washed with ethanol (60%).

3a: IR [KBr, ν (cm^{-1})]: 3446 (N–H), 3014 (=CH), 1693 (C=O). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 2.37 (3H, s, 6-CH_3), 2.44 (3H, s, 2-CH_3), 7.51–7.62 (3H, m, arylidene $C_{3,4,5}$–H), 7.90 (2H, d, $J = 7.59$ Hz, arylidene $C_{2,6}$–H), 9.21 (1H, s, CH=), 13.29 (1H, s, NH). EI-MS [m/z (%)]: 299 [M^+,100], 196 (94), 195 (38), 180 (1), 119 (2), 104 (30), 103 (50).

3b: IR [KBr, ν (cm^{-1})]: 3452 (N–H), 3013 (=CH), 1690 (C=O). ^{1}H-NMR [200 MHz, δ, ppm, DMSO-d_6]: 2.35 (3H, s, arylidene-CH_3), 2.39 (3H, s, 6-CH_3), 2.43 (3H, s, 2-CH_3), 7.36 (2H, d, $J = 7.98$ Hz, arylidene $C_{3,5}$–H), 7.78 (2H, d, $J = 8.06$ Hz, arylidene $C_{2,6}$–H), 8.84 (1H, s, CH=), 13.21 (1H, s, NH).

3c: IR [KBr, ν (cm^{-1})]: 3446 (N–H), 3014 (=CH), 1693 (C=O). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 2.35 (3H, s, 6-CH_3), 2.44 (3H, s, 2-CH_3), 6.96 (1H, t, $J =$ 7.44 Hz, arylidene C_5–H), 7.00 (1H, d, $J = 8.30$ Hz, arylidene C_3–H), 7.44 (1H, t, $J = 7.38$ Hz, arylidene C_4–H), 7.84 (1H, d, $J = 7.80$ Hz, arylidene C_6–H), 9.22 (1H, s, CH=), 10.40 (1H, s, ar-OH), 13.20 (1H, s, NH).

3d: IR [KBr, ν (cm^{-1})]: 3450 (N–H), 3011 (=CH), 1694 (C=O). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 2.34 (3H, s, 6-CH_3), 2.43 (3H, s, 2-CH_3), 3.86 (3H, s, ar-OCH_3), 7.11 (2H, d, $J = 8.14$ Hz, arylidene $C_{3,5}$–H),

7.86 (2H, d, $J = 8.18$ Hz, arylidene $C_{2,6}$–H), 8.77 (1H, s, CH=), 13.26 (1H, s, NH).

3e: IR [KBr, ν (cm^{-1})]: 3011 (=CH), 1693 (C=O). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 2.37 (3H, s, 6-CH_3), 2.43 (3H, s, 2-CH_3), 7.76 (2H, d, $J = 8.48$ Hz, arylidene $C_{3,5}$–H), 7.85 (2H, d, $J = 8.48$ Hz, arylidene $C_{2,6}$–H), 8.96 (1H, s, CH=).

3f: IR [KBr, ν (cm^{-1})]: 3447 (N–H), 3019 (=CH), 1695 (C=O). ^{1}H-NMR [200 MHz, δ, ppm, DMSO-d_6]: 2.37 (3H, s, 6-CH_3), 2.43 (3H, s, 2-CH_3), 7.63 (2H, d, $J = 8.39$ Hz, arylidene $C_{3,5}$–H), 7.93 (2H, d, $J = 8.55$ Hz, arylidene $C_{2,6}$–H), 8.96 (1H, s, CH=), 13.30 (1H, s, NH). EI-MS [m/z (%)]: 333 [M^+,5], 211 (100), 196 (7), 195 (15), 180 (1), 155 (2), 153 (4), 140 (4), 139 (2), 138 (3), 137 (3).

3g: IR [KBr, ν (cm^{-1})]: 3460 (N–H), 3013 (=CH), 1693 (C=O). ^{1}H-NMR [400 MHz, δ, ppm, DMSO-d_6]: 2.37 (3H, s, 6-CH_3), 2.44 (3H, s, 2-CH_3), 7.40 (2H, t, $J =$ 8.84 Hz, arylidene $C_{3,5}$–H), 7.98 (2H, dd, $J = 7.96$, 7.75 Hz, arylidene $C_{2,6}$–H), 8.93 (1H, s, CH=), 13.15 (1H, s, NH). EI-MS [m/z (%)]: 317 [M^+,100], 196 (75), 195 (59), 180 (2), 137 (19), 122 (28), 121 (29).

3h: IR [KBr, ν (cm^{-1})]: 3446 (N–H), 3015 (=CH), 1693 (C=O). ^{1}H-NMR [200 MHz, δ, ppm, DMSO-d_6]: 2.42 (3H, s, 6-CH_3), 2.45 (3H, s, 2-CH_3), 8.15 (2H, d, $J = 8.75$ Hz, arylidene $C_{2,6}$–H), 8.35 (2H, d, $J = 8.72$ Hz, arylidene $C_{3,5}$–H), 9.23 (1H, s, CH=), 13.27 (1H, s, NH).

4.2. In vitro anticancer screening

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 per 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 dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of the 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^{-1} 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 concentrations levels. Percentage growth inhibition was calculated as:

$$\frac{(\mathrm{Ti} - \mathrm{Tz})}{(C - \mathrm{Tz})} \times 100 \text{ for concentrations for which } \mathrm{Ti} \geq \mathrm{Tz}$$

$$\frac{(\mathrm{Ti} - \mathrm{Tz})}{Tz} \times 100 \text{ for concentrations for which } \mathrm{Ti} < \mathrm{Tz}$$

Three dose–response parameters were calculated for each experimental agent. Growth inhibition of 50% (GI_{50}) was calculated from $[(\mathrm{Ti}-\mathrm{Tz})/(C-\mathrm{Tz})] \times 100 =$ 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 $\mathrm{Ti} = \mathrm{Tz}$. Values were calculated for each of these 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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References

[1] A. Andreani, M. Rambaldi, F. Andreani, R. Bossa, I. Galatulas, Eur. J. Med. Chem. 23 (1988) 385–389.
[2] J. Chern, Y. Liaw, C. Chen, J. Rong, C. Huang, Heterocycles 36 (1993) 1091–1103.
[3] A. Andreani, M. Rambaldi, A. Leoni, A. Locatelli, R. Bossa, J. Med. Chem. 39 (1996) 2852–2855.
[4] H. Yoo, M. Suh, S. Park, J. Med. Chem. 41 (1998) 4716–4722.
[5] A. Andreani, A. Granaiola, A. Leoni, A. Locatelli, R. Morigi, M. Rambaldi, G. Giorgi, L. Salvini, V. Garaliene, Anti-Cancer Drug Des. 16 (2001) 167–174.
[6] J.J. Oleson, A. Sloboda, W.P. Troy, S.L. Halliday, M.J. Landes, R.B. Angier, J. Semb, K. Cyr, J.H. Williams, J. Am. Chem. Soc. 77 (1955) 6713–6714.
[7] A.K. Gadad, S.S. Karki, V.G. Rajurkar, B.A. Bhongade, Arzneim.-Forsch./Drug Res. 49 (1999) 858–863.
[8] A. Andreani, A. Leoni, A. Locatelli, R. Morigi, M. Rambaldi, M. Recanatini, V. Garaliene, Bioorg. Med. Chem. 8 (2000) 2359–2366.
[9] I.A.M. Khazi, C.S. Mahajanshetti, A.K. Gadad, A.D. Tarnalli, C.M. Sultanpur, Arzneim.-Forsch./Drug Res. 46 (1996) 949–952.
[10] A.K. Gadad, C.S. Mahajanshetti, S. Nimbalkar, A. Raichurkar, Eur. J. Med. Chem. 35 (2000) 853–857.
[11] A. Andreani, A. Leoni, A. Locatelli, R. Morigi, M. Rambaldi, W.A. Simon, J. Senn-Bilfinger, Arzneim.-Forsch./Drug Res. 50 (2000) 550–553.
[12] W.K. Amery, C.H. Hoerig, in: R.I. Fenichel, M.A. Chirigos (Eds.), Immune Modulation Agents and their Mechanism, Marcel Dekker, Newyork-Basel, 1984, pp. 383–408.
[13] S. Ban, J. Pharm. Soc. Jpn. 74 (1954) 658–661.
[14] A. Monks, D. Scudiero, P. Skehan, R. Shomaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo, M. Boyd, J. Nat. Cancer Inst. 83 (1991) 757–766.
[15] F. Leteurtre, G. Kohlhagen, K.D. Paull, Y. Pommier, J. Nat. Cancer Inst. 86 (1994) 1239–1244.
[16] S.-C. Kuo, H.-Z. Lee, J.-P. Juang, Y.-T. Lin, T.-S. Wu, J.-J. Chang, D. Lednicer, K.D. Paull, C.M. Lin, E. Hamel, K.-H. Lee, J. Med. Chem. 36 (1993) 1146–1156.