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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of new imidazo[2,1-*b*]thiazole derivatives as anticancer agents

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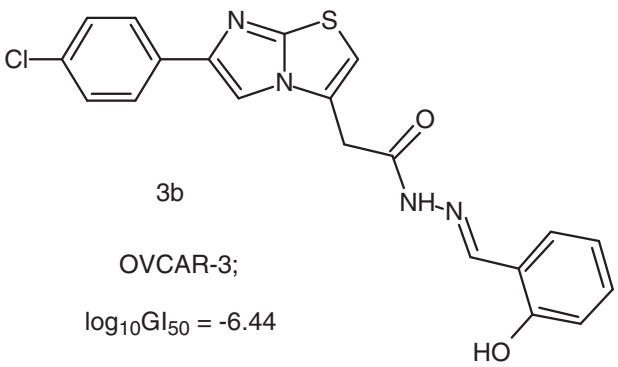
Abstract A series of arylidenehydrazide compounds (**3a**–**3j**) were synthesized from [6-(4-chlorophenyl)imidazo[2,1-*b*]thiazol-3-yl]acetic acid hydrazide. The newly synthesized compounds **3b** and **3h** were subjected to the National Cancer Institute's in vitro disease-oriented antitumor screening to be evaluated for antitumor activity. Compound **3b**, the most potent compound examined, displayed broad spectrum antiproliferative activity against all of the tested cell lines with $\log_{10}GI_{50}$ values between −4.41 and −6.44. The greatest growth inhibitions were observed against an ovarian cancer cell line(OVCAR-3), a colon cancer cell line (HCT-15), two renal cancer cell lines (CAKI-1 and UO-31) and two leukemia cell lines (CCRF-CEM and SR) with $\log_{10}GI_{10}$ values −6.44, −6.33, −6.11, −6.30, −6.13 and −6.22, respectively.



Keywords Anticancer activity · Imidazo[2 · 1-*b*]thiazole · Arylidenehydrazide

Introduction

Cancer constitutes a broad range of diseases diagnosed with abnormal cell growth. According to the World Health Organization, approximately one third of cancer deaths are related with poor diet and daily habits such as high fat diets, lack of exercise, low fiber intake, tobacco and alcohol use. On the other hand, in economically less developed countries one fifth of cancer deaths are engaged with viral infections like hepatitis B and C viruses and human papillomavirus (de Martel et al., 2012). GLOBOCAN reported 14.1 million new cases and 8.2 million deaths related with cancer in 2012 around the world (Torre et al., 2015). The design and development of novel molecules to treat cancer has spurred great interest in many industrial and academic research laboratories worldwide.

Among fused heterocyclic compounds, imidazothiazole derivatives attracted much attention owing to their

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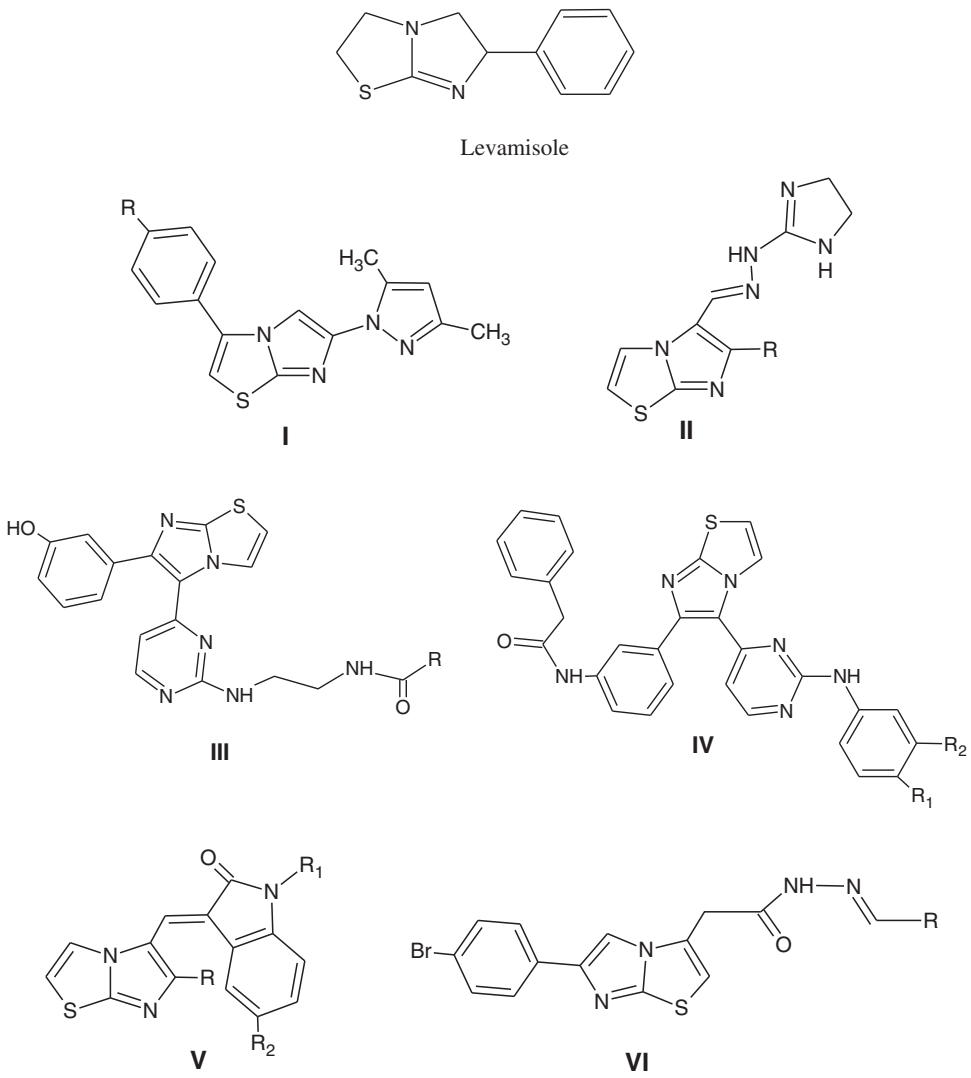
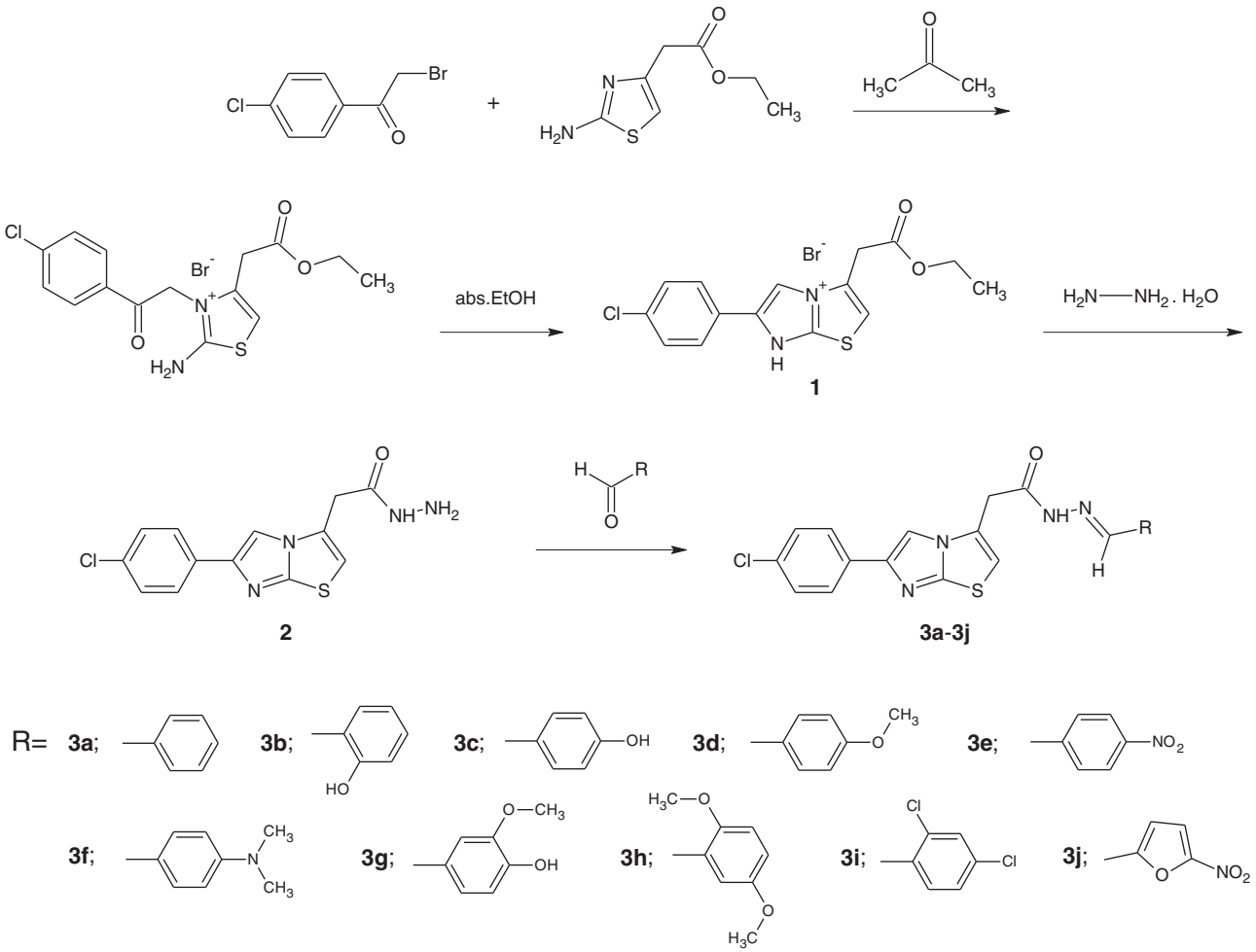


Fig. 1 The structures of some imidazo[2,1-*b*]thiazoles having antitumor activity

wide-range of biological properties such as antifungal (Malik et al., 2013), antitubercular (Samala et al., 2016), antipsychotic (Cole et al., 2007), antiviral (Wang et al., 2015) and antitumor activities (Romagnoli et al., 2015). Levamisole, 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*] thiazole has been one of the most extensively studied imidazothiazole derivative in the medicinal chemistry field (Fascio et al., 2015). Originally used for the treatment of parasitic infections, Levamisole has spurred great interest for its immunostimulating activities in cancer (Dillman, 2011). It appears that Levamisole can modulate the immune system through T-cell activation and proliferation (Chen et al., 2008), increasing the neutrophil mobility (Earhoudi et al., 1978) and stimulating the antibody formation. The combination of Levamisole with 5-fluorouracil was shown to be beneficial for patients with Dukes stage C colon cancer (Mutch and Hutson, 1991) and stage III colon cancer (Sun and Haller, 2005) following the surgical resection. Over the past years, a large number of imidazo[2,1-*b*]thiazole derivatives (Fig. 1) were recorded to possess antineoplastic activities (Ali et al., 2014 (I); Andreani et al., 2011 (II); Park et al., 2011 (III); Fidanze et al., 2010 (IV); Andreani et al., 2008 (V); Gürsoy and Ulusoy Güzeldemirci, 2007 (VI)).

Besides heterocylic ring systems, hydrazones that carry an azomethine –NHN=CH– proton constitutes a versitile class of compounds and are heavily used in drug design and development campaigns (Tripathi et al., 2011). Compounds with a hydrazide-hydrazone not only serve as intermediates in a synthesic pathway, but are in and of themselves also very effective organic compounds (Raj et al., 2015). For example, many of the hydrazones were reported to exhibit significant anticancer activities (Barbosa et al., 2011; Hayat et al., 2010; Cui et al., 2010).

One of the most promising approaches to treat cancer is to design hybrid molecules with two different biologically



Scheme 1 Synthesis of [6-(4-chlorophenyl)imidazo[2,1-*b*]thiazol-3-yl] acetic acid arylidenehydrazide derivatives (**3a–3j**)

active components that are built into one single molecule. In light of the above statement, we hypothesize that including both an imidazo[2,1-b]thiazole scaffold and a hyrazide-hydrazon core in our compounds should in return enhance the antitumoral properties of the two distinct drug entities. We previously demonstrated a successful application of such hybrid approach and showed that [6-(4-bromophenyl)imidazo[2,1-b]thiazol-3-yl]acetic acid hydrazide derivatives exhibit growth inhibitory effects on a broad range of human cancer cell lines (Gürsoy and Ulusoy Güzeldemirci, 2007). In continuation of our previous studies on the biological properties of imidazo[2,1-b]thiazole containing derivatives (Çapan et al., 1999; Ulusoy, 2002; Ulusoy et al., 2002; Ulusoy Güzeldemirci and Küçükbasmacı, 2010; Ulusoy Güzeldemirci et al., 2013) we have designed and synthesized a number of novel arylidenehyrazides bearing imidazo[2,1-*b*]thiazole moiety compounds as potential anticancer agents.

Results and discussion

Chemistry

In this study, we synthesized novel imidazo[2,1-*b*]thiazole derivatives bearing hydrazone moieties and investigated their anticancer activities. The target compounds were synthesized in four steps as shown in Scheme 1. The structures of the compounds were confirmed by analytical and spectral (UV, IR, ^{1}H NMR, ^{13}C NMR (APT), ^{13}C NMR (DEPT), HSQC-2D and ESI-MS/MS) data.

Firstly, ethyl 2-aminothiazole-4-acetate treated with 4-chloro-2′-bromoacetophenone in acetone to obtain ethyl 6-(4-chlorophenyl)imidazo[2,1-*b*]thiazole-3-acetate hydrobromide (**1**) The key intermediate 2-[6-(4-chlorophenyl) imidazo[2,1-*b*]thiazol-3-yl]acetohydrazide (**2**) was prepared by the reaction of hydrazine hydrate with **1**. Finally, condensation of **2** with appropriate aromatic aldehydes furnished the target hydrazones (**3a–3j**). The structures of the

compounds (**3a–3j**) were confirmed by UV, IR, ^{1}H NMR, ^{13}C-NMR (APT) (**3a**, **3d**, **3h** and **3i**), ^{13}C-NMR (DEPT) (**3a** and **3d**), HSQC-2D (**3a**, **3d**, **3e** and **3i**), ESI-MS spectral data and elemental analyses. Spectral data of compounds **3a**, **3b** and **3h** are presented in Supplementary Fig. S1–S28 (see Supporting Information). UV spectral data of compounds were consistent with literature values (Küçükgüzel et al., 2003). In the IR spectra exhibited the NH, C=O and C=N bands in the 3212–3135, 1687–1660 and 1622–1561 cm^{-1} regions, respectively. The absence of the NH_2 absorptions of hydrazide **2** (δ 4.33 ppm) in the ^{1}H NMR spectra of hydrazide hydrazones (**3a–j**) and the presence of new resonances for N=CH proton of **3a–j** confirmed the hydrazone formation. Furthermore, the ^{1}H NMR spectra of **3a–j** indicated the presence of two geometrical isomers in a ratio of 3:7 in DMSO-d_6 as deduced from the NH, N=CH and CH_2 protons resonating as double singlets at about δ 12.04–11.35/12.12–11.44, 8.39–7.91/8.57–8.07 and 3.97–3.85/4.39–4.27 ppm, respectively. The N=CH double bond restricted rotation is thought to give rise to the formation of E and Z isomers (Gürsoy et al., 1997; Gürsoy and Ulusoy Güzeldemirci, 2007; Çapan et al., 1999; Ayhan-Kılcıgil et al., 2012). The formation of the aimed hydrazide-hydrazone structure was approved by the ^{13}C NMR of **3a**, **3d**, **3e**, **3h** and **3i** which were selected as prototypes (Palagiano et al., 1995; Santos et al., 1998). Molecular weights of the selected compounds were confirmed using electrospray ionization tandem mass spectra (ESI-MS/MS) The cleavage of CH_2–CO, CO–NH and NH–N bonds of the hydrazide moiety was the major fragmentation pattern observed (Kingston et al., 1970; Ulusoy et al., 2001; Gürsoy and Ilhan, 1995).

Anticancer activity

Structures of the newly synthesized imidazo[2,1-*b*]thiazole derivatives (**3a-j**) were submitted to the National Cancer Institute (NCI), Bethesda, Maryland, USA (Alley et al., 1988; Grever et al., 1992; Boyd and Paull, 1995; Shoemaker, 2006) and compounds **3b** and **3h** were selected for the evaluation of their anticancer activity. The cell lines used in the NCI screen were leukemia, non-small cell lung cancer (NSCL), colon cancer, central nervous system (CNS) cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer cell lines.

The selected compounds **3b** and **3h** were initially tested at a single dose of 10^{-5} M concentration in the full NCI 60 cell panel and the percentages of the growth inhibition (GI) over the tested cell lines were determined. The results retrieved from the single dose screen of compounds **3b** and **3h** were reported as a mean graph in Figs. 2 and 3, respectively. The growth percentage was measured using a spectrophotometer and compared to controls. To estimate cell viability and growth, a 48-h continuous drug exposure protocol was followed and a sulphorhodamine B (SRB) protein assay was conducted. Bar graphs containing means were constructed for each effect and each bar was depicted with standard deviations of individual tumor cell lines. The center points on the mean graphs represent the mean of all growth inhibition percentages over all cell lines. Bars that point to the right are cell lines where the inhibition was greater than the average, while bars that point to the left are cell lines where the inhibition was less than the average. Preliminary results revealed that the lowest growth percentages of R-2,5-dimethoxyphenyl substituted compound **3h** were determined against two leukemia cell lines SR and K-562 and a prostate cancer cell line DU-145 with 32.07, 51.07 and 55.11 % growth, respectively (Fig. 3). On the other hand, R-2-hydroxyphenyl substituted **3b** showed a better antiproliferative activity profile than the compound **3h**. While compound **3b** exhibited a promising inhibitory activity over most of the cancer cell lines, the most remarkable effects were observed against leukemia cell lines SR and HL-60 (TB) and the prostate cancer cell line DU-145 with −11.51, −3.50 and −8.65 % growth, respectively (Fig. 2).

Compound **3b** was selected further for a five-dose assay and evaluated against 60 cell lines of nine tumor subpanels in 5 × 10-fold dilutions with the top dose being 10^{-4} M. The in vitro test results retrieved from five-dose assay for compound **3b** are summarized in Supplementary Table S1 (see Supporting Information). From the dose response curves illustrated in Supplementary Fig. S29 (see Supporting Information) three response parameters were calculated for each cell line. The GI_{50} value was defined as the negative $\log_{10}$ concentration that was required to inhibit 50 % of cell growth relative to untreated cells, while the TGI value was defined as the negative $\log_{10}$ minimum concentration resulting in total growth inhibition (TGI), and lastly the LC_{50} value was the negative $\log_{10}$ concentration required to kill 50 % of the total cell population. The larger negative values indicated the more sensitive cell lines. Therefore, compounds with $\log_{10}GI_{50}$ values −4 and greater were considered to be active. As can be seen from Table 1, the $\log_{10}GI_{50}$ values of compound **3b** was less than −4 for all tested cancer cell lines and thus, exhibited a broad spectrum of antitumor properties.

In our previous studies we presented that [6-(4-bromophenyl)imidazo[2,1-b]thiazol-3-yl]acetic acid arylidenehydrazides show pronounced effects against the full panel of 60 human tumor cell lines in the five-dose assay (Gürsoy and Ulusoy Güzeldemirci, 2007). Among the tested compounds, 4-hydroxybenzylidenehydrazide derivative was the most active member and the most remarkable antitumor effect of this compound was against the prostate cancer cell line PC-3 with a $\log_{10}GI_{50}$ value of < -8.00. Moreover,

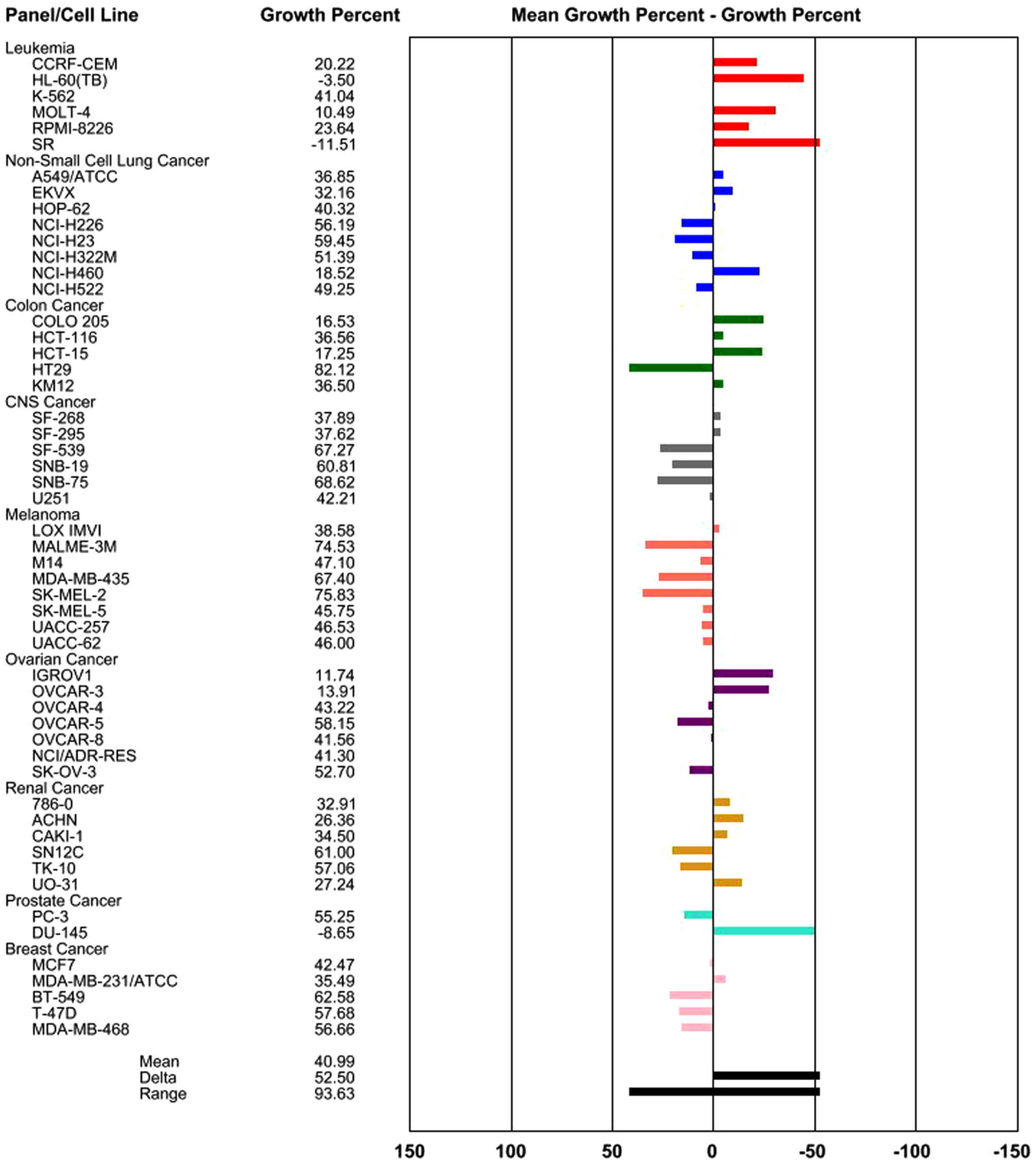


Fig. 2 One dose mean graph of **3b**

it was shown that 2-hydroxybenzylidenehydrazide derivative of 4-bromophenyl substituted imidazo[2,1-b]thiazole also displayed favorable in vitro growth inhibitory effects on all nine tumor subpanels with $\log_{10}GI_{50}$ values between −4.00 and −7.43. In the case of the newly synthesized compund **3b**, the only difference between the two compounds is the substitution of imidazo[2,1-b]thiazole moiety with 4-chlorophenyl moiety instead of 4-bromophenyl. The comparison of the tumor growth inhibiton effects of these two compounds revealed that the replacement of the bromo ion with chloro did not improve the overall cytotoxic effects of imidazo[2,1-b]thiazoles. Nevertheless, the chloro

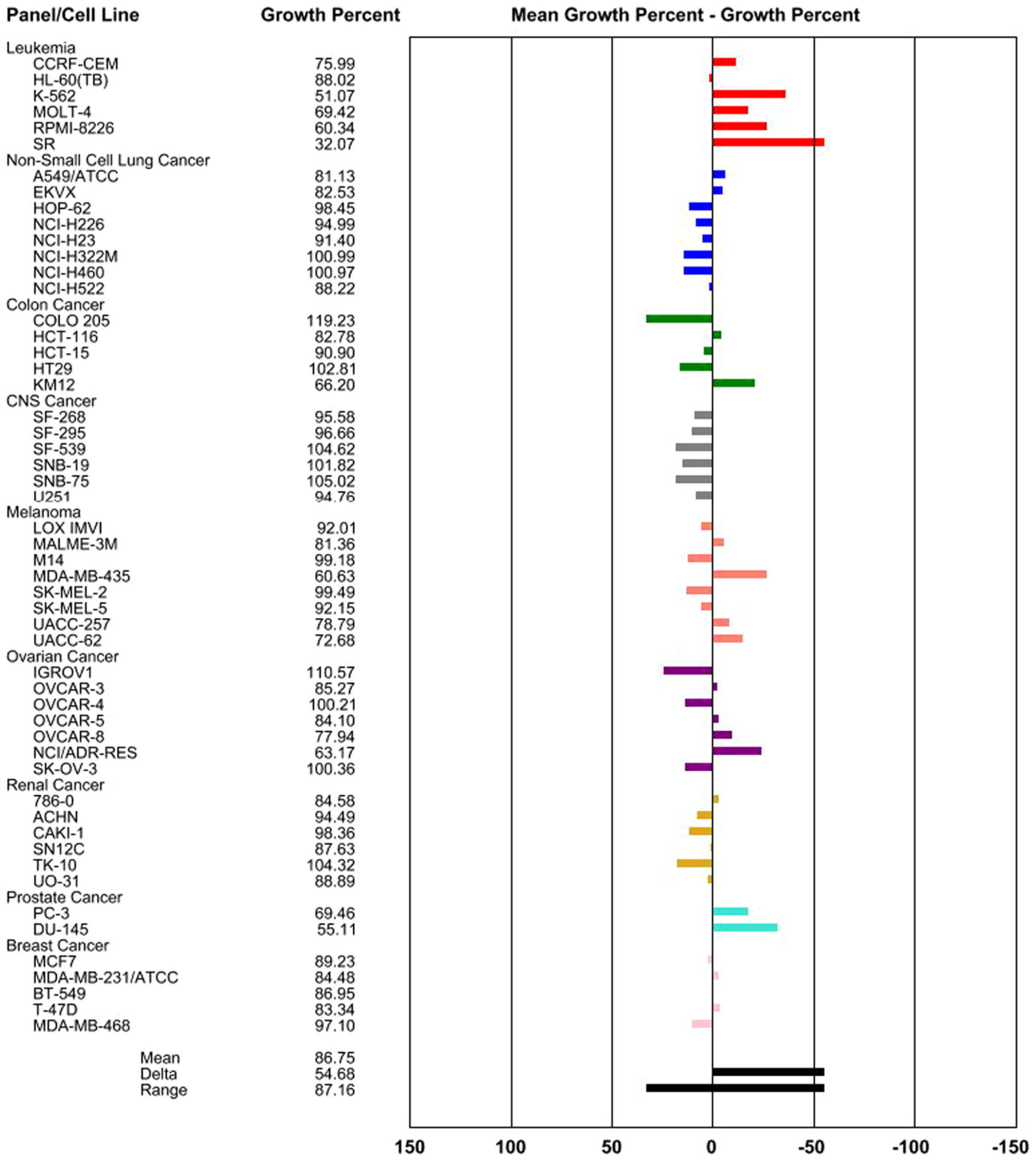


Fig. 3 One dose mean graph of **3h**

substitution improved the anticancer effect on particular cell lines such as CCRF-CEM ($log_{10}GI_{50}$ value −6.13) and MOLT-4 ($log_{10}GI_{50}$ value −5.66) from the leukemia subpanel, A549/ATCC ($log_{10}GI_{50}$ value −5.29), EKVX ($log_{10}GI_{50}$ value −5.49), NCI-H322M ($log_{10}GI_{50}$ value −5.81) and NCI-H460 ($log_{10}GI_{50}$ value −5.86) from the NSCL panel, COLO 205 ($log_{10}GI_{50}$ value −5.53), HCC-2998 ($log_{10}GI_{50}$ value −5.62), HCT-15 ($log_{10}GI_{50}$ value −6.33), KM-12 ($log_{10}GI_{50}$ value −5.55) from the colon cancer subpanel, SF-268 ($log_{10}GI_{50}$ value −5.90), SNB-75 ($log_{10}GI_{50}$ value −5.36), and U251 ($log_{10}GI_{50}$ value −5.75) from the CNS cancer subpanel, LOX IMVI ($log_{10}GI_{50}$

Table 1 Comparison of in vitro tumor cell GI of compound **3b**, Cisplatin and Sorafenib

Panel/cell line	3b			Cisplatin			Sorafenib		
	$Log_{10}GI_{50}$	$Log_{10}TGI$	$Log_{10}LC_{50}$	$Log_{10}GI_{50}$	$Log_{10}TGI$	$Log_{10}LC_{50}$	$Log_{10}GI_{50}$	$Log_{10}TGI$	$Log_{10}LC_{50}$
Leukemia									
CCRF-CEM	−6.13	> −4.00	> −4.00	−5.257	−4.215	−4.06	−5.665	−5.037	−4
HL-60(TB)	−5.78	−5.07	> −4.00	−5.18	−4.047	−4	−5.793	−4.595	−4
K-562	−5.47	> −4.00	> −4.00	−4.577	−4.062	−4	−5.547	−4	−4
MOLT-4	−5.66	> −4.00	> −4.00	−4.877	−4.186	−4	−5.538	−4	−4
RPMI-8226	−5.47	> −4.00	> −4.00	−4.774	−4.073	−4	−5.837	−5.319	−4
SR	−6.22	> −4.00	> −4.00	−5.113	−4.266	−4	−5.515	−4.885	−4
Non-small cell lung cancer									
A549/ATCC	−5.29	> −4.00	> −4.00	−4.664	−4.152	−4	−5.533	−4.867	−4.068
EKVX	−5.49	> −4.00	> −4.00	−4.452	−4.097	−4	−5.602	−4.91	−4.062
HOP-62	−5.33	> −4.00	> −4.00	−5.063	−4.349	−4.02	−5.719	−5.372	−4.995
HOP-92	−5.47	−4.49	> −4.00	−4.706	−4.156	−4.013	−5.796	−5.242	−4.303
NCI-H226	−5.33	> −4.00	> −4.00	−4.594	−4.235	−4.025	−5.721	−5.159	−4.442
NCI-H23	−5.27	> −4.00	> −4.00	−5.412	−4.413	−4	−5.748	−5.122	−4.197
NCI-H322M	−5.81	−4.23	> −4.00	−4.507	−4.103	−4	−5.552	−5.082	−4.314
NCI-H460	−5.86	> −4.00	> −4.00	−5.627	−4.493	−4.025	−5.634	−5.189	−4.521
Colon cancer									
COLO 205	−5.53	> −4.00	> −4.00	−4.162	−4.061	−4.03	−5.661	−5.273	−4.451
HCC-2998	−5.62	> −4.00	> −4.00	−4.746	−4.265	−4.081	−5.519	−5.003	−4.367
HCT-15	−6.33	> −4.00	> −4.00	−4.406	−4.113	−4.026	−5.607	−5.036	−4.348
KM12	−5.55	> −4.00	> −4.00	−4.405	−4.064	−4.022	−5.803	−5.229	−4.573
SW-620	−5.17	> −4.00	> −4.00	−4.715	−4.013	−4	−5.56	−5.059	−4.474
CNS cancer									
SF-268	−5.90	−4.26	> −4.00	−5.4	−4.531	−4	−5.598	−5.13	−4.573
SF-295	−5.28	> −4.00	> −4.00	−5.188	−4.232	−4.019	−5.786	−5.354	−4.667
SF-539	−5.10	> −4.00	> −4.00	−5.29	−4.618	−4.194	−5.787	−5.292	−4.733
SNB-19	−4.91	> −4.00	> −4.00	−4.755	−4.077	−4	−5.465	−4.923	−4.322
SNB-75	−5.36	> −4.00	> −4.00	−5.057	−4.341	−4.02	−5.529	−5.018	−4.52
U251	−5.75	−4.45	> −4.00	−4.947	−4.31	−4.127	−5.677	−5.236	−4.713
Melanoma									
LOX IMVI	−5.71	−4.44	> −4.00	−5.178	−4.413	−4.178	−5.784	−5.351	−4.865
MALME-3M	−5.26	−4.03	> −4.00	−4.908	−4.429	−4.208	−5.668	−4.86	−4
MDA-MB-435	−5.21	> −4.00	> −4.00	−4.609	−4.245	−4.05	−5.751	−5.326	−4.63
SK-MEL-28	−5.26	> −4.00	> −4.00	−4.658	−4.158	−4	−5.579	−5.127	−4.621
SK-MEL-5	−5.53	> −4.00	> −4.00	−4.957	−4.391	−4.163	−5.816	−5.495	−5.174
UACC-257	−5.32	> −4.00	> −4.00	−4.597	−4.196	−4.009	−5.668	−5.239	−4.632
UACC-62	−5.47	−4.41	> −4.00	−4.889	−4.353	−4.12	−5.773	−5.35	−4.868
Ovarian cancer									
IGROV1	−5.52	> −4.00	> −4.00	−5.032	−4.383	−4.152	−5.584	−5.063	−4.216
OVCAR-3	−6.44	−4.08	> −4.00	−4.99	−4.506	−4	−5.532	−5.014	−4.535
OVCAR-4	−5.82	> −4.00	> −4.00	−4.934	−4.259	−4	−5.455	−4.635	−4
OVCAR-5	−4.41	> −4.00	> −4.00	−4.799	−4.116	−4	−5.531	−4.811	−4.058
OVCAR-8	−5.52	−4.10	> −4.00	−4.553	−4.112	−4.042	−5.528	−4.675	−4
NCI/ADR-RES	−5.58	−4.09	> −4.00	−4.696	−4.083	−4	−5.596	−4.766	−4
SK-OV-3	−5.30	> −4.00	> −4.00	−4.632	−4.113	−4	−5.64	−5.214	−4.608

Table 1 continued

Panel/cell line	3b			Cisplatin			Sorafenib		
	$Log_{10}GI_{50}$	$Log_{10}TGI$	$Log_{10}LC_{50}$	$Log_{10}GI_{50}$	$Log_{10}TGI$	$Log_{10}LC_{50}$	$Log_{10}GI_{50}$	$Log_{10}TGI$	$Log_{10}LC_{50}$
Renal cancer									
A498	−5.08	> −4.00	> −4.00	−4.284	−4.146	−4.054	−5.645	−5.175	−4.654
ACHN	−5.75	> −4.00	> −4.00	−5.14	−4.489	−4.015	−5.553	−4.808	−4.029
CAKI-1	−6.11	−4.80	−4.80	−5.351	−4.316	−4	−5.544	−5.058	−4.261
RXF 393	−5.57	−4.40	−4.40	−4.691	−4.247	−4.027	−5.472	−5.012	−4.328
SN 12C	−4.96	> −4.00	> −4.00	−4.627	−4.082	−4	−5.628	−5.12	−4.555
UO-31	−6.30	−4.36	−4.36	−4.794	−4.28	−4.036	−5.59	−5.208	−4.337
Prostate cancer									
PC-3	−5.46	> −4.00	> −4.00	−4.608	−4.124	−4	−5.69	−5.104	−4
DU-145	−5.57	> −4.00	> −4.00	−5.165	−4.324	−4	−5.476	−4.899	−4.353
Breast cancer									
MCF7	−5.48	> −4.00	> −4.00	−4.908	−4	−4	−5.553	−4.999	−4.104
MDA-MB-231/ATCC	−4.88	> −4.00	> −4.00	−4.124	−4	−4	−5.899	−5.379	−4.743
HS 578T	−5.00	> −4.00	> −4.00	−4.634	−4.058	−4	−5.582	−4.708	−4
T-47D	−5.38	> −4.00	> −4.00	−4.301	−4	−4	−5.758	−5.158	−4.092
MDA-MB-468	−5.80	−5.31	−5.31	−5.196	−4.715	−4.194	−5.696	−5.117	−4.369

value −5.71), and SK-MEL-28 ($log_{10}GI_{50}$ value −5.26) from the melanoma subpanel, OVCAR-3 ($log_{10}GI_{50}$ value −6.44) and SK-OV-3 ($log_{10}GI_{50}$ value −5.30) from the ovarian cancer subpanel, RXF 393 ($log_{10}GI_{50}$ value −5.57) and UO-31 ($log_{10}GI_{50}$ value −6.30) from the renal cancer subpanel, PC3 ($log_{10}GI_{50}$ value −5.46) and DU-145 ($log_{10}GI_{50}$ value −5.57) from the prostate cancer subpanel, and finally MCF7 ($log_{10}GI_{50}$ value −5.48), HS 578T ($log_{10}GI_{50}$ value −5.00) and T-47D ($log_{10}GI_{50}$ value −5.38) from the breast cancer subpanel. On the same cancer cell lines, the $log_{10}GI_{50}$ values of the bromo substituted compound were −5.74, −5.63, −5.21, −5.41, −5.67, −4.80, −5.29, −5.46, −6.02, −5.44, > −4.00, −4.89, −5.44, −5.46, −4.92, −5.49, −4.89, −4.42, −5.15, −5.37, −5.54, −4.88, −4.87, and −5.28.

In the next step, we compared the cytotoxic effect of compound **3b** with the two well-known anticancer drugs Cisplatin (CAS no. 15663-27-1) and Sorafenib (CAS no. 475207-59), which differ in their molecular mechanism of action, but show remarkable cytotoxic activities on a wide range of human cancer cell lines such as compound **3b**. While Cisplatin is an alkylating agent that covalently binds to DNA and induces cellular apoptosis (Dasari and Tchounwou, 2014), Sorafenib targets several protein kinases such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) receptor families, and serine/threonine kinase Raf (Adnane et al., 2006). Cisplatin was first approved for the treatment of ovarian and testicular cancer by the US Food and Drug Administration (FDA) in 1978 (Kelland, 2007). Since that time, Cisplatin has been used in clinical trials alone or in combination with other drugs for the treatment of a variety of cancer types, including head and neck, bladder, cervical, lung, ovarian, and testicular cancers (Dasari and Tchounwou, 2014). In comparison to Cisplatin, Sorafenib is a much newer chemotherapeutic agent used for cancer treatment. Sorafenib received its first FDA approval in 2005 for the treatment of renal cell carcinoma (RCC) (Fishman et al., 2015), which was followed by two more FDA approvals in 2007 and 2013 for the treatment of hepatocellular and thyroid carcinomas, respectively (Cervello et al., 2012; Wang et al., 2013; White and Cohen, 2015).

Biological data of Cisplatin (NSC-119875) and Sorafenib (NSC-747971) was taken from publicly available NCI Anti-cancer Agent Mechanism Database (Weinstein et al., 1997; van Osdol et al., 1994) for comparison of three response parameters, GI_{50}, TGI and LC_{50} values, with that of compound **3b**. Compound **3b** demonstrated higher potencies against all human cancer cell lines except NCI-H23 (a NSCL cancer cell line), SF-539 (a CNS cancer cell line), and OVCAR-5 (an ovarian cancer cell line) than that of the reference compound, Cisplatin (Table 1). Nevertheless, compound **3b** showed only a higher cytotoxic effect on CCRF-CEM, MOLT-4, and SR out of 9 leukemia cell lines than that of Sorafenib. In addition, Sorafenib demonstrated a greater inhibitory effect on all melanoma cancer cell lines in comparison to compound **3b**. A similar trend could be seen for the prostate and breast cancer cell lines. Sorafenib inhibited the cell growth more effectively than

compound **3b** except for the prostate cancer cell line; PC-3 and the breast cancer cell line; MDA-MB-468. Among seven ovarian cancer cell lines, compound **3b** exhibited a higher anitumoral activity on OVCAR-3 and OVCAR-4 cell lines than Sorafenib did. Most surprisingly, compound **3b** showed a better inhibitory effect on tumor cell growth for all renal cancer cell lines except A498 and SN 12C than the well-known anti-RCC agent, Sorafenib.

Conclusion

In this work, we present a synthesis and primary cytotoxicity evaluation of a new series of imidazo[2,1-*b*]thiazoles. Among these compounds, R-2-hydroxyphenyl substituted compound **3b** showed significant antiproliferative activity towards the 60 human tumor cell lines in nine cancer types. The most pronounced effect of this compound was seen on the ovarian cancer cell line OVCAR-3 ($\log_{10}GI_{50}$ value −6.44). On the same cancer cell line, the $\log_{10}GI_{50}$ values of the two well-known anticancer drugs, Cisplatin and Sorafenib, were −4.99 and −5.53, respectively. In addition, compound **3b** also exhibited significant antiproliferation activity on CCRF-CEM and SR, two leukemia cell lines, NCI-H322M and NCI-H460, two NSCL cancer cell lines, HCT-15, a colon cancer cell line, SF-268, a CNS cancer cell line, CAKI-1 and UO-31, two renal cancer cell lines and MDA-MB-468, a breast cancer cell line with $\log_{10}GI_{50}$ values of −6.13, −6.22, −5.81, −5.86, −6.33, −5.90, −6.11, −6.30, and −5.80, respectively. Furthermore, compound **3b** inhibited the in vitro growth of ACHN ($\log_{10}GI_{50}$ value −5.75), CAKI-1 ($\log_{10}GI_{50}$ value −6.11), RXF-393 ($\log_{10}GI_{50}$ value −5.57) and UO-31 ($\log_{10}GI_{50}$ value −6.30) renal cancer cell lines more effectively than Sorafenib. Collectively, these findings suggest that compound **3b** could be a promising starting point to develop anticancer agents, especially for the treatment of ovarian and renal cell carcinomas. Currently, further synthesis and biological investigations for similar novel analogs are underway in our laboratory.

Experimental

Chemistry

All chemicals were purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, MO, USA) chemical companies. Using a Büchi B-540 melting point apparatus (Flawil, Switzerland) with open capillaries, melting points were determined and are uncorrected. Elemental analyses were performed on a Thermo Finnigan Flash EA 1112 elemental analyzer. IR spectra were recorded (in KBr) using a Perkin Elmer Spectrum 100 FT-IR spectrometer and Shimadzu IRAffinity-1 FTIR spectrophotometer. ^{1}H and ^{13}C-NMR spectra were obtained on Varian ^{UNITY} INOVA 500 MHz spectrometer using DMSO-d_6 as an internal standard. All chemical shifts were reported as δ (ppm) values and spin–spin couplings (J) were exposed in Hz. MS (ESI-) were determined on a Finnigan LCQ Advantage Max mass spectrometer.

General procedure for synthesis of compounds

*Ethyl 6-(4-chlorophenyl)imidazo[2,1-b]thiazole-3-acetate hydrobromide **(1)***

These compounds were obtained according to the procedure described by Robert et al. (1975).

*2-[6-(4-Chlorophenyl)imidazo[2,1-b]thiazol-3-yl] acetohydrazide **(2)***

These compounds were prepared according to the procedure described by Kühmstedt et al. (1983).

General synthesis of [6-(4-chlorophenyl)imidazo[2,1-b] thiazol-3-yl]acetic acid arylidenehydrazide derivatives 3a–3j

A solution of 0.005 mol of compound **2** and 0.005 mol of an appropriate aromatic aldehyde in 100 ml ethanol was heated under reflux for 5 h. The precipitate obtained was purified either by recrystallization from ethanol or by washing with hot ethanol.

*[6-(4-Chlorophenyl)imidazo[2,1-b]thiazol-3-yl]acetic acid benzylidenehydrazide **3a***

Yield 66 %, mp 265–266 °C, UV λ_{max} (nm): 226 (ε 22053), 277 (ε 40470). IR ν_{max} (cm^{-1}): 3151 (N–H stretching), 1685 (amide I C=O stretching), 1613 (hydrazone C=N stretching). ^{1}H NMR (500MHz, δ, ppm, DMSO-d_6): 3.93, 4.36 (2s, 2H, CH_2CO), 7.14, 7.15 (2s, 1H, imidazothiazole C_2-H), 7.43–7.46 (m, 5H, arylidene $C_{3,4,5}$-H and Cl–Ph $C_{3,5}$-H), 7.69, 7.73 (2d, 2H, $J = 7.81$, 7.81 Hz, arylidene $C_{2,6}$-H), 7.81, 7.84 (2d, 2H, $J = 8.30$, 8.29 Hz, Cl–Ph $C_{2,6}$-H), 8.07, 8.25 (2s, 1H, N=CH), 8.32, 8.35 (2s, 1H, imidazothiazole C_5-H), 11.66, 11.77 (2s, 1H, CONH). ^{13}C NMR (APT) (125 MHz, δ, ppm, DMSO-d_6): 32.52, 34.04 (CH_2), 109.75, 110.03 (imidazothiazole C_5), 111.29, 111.44 (imidazothiazole C_2), 127.01, 127.06 (Cl–Ph $C_{2,6}$), 127.12, 127.54 (imidazothiazole C_3), 127.62, 127.81 (arylidene $C_{2,6}$), 129.36, 129.38 (Cl–Ph $C_{3,5}$), 129.52 (arylidene $C_{3,5}$), 130.66, 130.84 (arylidene C_4), 132.09, 132.14 (arylidene C_1),

133.54 (Cl–Ph C_1), 134.76, 134.79 (Cl–Ph C_4), 144.43, 147.80 (N=CH), 144.89, 145.16 (imidazothiazole C_6), 149.38, 149.49 (imidazothiazole C_{7a}), 163.98, 169.70 (CONH). ^{13}C NMR (DEPT) (125 MHz, δ, ppm, DMSO-d_6): 32.51, 34.01 (CH_2), 109.81, 110.10 (imidazothiazole C_5), 111.45, 111.57 (imidazothiazole C_2), 127.03, 127.08 (Cl–Ph $C_{2,6}$), 127.62, 127.81 (arylidene $C_{2,6}$), 129.38 (Cl–Ph $C_{3,5}$), 129.53 (arylidene $C_{3,5}$), 130.66, 130.84 (arylidene C_4), 144.42, 147.78 (N=CH). ^{13}C NMR (HSQC) (125 MHz, δ, ppm, DMSO-d_6): 32.52, 34.04 (CH_2), 109.75, 110.03 (imidazothiazole C_5), 111.29, 111.45 (imidazothiazole C_2), 127.01, 127.07 (Cl–Ph $C_{2,6}$), 127.12, 127.54 (imidazothiazole C_3), 127.62, 127.81 (arylidene $C_{2,6}$), 129.36, 129.38 (Cl–Ph $C_{3,5}$), 129.50, 129.52 (arylidene $C_{3,5}$), 130.66, 130.84 (arylidene C_4), 132.10, 132.15 (arylidene C_1), 133.53, 133.55 (Cl–Ph C_1), 134.76, 134.79 (Cl–Ph C_4), 144.43, 147.80 (N=CH), 144.88, 145.15 (imidazothiazole C_6), 149.38, 149.48 (imidazothiazole C_{7a}), 163.97, 169.70 (CONH). MS (ESI-) *m/z* (rel intensity %): 395 ([M–H+2]$^-$, 31), 393 ([M–H]$^-$, 100). MS2 (ESI-) *m/z* (rel intensity %): 393 ([M–H]$^-$, 30), 289 (100), 273 (1), 247 (10). Anal. for $C_{20}H_{15}ClN_4OS.H_2O$, Calcd. C, 58.18 H, 4.15 N, 13.57 Found C, 57.93 H, 3.74 N, 13.29.

[6-(4-Chlorophenyl)imidazo[2,1-b]thiazol-3-yl]acetic acid 2-hydroxybenzylidenehydrazide ***3b***

Yield 85 %, mp 257–258 °C, UV λ_{max} (nm): 228 (ε 23374), 279 (ε 31437), 291 (ε 26785), 323 (ε 9613). IR ν_{max} (cm^{-1}): 3138 (N-H stretching), 1685 (amide I C=O stretching), 1622 (hydrazone C=N stretching). ^{1}H NMR (500 MHz, δ, ppm, DMSO-d_6): 3.93, 4.31 (2s, 2H, CH_2CO), 6.83-6.91 (m, 2H, arylidene $C_{3,5}$-H), 7.09, 7.12 (2s, 1H, imidazothiazole C_2-H), 7.23–7.29 (m, 1H, arylidene C_4-H), 7.41–7.45 (m, 2H, Cl–Ph $C_{3,5}$-H), 7.54, 7.74 (2d, 1H, $J = 7.81$, 7.32 Hz, arylidene C_6-H), 7.81, 7.84 (2d, 2H, $J = 7.81$, 8.30 Hz, Cl–Ph $C_{2,6}$-H), 8.28, 8.29 (2s, 1H, imidazothiazole C_5-H), 8.36, 8.45 (2s, 1H, N=CH), 10.05, 10.97 (2s, 1H, arylidene C_2-OH), 11.58, 11.95 (2s, 1H, CONH). MS (ESI-) *m/z* (rel intensity %): 411 ([M–H+2]$^-$, 32), 409 ([M–H]$^-$, 100), 273 (3). MS2 (ESI-) *m/z* (rel intensity %): 409 ([M–H]$^-$, 51), 290 (1), 273 (100). Anal. for $C_{20}H_{15}ClN_4O_2S$, Calcd. C, 58.46 H, 3.68 N, 13.64 Found C, 58.30 H, 3.82 N, 13.44.

[6-(4-Chlorophenyl)imidazo[2,1-b]thiazol-3-yl]acetic acid 4-hydroxybenzylidenehydrazide ***3c***

Yield 75 %, mp 278–278 °C, UV λ_{max} (nm): 228 (ε 37801), 284 (ε 38253). IR ν_{max} (cm^{-1}): 3149 (N-H stretching), 1667 (amide I C=O stretching), 1607 (hydrazone C=N stretching). ^{1}H NMR (500 MHz, δ, ppm, DMSO-d_6): 3.86, 4.28 (2s, 2H, CH_2CO), 6.81 (d, 2H, $J = 8{,}30$ Hz, arylidene $C_{3,5}$-H), 7.01, 7.07 (2s, 1H, imidazothiazole C_2-H), 7.40–7.45 (m, 2H, Cl–Ph $C_{3,5}$-H), 7.51–7.55 (m, 2H, arylidene $C_{2,6}$-H), 7.80–7.85 (m, 2H, Cl–Ph $C_{2,6}$-H), 7.95, 8.12 (2s, 1H, N=CH), 8.25, 8.27 (2s, 1H, imidazothiazole C_5-H), 9.88, 9.89 (2s, 1H, arylidene C_4-OH), 11.43, 11.52 (2s, 1H, CONH). MS (ESI-) *m/z* (rel intensity %): 411 ([M–H+2]$^-$, 16), 409 ([M–H]$^-$, 100). MS2 (ESI-) *m/z* (rel intensity %): 409 ([M–H]$^-$, 17), 289 (52), 288 (100), 274 (1), 273 (3), 247 (10). Anal. for $C_{20}H_{15}ClN_4O_2S$, Calcd. C, 58.46 H, 3.68 N, 13.64 Found C, 58.05 H, 3.78 N, 13.74.

[6-(4-Chlorophenyl)imidazo[2,1-b]thiazol-3-yl]acetic acid 4-methoxybenzylidenehydrazide ***3d***

Yield 88 %, mp 239–240 °C, UV λ_{max} (nm): 228 (ε 36033), 290 (ε 43979). IR ν_{max} (cm^{-1}): 3212, 3138 (N–H stretching), 1660 (amide I C=O stretching), 1608 (hydrazone C=N stretching). ^{1}H NMR (500 MHz, δ, ppm, DMSO-d_6): 3.78, 3.79 (2s, 3H, arylidene C_4-OCH_3), 3.88, 4.30 (2s, 2H, CH_2CO), 6.97–7.00 (m, 2H, arylidene $C_{3,5}$-H), 7.08 (s, 1H, imidazothiazole C_2-H), 7.40–7.44 (m, 2H, Cl–Ph $C_{3,5}$-H), 7.63–7.67 (m, 2H, arylidene $C_{2,6}$-H), 7.81–7.85 (m, 2H, Cl–Ph $C_{2,6}$-H), 8.00, 8.17 (2s, 1H, N=CH), 8.26, 8.28 (2s 1H, imidazothiazole C_5-H), 11.51, 11.60 (2s, 1H, CONH). ^{13}C NMR (APT) (125 MHz, δ, ppm, DMSO-d_6): 32.56, 34.10 (CH_2), 56.00 (arylidene OCH_3), 109.61, 109.89 (imidazothiazole C_5), 110.84, 111.09 (imidazothiazole C_2), 115.02 (arylidene $C_{3,5}$), 126.97, 127.03 (Cl–Ph $C_{2,6}$), 127.14, 127.50 (imidazothiazole C_3), 127.33, 127.37 (arylidene C_1), 129.21, 129.42 (arylidene $C_{2,6}$), 129.29, 129.34 (Cl–Ph $C_{3,5}$), 131.91, 132.01 (Cl–Ph C_1), 133.89, 133.98 (Cl–Ph C_4), 144.30, 147.72 (N=CH), 145.37, 145.52 (imidazothiazole C_6), 149.46, 149.55 (imidazothiazole C_{7a}), 161.43, 161.61 (arylidene C_4), 163.69, 169.44 (CONH). ^{13}C NMR (DEPT) (125 MHz, δ, ppm, DMSO-d_6): 32.55, 34.08 (CH_2), 56.00 (arylidene OCH_3), 109.92, 109.89 (imidazothiazole C_5), 110.85 (imidazothiazole C_2), 115.02 (arylidene $C_{3,5}$), 126.96, 127.02 (Cl–Ph $C_{2,6}$), 129.21, 129.42 (arylidene $C_{2,6}$), 129.30, 129.35 (Cl–Ph $C_{3,5}$), 144.27, 147.67 (N=CH). ^{13}C NMR (HSQC) (125 MHz, δ, ppm, DMSO-d_6): 32.55, 34.10 (CH_2), 55.99 (arylidene OCH_3), 109.61, 109.90 (imidazothiazole C_5), 110.84, 111.09 (imidazothiazole C_2), 115.02 (arylidene $C_{3,5}$), 126.97, 127.03 (Cl–Ph $C_{2,6}$), 127.13, 127.50 (imidazothiazole C_3), 127.33, 127.37 (arylidene C_1), 129.21, 129.42 (arylidene $C_{2,6}$), 129.29, 129.34 (Cl–Ph $C_{3,5}$), 131.90, 132.01 (Cl–Ph C_1), 133.89, 133.99 (Cl–Ph C_4), 144.29, 147.71 (N=CH), 145.37, 145.52 (imidazothiazole C_6), 149.45, 149.55 (imidazothiazole C_{7a}), 161.43, 161.60 (arylidene C_4), 163.69, 169.44 (CONH). MS (ESI-) *m/z* (rel intensity %): 425 ([M–H+2]$^-$, 32); 423 ([M–H]$^-$, 100). MS2 (ESI-) *m/z* (rel intensity %): 423 ([M–H]$^-$, 8), 290 (20), 289 (97), 247 (47), 208 (100). Anal. for $C_{21}H_{17}ClN_4O_2S$, Calcd. C, 59.36 H, 4.03 N, 13.19 Found C, 59.25 H, 3.94 N, 13.18.

[6-(4-Chlorophenyl)imidazo[2,1-b]thiazol-3-yl]acetic acid 4-nitrobenzylidenehydrazide ***3e***

Yield 86 %, mp 260–261 °C, UV λ_{max} (nm): 233 (ε 32431), 258 (ε 35406), 321 (ε 25013). IR ν_{max} (cm^{-1}): 3206, 3146 (N-H stretching), 1682 (amide I C=O stretching), 1608 (hydrazone C=N stretching). ^{1}H NMR (500 MHz, δ, ppm, DMSO-d_6): 3.96, 4.39 (2s, 2H, CH_2CO) 7.11 (s, 1H, imidazothiazole C_2-H), 7.41–7.45 (m, 2H, Cl–Ph $C_{3,5}$-H), 7.82, 7.85 (2d, 2H, J = 8.79, 8.78 Hz, Cl–Ph $C_{2,6}$-H), 7.96, 8.00 (2d, 2H, J = 8.79, 8.79 Hz, arylidene $C_{2,6}$-H), 8.16, 8.34 (2s, 1H, N=CH), 8.26–8.29 (m, 3H, arylidene $C_{3,5}$-H and imidazothiazole C_5-H), 11.94, 12.04 (2s, 1H, CONH). ^{13}C NMR (HSQC) (125 MHz, δ, ppm, DMSO-d_6): 32.58, 34.09 (CH_2), 109.64, 109.89 (imidazothiazole C_5), 111.14, 111.35 (imidazothiazole C_2), 124.70 (arylidene $C_{3,5}$), 126.79, 127.17 (imidazothiazole C_3), 126.97, 127.03 (Cl–Ph $C_{2,6}$), 128.56, 128.75 (arylidene $C_{2,6}$), 129.31, 129.34 (Cl–Ph $C_{3,5}$), 131.93, 132.02 (Cl–Ph C_1), 133.87, 133.96 (Cl–Ph C_4), 141.07, 141.14 (arylidene C_1), 142.01, 145.34 (N=CH), 145.42, 145.55 (imidazothiazole C_6), 148.49, 148.64 (arylidene C_4), 149.47, 149.54 (imidazothiazole C_{7a}), 164.49, 170.17 (CONH). MS (ESI-) *m/z* (rel intensity %): 440 ([M–H+2]$^-$, 30), 438 ([M–H]$^-$, 100). MS2 (ESI-) *m/z* (rel intensity %): 438 ([M–H]$^-$, 49), 290 (2), 273 (100). Anal. for $C_{20}H_{14}ClN_5O_3S$, Calcd. C, 54.61 H, 3.21 N, 15.92 Found C, 54.98 H, 3.39 N, 15.89.

[6-(4-Chlorophenyl)imidazo[2,1-b]thiazol-3-yl]acetic acid 4-dimethylaminobenzylidenehydrazide ***3f***

Yield 88 %, mp 257–259 °C, UV λ_{max} (nm): 255 (ε 23401), 346 (ε 27780). IR ν_{max} (cm^{-1}): 3149 (N–H stretching), 1674 (amide I C=O stretching), 1613 (hydrazone C=N stretching). ^{1}H NMR (500 MHz, δ, ppm, DMSO-d_6): 2.95 (s, 6H, arylidene C_4-N$(CH_3)_2$), 3.85, 4.27 (2s, 2H, CH_2CO), 6.70, 6.72 (2d, 2H, J = 8.78, 8.79 Hz, arylidene $C_{3,5}$-H), 7.07 (s, 1H, imidazothiazole C_2-H), 7.40, 7.43 (2d, 2H, J = 8.78, 8.78 Hz, Cl–Ph $C_{3,5}$-H), 7.49, 7.51 (2d, 2H, J = 7.32, 8.79 Hz, arylidene $C_{2,6}$-H), 7.81, 7.84 (2d, 2H, J = 8.29, 8.78 Hz, Cl–Ph $C_{2,6}$-H), 7.91, 8.07 (2s, 1H, N=CH), 8.26, 8.28 (2s, 1H, imidazothiazole C_5-H), 11.35, 11.44 (2s, 1H, CONH). MS (ESI-) *m/z* (rel intensity %): 438 ([M–H+2]$^-$, 41), 436 ([M–H]$^-$, 100). MS2 (ESI-) *m/z* (rel intensity %): 436 ([M–H]$^-$, 31), 288 (100), 247 (66), 208 (88). Anal. for $C_{22}H_{20}ClN_5OS$, calcd. C, 60.34 H, 4.60 N, 15.99 found C, 60.35 H, 4.46 N, 15.75.

[6-(4-Chlorophenyl)imidazo[2,1-b]thiazol-3-yl]acetic acid 3-methoxy-4-hydroxybenzylidenehydrazide ***3g***

Yield 82 %, mp 256–257 °C, UV λ_{max} (nm): 230 (ε 25735), 282 (ε 29635), 317 (ε 23148). IR ν_{max} (cm^{-1}): 3187 (N–H stretching), 1683 (amide I C=O stretching), 1601 (hydrazone C=N stretching). ^{1}H NMR (500 MHz, δ, ppm, DMSO-d_6): 3.78, 3.79 (2s, 3H, arylidene C_3-OCH_3), 3.87, 4.31 (2s, 2H, CH_2CO), 6.82 (d, 1H, J = 8.30 Hz, arylidene C_5-H), 7.07, 7.08 (2s, 1H, imidazothiazole C_2-H), 7.10, 7.11 (2d, 1H, J = 8.05, 1.71 Hz, arylidene C_6-H), 7.26, 7.29 (2d, 1H, J = 1.96, 1.95 Hz, arylidene C_2-H), 7.41, 7.43 (2d, 2H, J = 8.79, 8.78 Hz, Cl–Ph $C_{3,5}$-H), 7.80, 7.84 (2d, 2H, J = 8.78, 8.78 Hz, Cl–Ph $C_{2,6}$-H), 7.94, 8.11 (2s, 1H, N=CH), 8.25, 8.27 (2s, 1H, imidazothiazole C_5-H), 9.48, 9.51 (2s, 1H, arylidene C_4-OH), 11.47, 11.55 (2s, 1H, CONH). MS (ESI-) *m/z* (rel intensity %): 441 ([M–H+2]$^-$, 40), 439 ([M–H]$^-$, 100). MS2 (ESI-) *m/z* (rel intensity %): 439 ([M–H]$^-$, 19), 289 (43), 288 (100), 273 (2), 208 (92). Anal. for $C_{21}H_{17}ClN_4O_3S$, calcd. C, 57.21 H, 3.89 N, 12.71 found C, 56.98 H, 4.27 N, 12.83.

[6-(4-Chlorophenyl)imidazo[2,1-b]thiazol-3-yl]acetic acid 2,5-dimethoxybenzylidenehydrazide ***3h***

Yield 90 %, mp 240–242 °C, UV λ_{max} (nm): 229 (ε 18554), 270 (ε 30284), 345 (ε 9322). IR ν_{max} (cm^{-1}): 3172, 3142 (N–H stretching), 1671 (amide I C=O stretching), 1599 (hydrazone C=N stretching). ^{1}H NMR (500 MHz, δ, ppm, DMSO-d_6): 3.71 (s, 3H, arylidene C_5-OCH_3), 3.79, 3.80 (2s, 3H, arylidene C_2-OCH_3), 3.88, 4.33 (2s, 2H, CH_2CO), 6.99 (dd, 1H, J = 9.27, 2.92 Hz, arylidene C_4-H), 7.03–7.09 (m, 2H, imidazothiazole C_2-H and arylidene C_3-H), 7.28–7.44 (m, 3H, arylidene C_6-H and Cl–Ph $C_{3,5}$-H), 7.81, 7.84 (2d, 2H, J = 8.30, 7.80 Hz, Cl–Ph $C_{2,6}$-H), 8.26, 8.27 (2s, 1H, imidazothiazole C_5-H), 8.36, 8.54 (2s, 1H, N=CH), 11.60, 11.75 (2s, 1H, CONH). ^{13}C NMR (APT) (125 MHz, δ, ppm, DMSO-d_6): 32.64, 34.12 (CH_2), 56.15, 56.17 (arylidene 5-OCH_3), 56.94, 56.99 (arylidene 2-OCH_3), 109.63, 109.85 (imidazothiazole C_5), 110.47, 110,87 (imidazothiazole C_2), 114.03 (arylidene C_3), 114.17 (arylidene C_6), 117.78, 118.47 (arylidene C_4), 123.29, 123.45 (arylidene C_1), 126.96, 127.02 (Cl–Ph $C_{2,6}$), 127.00, 127.51 (imidazothiazole C_3), 129.28, 129.34 (Cl–Ph $C_{3,5}$), 131.90, 132.01 (Cl–Ph C_1), 133.88, 133.98 (Cl–Ph C_4), 139.88, 143.16 (N=CH), 145.40, 145.53 (imidazothiazole C_6), 149.47, 149.55 (imidazothiazole C_{7a}), 152.88, 153.01 (arylidene C_5), 153.97, 154.00 (arylidene C_2), 163.79, 169.66 (CONH). MS (ESI-) *m/z* (rel intensity %): 455 ([M–H+2]$^-$, 21), 453 ([M–H]$^-$, 100). MS2 (ESI-) *m/z* (rel intensity %): 455 ([M–H+2]$^-$, 3), 453 ([M–H]$^-$, 30), 290 (10), 289 (100), 273 (18), 247 (7), 208 (97). Anal. for $C_{22}H_{19}ClN_4O_3S$, calcd. C, 58.08 H, 4.21 N, 12.32 found C, 57.72 H, 4.21 N, 12.31.

[6-(4-Chlorophenyl)imidazo[2,1-b]thiazol-3-yl]acetic acid 2,4-dichlorobenzylidenehydrazide ***3i***

Yield 91 %, mp 254–255 °C, UV λ_{max} (nm): 231 (ε 16051), 286 (ε 30100), 319 (ε 11367). IR ν_{max} (cm^{-1}): 3135

(N–H stretching), 1687 (amide I C=O stretching), 1594 (hydrazone C=N stretching). ^{1}H NMR (500 MHz, δ, ppm, DMSO-d_6): 3.93, 4.35 (2s, 2H, CH_2CO), 7.09, 7.10 (2s, 1H, imidazothiazole C_2-H), 7.41–7.44 (m, 2H, Cl–Ph $C_{3,5}$-H), 7.48 (dd, 1H, $J = 8.78$, 1.95 Hz, arylidene C_5-H), 7.71, 7.72 (2d, 1H, $J = 1.95$, 1.95 Hz, arylidene C_3-H), 7.80–7.86 (m, 2H, Cl–Ph $C_{2,6}$-H), 7.93, 8.04 (2d, 1H, $J = 8.79$, 8.30 Hz, arylidene C_6-H), 8.27, 8.28 (2s, 1H, imidazothiazole C_5-H), 8.39, 8.57 (2s, 1H, N=CH), 11.87, 12.02 (2s, 1H, CONH). ^{13}C NMR (APT) (125 MHz, δ, ppm, DMSO-d_6): 32.55, 34.07 (CH_2), 109.91 (imidazothiazole C_5), 111.09 (imidazothiazole C_2), 126.77, 127.21 (imidazothiazole C_3), 126.96, 127.03 (Cl–Ph $C_{2,6}$), 128.65 (arylidene C_5), 128.79, 128.86 (arylidene C_6), 129.31, 129.34 (Cl–Ph $C_{3,5}$), 130.10 (arylidene C_3), 131.13, 131.18 (arylidene C_1), 131.92, 132.01 (Cl–Ph C_1), 133.88, 133.97 (arylidene C_2), 134.38, 134.54 (Cl–Ph C_4), 135.68, 135.90 (arylidene C_4), 139.37, 142.60 (N=CH), 145.39, 145.54 (imidazothiazole C_6), 149.45 (imidazothiazole C_{7a}), 164.25, 169.96 (CONH). ^{13}C NMR (HSQC) (125 MHz, δ, ppm, DMSO-d_6): 32.55, 34.07 (CH_2), 109.64, 109.89 (imidazothiazole C_5), 111.09, 111.39 (imidazothiazole C_2), 126.77, 127.20 (imidazothiazole C_3), 126.96, 127.03 (Cl–Ph $C_{2,6}$), 128.64, 128.76 (arylidene C_5), 128.79, 128.85 (arylidene C_6), 129.30, 129.33 (Cl–Ph $C_{3,5}$), 130.09 (arylidene C_3), 131.13, 131.17 (arylidene C_1), 131.93, 132.01 (Cl–Ph C_1), 133.87, 133.96 (arylidene C_2), 134.38, 134.54 (Cl–Ph C_4), 135.67, 135.90 (arylidene C_4), 139.38, 142.61 (N=CH), 145.39, 145.54 (imidazothiazole C_6), 149.46, 149.53 (imidazothiazole C_{7a}), 164.26, 169.96 (CONH). MS (ESI-) *m/z* (rel intensity %): 465 ($[M–H+4]^-$, 28), 463 ($[M–H+2]^-$, 100), 461 ($[M–H]^-$ 81). MS2 (ESI-) *m/z* (rel intensity %): 463 ($[M–H+2]^-$, 70), 462 (6), 290 (5), 289 (11), 275 (45), 273 (100), 247 (7), 247 (1), 208 (10). Anal. for $C_{20}H_{13}Cl_3N_4OS$, calcd. C, 51.80 H, 2.83 N, 12.08 found C, 51.69 H, 3.25 N, 12.31.

[6-(4-Chlorophenyl)imidazo[2,1-b]thiazol-3-yl]acetic acid 5-nitro-2-furfurylidenehydrazide ***3j***

Yield 41 %, mp 254–256 °C, UV λ_{max} (nm): 233 (ε 24501), 260 (ε 39632), 349 (ε 16893). IR ν_{max} (cm^{-1}): 3203, 3147 (N–H stretching), 1670 (amide I C=O stretching), 1561 (hydrazone C=N stretching). ^{1}H NMR (500 MHz, δ, ppm, DMSO-d_6): 3.97, 4.33 (2s, 2H, CH_2CO), 7.11 (s, 1H, imidazothiazole C_2-H), 7.24, 7.30 (2d, 1H, $J = 3.90$, 3.90 Hz, furan C_3-H), 7.41–7.44 (m, 2H, Cl–Ph $C_{3,5}$-H), 7.77–7.85 (m, 3H, Cl–Ph $C_{2,6}$-H and furan C_4-H), 8.01, 8.21 (2s, 1H, N=CH), 8.24, 8.27 (2s, 1H, imidazothiazole C_5-H), 12.04, 12.12 (2s, 1H, CONH). MS (ESI-) *m/z* (rel intensity %): 430 ($[M–H+2]^-$, 31); 428 ($[M–H]^-$, 100). MS2 (ESI-) *m/z* (rel intensity %): 428 ($[M–H]^-$, 18), 290 (1), 274 (8), 273 (100), 208 (5). Anal. for $C_{18}H_{12}ClN_5O_4S$, calcd. C, 50.30 H, 2.81 N, 16.29 found C, 50.09 H, 3.13 N, 16.54.

In vitro evaluation of anticancer activity

All in vitro cell line screenings were conducted at the NCI in Bethesda, Maryland, USA (Alley et al., 1988; Grever et al., 1992; Boyd and Paull, 1995; Shoemaker, 2006). RPMI 1640 medium containing 5 % fetal bovine serum and 2 mM L-glutamine was used for growth of the human tumor cell lines of the cancer screening panel. A typical screening experiment consisted of cells that were inoculated in 96-well microtiter plates, each well contained a 100 µL, at plating densities ranging from 5000 to 40,000 cells per well. This range depended on the growth and doubling time of individual cell lines. The microtiter plates were incubated at 37 °C, 5 % CO_2, 95 % air and 100 % relative humidity (RH), after cell inoculation, for 24 h prior to addition of experimental drugs.

Two plates of each cell line were then fixed in situ with TCA, which represented a direct measure of cell populations for each cell line at the time of drug addition (Tz). Experimental drugs were frozen and stored until use, but first were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration. Before adding the drug, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration. The complete medium contained 50 µg/ml gentamicin. There were a total of five drug concentrations plus a control. The concentrations were 10-fold or ½ log serial dilutions and aliquots of 100 µl of these different drug dilutions were added to the appropriate microtiter wells containing 100 µl of medium. Finally, each well contained the desired final drug concentrations.

The plates were then incubated for an additional 48 h at 37 °C, 5 % CO_2, 95 % air, and 100 % RH. Cold TCA was added to terminate the assay for adherent cells. Cells were fixed in situ by the careful and slow addition of 50 µl cold 50 % (w/v) TCA (10 % TCA, final concentration) that was then incubated for 60 min at 4 °C. First, the supernatant was discarded, and then the plates were washed five times with tap water, followed by air drying. The plates were incubated for 10 min at room temperature after adding SRB solution (100 µl) at 0.4 % (w/v) in 1 % acetic acid to each well. Unbound dye was removed after staining by washing five times with 1 % acetic acid. Then the plates were air dried once again. A 10 mM trizma base was used to solubilize any bound stain, and the absorbance was read using an automated plate reader set for maximum absorption at 515 nm. The same methods were used for suspension cells, except that the assay was terminated by fixing settled cells at the bottom of the wells with the addition of 50 µl of 80 % TCA (final concentration, 16 % TCA). The percentage of growth was calculated at each drug concentration by using seven absorbance measurements time zero, (Tz), control growth, (C), and amount of growth in the presence of the

five drug concentration levels (Ti). Percent GI was calculated as follows:

$$\left[\frac{\mathrm{Ti}-\mathrm{Tz}}{\mathrm{C}-\mathrm{Tz}}\right] \times 100 \text{ for concentrations when } \mathrm{Ti} >/ = \mathrm{Tz}$$

$$\left[\frac{\mathrm{Ti}-\mathrm{Tz}}{\mathrm{Tz}}\right] \times 100 \text{ for concentrations for when } \mathrm{Ti} < \mathrm{Tz}.$$

For each experimental agent three dose response parameters were calculated. The GI_{50} defined as 50 % GI, was calculated from the following equation $[(\mathrm{Ti} - \mathrm{Tz})/(\mathrm{C} - \mathrm{Tz})] \times 100 = 50$. The parameters for the equation were obtained from the drug concentration resulting in a 50 % reduction in control cell net protein increase (as measured by SRB staining) during the drug incubation. The drug concentration resulting in TGI was determined by calculating $\mathrm{Ti} = \mathrm{Tz}$. The LC_{50}, which is defined as the concentration of drug resulting in a decline of 50 % protein at the end of the drug treatment relative to the beginning of the treatment. This indicated a net loss of cells after treatment and was calculated using the following equation $[(\mathrm{Ti} - \mathrm{Tz})/\mathrm{Tz}] \times 100 = -50$. Only if the level of activity was reached were the values calculated for each of these three parameters. However, the value for that particular parameter was expressed as either greater or less than the maximum or minimum concentration tested, if the effect was not reached or was exceeded.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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