European Journal of Medicinal Chemistry 75 (2014) 492–500

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis, *in vitro* anticancer evaluation and *in silico* studies of novel imidazo[2,1-*b*]thiazole derivatives bearing pyrazole moieties

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ARTICLE INFO

Article history:
Received 3 August 2013
Received in revised form
22 October 2013
Accepted 8 December 2013
Available online 23 January 2014

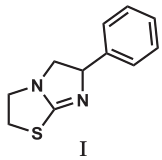
Keywords:
Aminothiazole
Imidazo[2,1-*b*]thiazole
Pyrazole
Anticancer evaluation
In silico studies

ABSTRACT

A series of imidazo[2,1-*b*]thiazoles bearing pyrazole moieties **4**–**6**(**a**–**c**) was synthesized through the reaction of 6-hydrazinylimidazo[2,1-*b*]thiazoles **3a**–**c** with different β-dicarbonyl compounds. Eleven compounds were screened at the National Cancer Institute (NCI), USA for anticancer activity at a single dose (10 μM). The *in vitro* anticancer evaluation revealed that compounds **2a** and **4**–**6**(**a**) exhibited increased potency towards *CNS SNB-75* and *Renal UO-31* cancer cell lines. COMPARE analyses showed strong to considerable correlations with rapamycin (mTOR inhibitor). The results of assessment of toxicities, druglikeness, and drug score profiles of compounds **2a** and **4**–**6**(**a**) are promising. Some of the target compounds showed good docking scores with potential anticancer targets, chosen based on pharmacophore mapping of the established derivatives.

1. Introduction

The global burden of cancer continues to increase largely because of aging and growth of the world population. Based on the GLOBOCAN estimates, about 12.7 million cancer cases and 7.6 million cancer deaths have occurred in 2008 [1]. The development of new anticancer agents is becoming the major interest in many academic and industrial research laboratories all over the world with the aim to develop more potent molecules with higher specificity and reduced toxicity. Levamisole **I**, the imidazo[2,1-*b*]thiazole derivative, was reported as a potential antitumor agent in patients with small tumor burdens [2]. In addition, numerous imidazo[2,1-*b*]thiazole derivatives were reported to possess antitumor activities [3–7]. Furthermore, it was found that the incorporation of pyrazole ring into different aryl or heteroaryl ring systems was reported to exhibit significant anticancer activities [8–13].



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Prompted by the above considerations, and in view of the need for new antitumor agents, it was of interest to prepare imidazo[2,1-*b*]thiazoles bearing different pyrazole moieties to be evaluated for their antitumor activity.

2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds **2**–**6**(**a**–**c**) is outlined in Scheme 1. 2-Amino-4-arylthiazoles **1a**–**c** were prepared utilizing either phenacyl chloride or phenacyl bromide according to a reported procedure [14] which is considered to be an easy, rapid and purification-free procedure. From literature survey, it was reported that a variety of aminoheterocyclic systems could yield fused ring systems containing keto group through reaction with chloroacetyl chloride [15,16], 4-chlorobutyryl chloride [17], ethyl chloroacetate [18,19], or 3-bromopropionic acid [20]. Recently, it was demonstrated that the reaction between 2-amino-4-phenylthiazole and chloroacetic acid could be furnished in ethanol yielding fused imidazothiazole derivatives [21]. In the present study, 2-amino-4-arylthiazoles **1a**–**c** were reacted with chloroacetic acid in glacial acetic acid in the presence of anhydrous sodium acetate *via* prolonged heating up to 40 h, and the products were obtained in 72–82% yield [22]. The structures of compounds **2a**–**c** were confirmed on the basis of spectral data. The ^{1}H NMR spectra showed a broad singlet at 3.48–3.85 ppm for the imidazo–CH_2 protons. Heating

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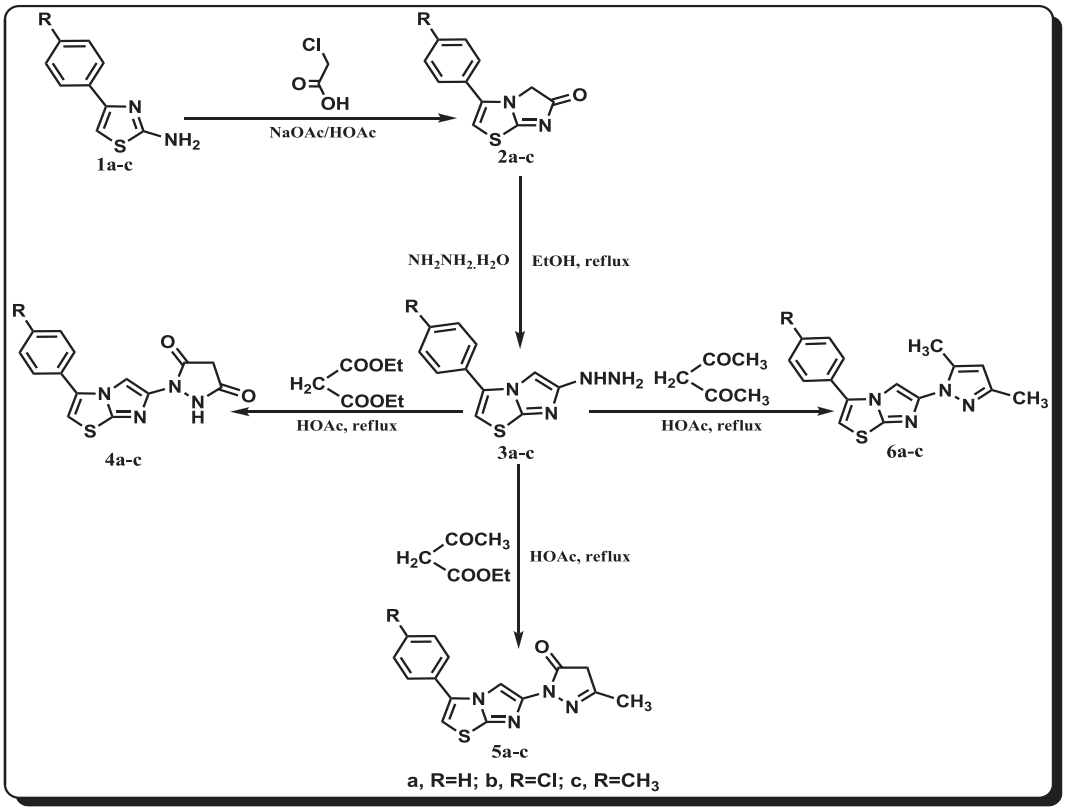
http://dx.doi.org/10.1016/j.ejmech.2013.12.010



Scheme 1. Synthesis of the title compounds **2**–**6** (**a**–**c**).

compounds **2a**–**c** in ethanol with hydrazine hydrate afforded 6-hydrazinyl-3-(un)substituted phenylimidazo[2,1-*b*]thiazoles **3a**–**c** in 56–65% yield. Compounds **3a**–**c** were confirmed by their ^{1}H NMR spectra, which showed CH–imidazole proton signal as a singlet peak at the expected region with D_2O exchangeable peaks for the NH_2 and NH protons. It was reported that the reaction between hydrazinoheterocycles and diethyl malonate, ethyl acetoacetate or acetylacetone could be performed through refluxing in ethanol [11,23,24], DMF and fused sodium acetate [25] and glacial acetic acid [26]. Compounds **3a**–**c** were refluxed with diethyl malonate, ethyl acetoacetate or acetylacetone in glacial acetic acid. The structures of the synthesized compounds **4**–**6**(**a**–**c**) were confirmed by microanalyses and spectral data (IR, ^{1}H NMR, ^{13}C NMR and EI-MS) which showed full agreement with their structures (Experimental section).

2.2. Biological evaluation

2.2.1. In vitro anticancer screening

The synthesized compounds **2a**, **b** and **4**–**6**(**a**–**c**) were selected by the National Cancer Institute (NCI) [27], Bethesda, Maryland, USA, under the Developmental Therapeutic Program (DTP) which is designed to screen up to 3000 compounds per year for potential anticancer activity. The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10 μM. The output from the single dose screen is reported as a mean graph and is available for analysis by the COMPARE program. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration levels. Compounds with drug-like properties, based on computer-aided design, are to be prioritized in the NCI screening service. Eleven compounds were selected for screening based on their ability to add diversity to the NCI small molecule compound collection. The operation of this screen utilizes 60 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney. The compounds were added at single high dose (10 μM) and the culture was incubated for 48 h. End point determinations were made with a protein binding dye, Sulforhodamine B [28–30].

Results for each compound are reported as a mean graph of the percent growth of the treated cells when compared to the untreated control cells. The percentage growth of the tested compounds against the full 60-cell line panel is illustrated in Table 1.

The mean percentage growth against the full 60-cell line panel and the screening data of the tested compounds against the most sensitive cell lines are illustrated in Table 2.

In light of the NCI-60 results, the following could be considered:

In this study, compounds **5b** and **6b** exhibited the lowest mean percentage growth against the full 60-cell line panel. Regarding sensitivity against individual cell lines, both compounds **5b** and **6b** showed observed low cell growth promotion against several *Leukemia* and *Non-Small Cell Lung* cancer cell lines, while compound **4a** decreased growth promotion with several *Non-Small Cell Lung* and *Renal* cancer cell lines. By comparing the results from different series, it was found that introduction of methyl pyrazolone moiety in compounds **5a**–**c** proved to enhance the potency towards *Renal UO-31* cancer cell line. It is worth mentioning that compounds **2a** and **4**–**6**(**a**) showed increased potency towards *CNS SNB-75* and *Renal UO-31* cancer cell lines with growth percentages ranging from 58.95 to 64.07%. In particular, compound **5a**, bearing methyl pyrazolone moiety, exhibited considerable potency with *Non-Small Cell Lung HOP-92*, *CNS SNB-75* and *Renal UO-31* cancer cell lines. In addition, compounds **2b** and **4**–**6**(**b**) demonstrated considerable

Table 1
Percentage cell growth of sixty human tumor cell line anticancer screening data of the tested compounds at single dose assay (10^{-5} M concentration).

Subpanel tumor cell lines	Percentage cell growth										
	2a	**2b**	**4a**	**4b**	**4c**	**5a**	**5b**	**5c**	**6a**	**6b**	**6c**
Leukemia											
CCRF-CEM	96.26	91.53	98.69	89.47	95.64	95.90	84.40	94.05	95.39	87.79	96.02
HL-60(TB)	114.37	94.17	119.92	88.13	107.29	89.19	92.53	109.67	104.66	90.21	105.89
K-562	88.78	79.72	94.24	88.10	94.74	83.39	71.37	80.53	76.85	83.10	108.61
MOLT-4	95.38	79.10	101.34	78.97	90.30	84.36	65.78	78.88	93.87	81.32	98.39
RPMI-8226	94.40	89.20	93.00	84.97	98.13	94.19	75.94	93.56	93.05	68.15	90.26
SR	85.96	77.82	82.99	74.04	84.39	84.55	73.28	73.33	80.88	65.83	81.05
Non-small cell lung cancer											
A549/ATCC	98.34	92.50	92.87	88.88	101.84	100.61	79.56	98.30	98.99	85.77	98.61
HOP-62	93.62	107.38	85.91	104.11	104.83	95.34	97.48	99.16	91.39	97.10	103.83
HOP-92	77.98	111.42	63.49	99.44	75.09	66.54	73.63	65.52	86.04	74.69	77.99
NCI–H226	101.74	110.36	89.96	102.56	94.39	100.72	95.36	89.74	88.30	81.47	94.85
NCI–H23	94.88	103.57	91.46	97.79	96.87	91.58	91.97	95.54	95.42	91.04	93.33
NCI–H322M	115.64	93.84	98.07	83.28	108.04	92.13	99.77	104.88	91.13	90.97	96.36
NCI–H460	105.38	106.25	104.31	104.17	110.69	106.96	103.29	104.79	103.73	101.28	101.67
NCI–H522	94.28	87.50	74.91	74.09	95.90	89.17	84.55	88.86	88.40	75.28	91.76
Colon cancer											
COLO 205	102.01	107.90	100.40	100.54	106.73	102.28	102.89	106.35	105.84	100.19	105.61
HCC-2998	97.85	101.15	103.09	102.03	101.53	97.33	100.25	102.92	105.33	102.08	106.71
HCT-116	97.59	97.12	95.51	93.24	107.73	96.53	97.07	94.42	101.22	89.79	96.97
HCT-15	95.89	93.29	91.24	92.86	100.09	96.68	88.73	103.34	94.95	95.55	101.14
HT29	109.07	88.41	103.85	91.35	111.21	106.26	85.68	117.53	97.58	87.04	103.52
KM12	111.45	100.39	101.16	106.11	114.88	102.38	104.52	104.05	103.54	99.93	107.83
SW-620	94.81	100.65	106.63	103.83	102.67	103.50	99.31	105.30	101.74	100.40	106.17
CNS cancer											
SF-268	110.43	100.09	99.50	105.43	105.75	99.29	96.32	111.30	98.39	101.66	111.50
SF-295	NT[a]	80.02	NT[a]	84.42	91.21	69.36	85.56	NT[a]	77.49	82.48	88.51
SF-539	97.50	94.76	88.22	101.26	101.39	89.71	94.70	102.43	94.00	104.27	108.66
SNB-19	99.66	100.32	90.95	90.22	102.29	90.75	88.01	100.49	96.51	92.78	100.35
SNB-75	61.00	75.77	63.13	74.16	73.33	59.39	65.32	79.10	64.07	69.22	85.89
U251	93.89	94.33	84.15	91.89	98.07	90.73	91.72	94.06	94.69	87.79	94.95
Melanoma											
LOX IMVI	89.40	98.88	86.89	96.45	96.89	90.77	96.76	93.01	91.90	91.91	92.77
MALME-3M	101.88	99.35	141.10	97.08	118.22	107.15	102.05	107.40	94.08	93.14	113.53
M14	99.94	97.04	100.94	100.10	105.40	100.26	96.23	99.25	95.36	101.37	103.67
MDA-MB-435	95.09	87.82	99.88	93.72	90.01	92.94	87.44	83.58	96.89	94.49	94.60
SK-MEL-2	113.94	98.15	101.90	101.00	116.24	101.88	103.79	114.42	97.61	113.34	125.13
SK-MEL-28	104.72	95.70	96.41	95.71	101.85	102.44	96.35	98.52	102.42	97.94	94.82
SK-MEL-5	99.82	105.10	89.58	99.65	95.89	101.76	92.42	90.04	94.18	85.48	98.86
UACC-257	102.96	96.31	97.60	102.61	109.47	101.53	94.93	100.48	104.85	104.07	108.31
UACC-62	90.29	88.66	85.00	90.13	91.76	91.11	81.88	74.11	89.44	86.86	90.51
Ovarian cancer											
IGROV1	103.87	95.21	91.47	117.44	105.27	88.02	99.28	101.33	76.48	107.51	106.95
OVCAR-3	110.53	105.26	98.97	109.55	113.24	99.81	105.92	109.76	100.66	107.01	114.96
OVCAR-4	97.73	90.35	81.20	87.39	99.12	98.04	77.19	99.90	93.01	81.89	97.77
OVCAR-5	113.22	97.04	103.88	115.07	107.89	96.68	105.89	124.33	95.40	109.27	112.78
OVCAR-8	97.86	95.97	89.77	100.64	99.34	104.97	94.22	102.01	95.88	91.51	96.05
NCI/ADR-RES	99.61	101.78	94.33	99.31	107.97	92.50	100.28	99.30	95.47	100.05	98.47
SK-OV-3	89.46	98.24	86.83	103.15	101.17	88.91	100.47	95.50	89.91	93.44	100.81
Renal cancer											
786-0	104.48	93.96	103.52	96.40	103.12	96.64	94.09	101.01	98.00	101.78	104.52
A498	111.20	70.24	78.40	75.20	97.97	96.57	74.06	99.21	111.15	85.72	102.68
ACHN	97.86	94.99	81.46	101.79	90.53	91.20	89.14	96.88	83.84	91.00	90.72
CAKI-1	78.74	81.31	75.88	78.89	90.13	79.90	79.28	84.53	77.38	78.63	83.54
RXF 393	101.55	111.06	89.67	111.56	100.77	108.59	104.15	95.88	93.38	93.47	104.57
SN12C	91.53	94.89	92.23	88.42	103.92	96.04	90.87	96.40	96.47	88.99	98.14
TK-10	124.72	79.93	130.54	94.95	139.24	124.73	98.75	134.53	124.03	128.30	140.69
UO-31	61.27	92.67	59.58	110.76	74.68	58.95	80.94	71.63	59.16	89.64	94.43
Prostate cancer											
PC-3	82.43	101.36	76.37	89.98	87.33	77.57	79.39	75.26	88.65	79.09	84.73
DU-145	112.39	101.64	105.83	104.83	118.36	106.34	105.05	113.22	104.76	112.85	115.53
Breast cancer											
MCF78	88.54	90.89	85.84	88.99	93.87	89.58	93.54	91.82	88.70	85.06	89.72
MDA-MB-231/ATCC	96.67	102.16	85.68	106.75	103.79	89.89	96.46	79.03	86.29	86.43	88.80
HS 578T	103.17	110.60	100.93	98.28	107.23	105.52	101.13	108.67	105.70	94.30	97.03
BT-549	111.82	89.05	97.61	95.35	112.56	89.72	101.20	111.26	95.32	113.37	125.41
T-47D	84.40	92.10	82.73	87.34	85.68	89.39	73.71	80.63	87.19	80.72	98.15
MDA-MB-468	106.39	106.41	102.08	104.39	105.50	114.34	97.94	102.05	102.40	88.55	111.59

[a] NT: not tested.

Table 2
Mean percentage growth and screening data of the tested compounds with the most sensitive cell lines represented as percent cell growth.

Comp. No.	NSC code	Mean percentage growth	Leukemia SR	Non-Small cell lung cancer HOP-92	CNS cancer SNB-75	Renal cancer UO-31
2a	768188	98.20	85.96	77.98	**61.00**[a]	**61.27**[a]
2b	768168	95.40	77.82	111.42	75.77	92.67
4a	768190	93.33	82.99	**63.49**[a]	**63.13**[a]	**59.58**[a]
4b	768169	95.56	74.04	99.44	74.16	110.76
4c	768179	100.84	84.39	75.09	73.33	74.68
5a	768170	94.06	84.55	**66.54**[a]	**59.39**[a]	**58.95**[a]
5b	768171	91.25	73.28	73.63	**65.32**[a]	80.94
5c	768180	97.12	73.33	**65.52**[a]	79.10	71.63
6a	768176	93.89	80.88	86.04	**64.07**[a]	**59.16**[a]
6b	768177	92.28	**65.83**[a]	74.69	**69.22**	89.64
6c	768185	100.62	81.05	77.99	85.89	94.43

[a] Underlined values are those below 70.00%.

potency towards *Leukemia MOLT-4* and *SR* and *Renal A498* cancer cell lines than other compounds in the same series.

We performed COMPARE [31] analyses for compounds **2a**, **b** and **4**–**6**(**a**–**c**) in order to investigate the similarity of their cytotoxicity pattern (mean graph fingerprints) with those of known anticancer standard agents, NCI active synthetic compounds and natural extracts, which are present in public available databases. Such analysis is based on comparing the patterns of differential growth inhibition for cultured cell lines and can potentially gain insight into the mechanism of the cytotoxic action. If the data pattern correlates well with that of compounds belonging to a standard agent database (Pearson's correlation coefficient (PCC $>$ 0.6)), the compound of interest may have the same mechanism of action [32,33]. On the other hand, if the activity pattern does not correlate with any standard agent, it is possible that the compound has a novel mechanism of action. Standard COMPARE analyses were performed at the GI_{50} level.

It was established that compounds **4c** and **6a** demonstrated high correlation levels with rapamycin (NSC S226080) with PCC values of 0.615 and 0.648, respectively. Considerable correlations between compounds **2a**, **4a**, **5a**, **5b**, **5c** and **6b**, and rapamycin were noted with PCC values of 0.574, 0.572, 0.58, 0.587, 0.557 and 0.514, respectively. Such similarity in COMPARE results could indicate the resemblance in mechanisms of action with rapamycin. Rapamycin is reported to be mTOR inhibitor which is considered to be a key enzyme in regulation of cellular metabolism, growth, and proliferation [34–36]. In addition, compound **5c** exhibited a considerable correlation with merbarone (NSC S336628) with PCC value of 0.563. Merbarone is a catalytic inhibitor of topoisomerase II and so inhibit DNA cleavage [37]. Compounds **2b**, **4b** and **6c** did not display high correlation levels with the NCI tested drugs or other biological active substances. It can be assumed that this compound may have a unique mechanism of action that differs from other known anticancer agents.

2.2.2. Total polar surface area and Lipinski's rule of five

It is well established that more than 80% of the drugs on the market have an estimated log S value greater than −4. Typically, a low solubility goes along with a bad absorption and therefore the general aim is to avoid poorly soluble compounds. As shown in Table 3, the entire target compounds **2a**–**c** and **4**–**6**(**a**–**c**), having log *S* values above −4, are expected to have good aqueous solubility which significantly affects its absorption and distribution characteristics.

The total polar surface area (TPSA) was calculated using Canvas [38] program since it is a key property that has been linked to drug bioavailability. Thus, passively absorbed molecules with a TPSA $>$140 are thought to have low oral bioavailability [39]. Since all the target compounds **2a**–**c** and **4**–**6**(**a**–**c**) have TPSA value ranging from 26.50 to 43.17 (Table 3), they theoretically should present good passive oral absorption.

Based on the reported data that nearly 40% of drug candidates fail in clinical trials because of poor ADME [40], we evaluated the compliance of the designed compounds to the Lipinski's rule of five, calculated by Canvas [38] and Osiris [41] programs. Molecules violating more than one of these rules may have problems with bioavailability. Predictions of ADME properties for the studied compounds are given in Table 3. The results showed that all the targeted compounds comply with these rules suggesting that the synthesized compounds would be possible drug molecules.

2.2.3. Assessment of toxicities, druglikeness, and drug score profiles

Osiris program [41] was used for prediction of the overall toxicity of the designed derivatives as the prediction process relies on a predetermined set of structural fragments that give rise to toxicity alerts in case they are encountered in the structure. All target compounds **2a**–**c** and **4**–**6**(**a**–**c**) showed low *in silico* possible toxicity risks as shown in Table 4. Osiris program was also used for calculating the fragment-based druglikeness of the designed compounds and a positive value indicates that the designed molecule contains fragments which are frequently present in commercial drugs.

Table 3
Solubility, total polar surface area, and calculated Lipinski's rule of five for tested compounds.

Comp. No.	Log *S*[a]	TPSA[b]	MW[c]	*c*Log *P*[d]	nHBA[e]	nHBD[f]	RB[g]	nVio[h]
2a	−2.46	26.50	216.26	1.56	3	0	1	**0**
2b	−3.38	28.47	250.70	2.18	3	0	1	**0**
2c	−2.99	28.34	230.29	1.88	3	0	1	**0**
4a	−2.55	36.62	298.32	0.41	3	1	2	**0**
4b	−3.29	38.59	332.76	1.03	3	1	2	**0**
4c	−2.89	38.45	312.35	0.73	3	1	2	**0**
5a	−3.10	37.95	296.35	2.64	3	0	2	**0**
5b	−3.83	39.92	330.79	3.25	3	0	2	**0**
5c	−3.44	39.78	310.37	2.95	3	0	2	**0**
6a	−1.73	41.20	294.37	3.27	2	0	2	**0**
6b	−2.47	43.17	328.82	3.89	2	0	2	**0**
6c	−2.08	43.04	308.40	3.59	2	0	2	**0**

[a] Solubility parameter.
[b] Total polar surface area (Å).
[c] Molecular weight.
[d] Calculated lipophilicity.
[e] Number of hydrogen bond acceptors.
[f] Number of hydrogen bond donors.
[g] Rotatable bonds.
[h] Number of violations to Lipinski's rule of five.

Table 4
Toxicity risks, druglikeness and drug scores of the designed compounds.

Comp. No.	Toxicity risks (Mutagenicity, Tumorigenicity, Irritancy, Reproductive effects)	Druglikeness	Drug score
2a	–[a]	3.39	**0.91**[b]
2b	–[a]	**4.14**[b]	**0.86**[b]
2c	–[a]	2.12	**0.86**[b]
4a	–[a]	0.66	0.61
4b	–[a]	1.60	0.63
4c	–[a]	−0.70	0.48
5a	–[a]	3.67	0.68
5b	–[a]	**4.57**[b]	0.61
5c	–[a]	2.37	0.63
6a	–[a]	0.16	0.54
6b	–[a]	1.55	0.58
6c	–[a]	−1.14	0.42

[a] No indication for toxic effects.
[b] Underlined values represent the highest results in each parameter.

The drug score combines druglikeness, *c*Log *P*, Log *S*, molecular weight and toxicity risks in one handy value. A value of 0.5 or more makes a compound a promising lead for future development of a safe and efficient drug. Predictions of potential toxicity, druglikeness and drug score for the studied compounds are given in Table 4. Almost all of the synthesized compounds, except **4c** and **6c**, possess good values of druglikeness and drug score.

2.2.4. Target fishing

An attempt was made to investigate the potential targets involved in observed inhibition displayed by the synthesized compounds against NCI 60 cell panel. PharmMapper server is a freely accessed web server designed to identify potential target candidates for the given small molecules using reverse pharmacophore mapping approach. The server hosts a large, in-house repertoire of pharmacophore database annotated from all the targets information in potential drug target databases, including over 7000 receptor-based pharmacophore models. PharmMapper finds the best mapping poses of the user uploaded molecules against all the targets in PharmTarget Database [42].

PharmMapper is available at http://59.78.96.61/pharmmapper. The server demonstrated a variety of putative targets that might exhibit considerable binding affinity to the target compounds. Eight targets, involved in cancer therapy, are common between the tested compounds. These targets might explain the observed antiproliferative activity. Table 5 lists the scores with the top eight targets proposed by PharmMapper.

2.2.5. Docking study

Docking simulations were carried out with the aid of Docking Server [43], a web-based interface that utilizes a number of computational chemistry software specifically aimed at correctly calculating accurate ligand geometry optimization, energy minimization, charge calculation, docking calculation and protein-ligand complex representation. Molecular docking simulations were performed for the target compounds to evaluate their recognition profile at the binding pocket of the proposed targets. The binary complex of the target coupled with its natural ligand was used as a reference for docking and modeling in this investigation.

The eight potential targets proposed by pharmacophore mapping approach were used to investigate their interaction with the designed compounds. The target compounds **2a**–**c** and **4**–**6**(**a**–**c**) were comparatively evaluated in terms of estimated free energy of binding (kcal/mol), and inhibition constant K_i (uM) to the eight proposed enzymes and the results are listed in Table 6.

Compounds **5b** and **6b** showing the lowest mean percentage growth against the full 60-cell line panel demonstrated the best docking score with the proposed targets.

3. Conclusion

On the basis of the results obtained from *in vitro* anticancer evaluation, it was found that compounds **5b** (NSC 768171) and **6b**

Table 5
Fit score of the synthesized compounds against the top eight targets.

Comp. No.	Fit scores with the top eight targets (PDB-Id)							
	Cathepsin K (1TU6)	Vitamin D_3 receptor (1DB1)	Dual specificity mitogen-activated protein kinase kinase 1 (1S9J)	Proto-oncogene tyrosine-protein kinase Src (1Y57)	Thymidylate synthase (1JU6)	Epidermal growth factor receptor (1XKK)	Epidermal growth factor receptor (2ITO)	Leukotriene A_4 hydrolase (1GW6)
2a	2.55	–[a]	3.22	**2.83**[b]	**2.96**[b]	–[a]	**2.72**[b]	–[a]
2b	**3.74**[b]	3.34	3.22	**2.83**[b]	**2.96**[b]	2.65	2.59	2.75
2c	**3.72**[b]	2.87	3.20	**2.83**[b]	**2.96**[b]	–[a]	**2.72**[b]	2.75
4a	3.27	2.70	2.88	–[a]	**3.12**[b]	–[a]	2.65	–[a]
4b	3.31	3.37	2.95	–[a]	**3.12**[b]	–[a]	–[a]	–[a]
4c	3.31	2.88	3.18	**2.82**[b]	**3.12**[b]	–[a]	**2.81**[b]	–[a]
5a	3.42	2.85	3.13	**2.82**[b]	–[a]	2.93	–[a]	**2.92**[b]
5b	3.42	3.37	2.99	–[a]	–[a]	–[a]	–[a]	–[a]
5c	3.39	2.94	3.04	–[a]	–[a]	–[a]	–[a]	**2.91**[b]
6a	–[a]	3.44	3.44	–[a]	–[a]	2.98	–[a]	–[a]
6b	–[a]	**3.48**[b]	**3.47**[b]	–[a]	–[a]	**3.31**[b]	–[a]	–[a]
6c	–[a]	**3.46**[b]	**3.55**[b]	–[a]	–[a]	**3.16**[b]	–[a]	–[a]

- [a] This target is not included in the top 300 targets for this compound.
- [b] Underlined values represent the highest fit score to each target.

Table 6
Estimated free energy of binding and inhibition constants of the synthesized compounds with the top eight targets.

Comp. No.	Est. free energy of binding with different targets (kcal/mol) (Est. inhibition constant K_i(uM))							
	Cathepsin K (1TU6)	Vitamin D_3 receptor (1DB1)	Dual specificity mitogen-activated protein kinase kinase 1 (1S9J)	Proto-oncogene tyrosine-protein kinase Src (1Y57)	Thymidylate synthase (1JU6)	Epidermal growth factor receptor (1XKK)	Epidermal growth factor receptor (2ITO)	Leukotriene A_4 hydrolase (1GW6)
2a	-4.76 (321.7)	-6.11 (33.26)	-6.02 (38.43)	-5.56 (84.20)	-5.38 (113.8)	-7.14 (5.89)	-6.17 (29.85)	-6.87 (9.18)
2b	-5.41 (109.1)	-7.15 (5.74)	-6.63 (13.87)	-6.51 (16.87)	-6.44 (19.18)	-6.76 (11.04)	-7.11 (6.13)	-8.09 (1.17)
2c	-5.05 (197.3)	-6.42 (19.76)	-6.22 (27.56)	-5.97 (42.27)	-5.62 (76.38)	-6.68 (12.61)	-6.34 (22.65)	-7.25 (4.82)
4a	-5.05 (199.2)	-5.54 (87.12)	-6.89 (8.90)	-5.84 (52.19)	-6.98 (7.61)	-6.87 (9.14)	-7.48 (3.31)	-8.69 (0.43)
4b	-5.84 (52.33)	-6.50 (17.07)	-7.23 (5.01)	-5.97 (42.34)	**-7.36 (4.02)**[a]	-6.40 (20.53)	**-9.34 (0.144)**[a]	-8.45 (0.635)
4c	-5.29 (132.3)	-5.82 (53.79)	-6.66 (13.08)	-5.79 (57.42)	-6.57 (15.37)	-6.28 (24.90)	**-8.21 (0.968)**[a]	-8.13 (1.09)
5a	-6.37 (21.55)	-6.30 (24.14)	-7.13 (5.95)	-6.61 (14.37)	-6.66 (13.10)	**-8.09 (1.18)**[a]	-6.44 (18.91)	-7.84 (1.80)
5b	**-6.71 (12.04)**[a]	**-7.16 (5.62)**[a]	**-7.72 (2.19)**[a]	**-6.85 (9.52)**[a]	**-7.34 (4.16)**[a]	-6.39 (20.62)	-7.17 (5.59)	**-9.46 (0.116)**[a]
5c	-6.25 (26.34)	-6.50 (17.26)	-7.20 (5.29)	-6.76 (11.07)	-6.75 (11.24)	-6.46 (18.42)	-6.91 (8.64)	-9.07 (0.226)
6a	-6.09 (34.53)	-6.35 (22.23)	-6.81 (10.24)	-6.65 (13.26)	-6.59 (14.77)	**-8.13 (1.09)**[a]	-6.56 (15.44)	**-9.38 (0.132)**[a]
6b	**-6.62 (13.93)**[a]	**-7.35 (4.13)**[a]	**-7.38 (3.91)**[a]	**-7.09 (6.33)**[a]	-7.30 (4.48)	-6.45 (18.68)	-7.28 (4.58)	-9.24 (0.169)
6c	-6.29 (24.65)	-6.64 (13.51)	-6.91 (8.66)	-6.72 (11.78)	-6.84 (9.66)	-8.03 (1.29)	-6.91 (8.57)	-9.09 (0.215)
Ref. Lig.	-3.69 (1990)	-8.66 (0.452)	-5.15 (166.8)	-7.95 (1.49)	-6.49 (17.37)	-11.11 (0.007)	-7.28 (4.58)	-7.89 (1.64)

- [a] Underlined values represent the highest affinity to each target.

(NSC 768177) demonstrated the lowest mean percentage growth against the full 60-cell line panel. They also manifested the lowest inhibition constants with the targets proposed by PharmMapper. Concerning the sensitivity against individual cell lines, compounds **2a** and **4**–**6**(**a**) exhibited increased potency towards *CNS SNB-75* and *Renal UO-31* cancer cell lines. *In vitro* anticancer evaluation, together with *in silico* studies, revealed that compounds **2a** and **4**–**6**(**a**) could be considered as promising leads for further development of more potent anticancer agents.

4. Experimental

4.1. General

2-Amino-4-arylthiazoles **1a**–**c** were prepared following the procedure reported by Dighe [14]. All the reagents and solvents were obtained from commercial suppliers, and used without purification. TLC was monitored on Fluka silica gel TLC aluminum cards (0.2 mm thickness) with fluorescent indicator 254 nm using a mixture of petroleum ether/ethyl acetate in various proportions.

Melting points (°C) were recorded using a Fischer–Johns melting point apparatus and are uncorrected. The IR spectra (KBr) were recorded on Mattson 5000 FT IR spectrophotometer (ν in cm^{-1}) in the Microanalytical Unit, Faculty of Science, Mansoura University. ^1H and ^{13}C NMR for compounds **3**–**6**(**a**) were recorded on Bruker 500 MHz FT NMR spectrometer and ^1H NMR spectra for remaining compounds were carried out at the National Research Centre using a Varian Gemini 500 MHz FT NMR. Deuteriodimethylsulfoxide (DMSO-d_6) is used as a solvent with the chemical shift being expressed in δ (ppm) and downfield from tetramethylsilane (TMS) as internal standard.

Electron impact mass spectra (EI-MS), recorded on a Shimadzu GC/MS QP-2010 Plus mass spectrometer, and elemental analysis (in accord with the calculated values) were carried out in the Microanalytical Unit, Faculty of Science, Cairo University. Anticancer evaluation was performed at National Cancer Institute (NCI), Bethesda, Maryland, USA.

4.2. General procedure for the synthesis of compounds (**2a**–**c**) [22]

A mixture of 2-amino-4-arylthiazole **1a**–**c** (10 mmol), chloroacetic acid (1.89 g, 20 mmol) and anhydrous sodium acetate (1.64 g, 20 mmol) in glacial acetic acid (10 mL) was refluxed for 40 h. The reaction mixture was cooled and poured onto ice water with stirring. The solid formed was filtered and crystallized from ethanol.

4.2.1. 3-Phenylimidazo[2,1-b]thiazol-6(5H)-one (**2a**)

Yield: 82%; mp 212–214 °C [21]; IR (KBr, ν, cm^{-1}): 3169 (CH aromatic), 3023, 2987 (CH aliphatic), 1654, 1644 (C=O), 1599, 1584 cm^{-1} (C=N); EI-MS (70 eV) *m/z* (Rel. Int.): 216 (M^+, 3.24), 199 (20.06), 176 (100.00), 134 (31.72), 98 (21.04), 77 (11.00).

*4.2.2. 3-(4-Chlorophenyl)imidazo[2,1-b]thiazol-6(5H)-one (**2b**)*

Yield: 80%; mp 254–256 °C [22]; ^{1}H NMR (δ, ppm, DMSO-d_6): 3.48 (s, 2H, CH_2), 7.46 (d, 2H, Ar–H), 7.63 (s, 1H, H–thiazole), 7.86 (d, 2H, Ar–H); EI-MS (70 eV) *m*/*z* (Rel. Int.): 252 (M^+ + 2, 30.47), 250 (M^+, 2.57), 210 (100.00), 168 (32.13), 132 (6.05), 111 (11.57); Anal. for $C_{11}H_7ClN_2OS$ (250.70) C, H, N.

*4.2.3. 3-p-Tolylimidazo[2,1-b]thiazol-6(5H)-one (**2c**)*

Yield: 70%; mp 132–134 °C [22]; ^{1}H NMR (δ, ppm, DMSO-d_6): 2.29 (s, 3H, $-CH_3$), 3.85 (s, 2H, CH_2), 7.22 (d, 2H, Ar–H), 7.31 (s, 1H, H–thiazole), 7.62 (d, 2H, Ar–H); EI-MS (70 eV) *m*/*z* (Rel. Int.): 230 (M^+, 47.37), 198 (100), 176 (41.17); Anal. for $C_{12}H_{10}N_2OS$ (230.29) C, H, N.

*4.3. General procedure for the synthesis of compounds (**3a–c**)*

Equimolar quantities of 3-(un)substituted phenylimidazo[2,1-*b*]thiazol-6(5*H*)-one **2a–c** (10 mmol) and hydrazine hydrate (99%) (0.6 mL, 10 mmol) were dissolved in warm ethanol (10 mL) and refluxed for 8 h. After standing for approximately 24 h at room temperature, the solvent was distilled under reduced pressure and the obtained solid was crystallized from aqueous ethanol.

*4.3.1. 6-Hydrazinyl-3-phenylimidazo[2,1-b]thiazole (**3a**)*

Yield: 61%; mp 140–142 °C; IR (KBr, ν, cm^{-1}): 3433, 3251 cm^{-1} (NH, NH_2), 3112 (CH aromatic), 1599, 1584 cm^{-1} (C=N); ^{1}H NMR (δ, ppm, DMSO-d_6): 4.34 (s, 2H, NH_2), 7.04 (t, 2H, Ar–H), 7.25 (t, 1H, Ar–H), 7.36 (s, 1H, H–thiazole), 7.75 (s, 1H, CH–imidazole), 7.80 (d, 2H, Ar–H), 8.67 (s, 1H, NH); ^{13}C NMR (δ, ppm, DMSO-d_6): 101.48 (CH–imidazole, –S–CH–thiazole), 125.50, 127.15, 128.44 (Ar–CH), 134.87 (quaternary Ar–C, thiazole–C), 149.82 (C_6–imidazothiazole), 168.22 (–S–C(N)=*N*-imidazothiazole); EI-MS (70 eV) *m*/*z* (Rel. Int.): 230 (M^+, 3.75), 214 (3.32), 198 (1.85), 176 (100.00), 134 (82.10), 112 (1.57); Anal. for $C_{11}H_{10}N_4S$ (230.29) C, H, N.

*4.3.2. 3-(4-Chlorophenyl)-6-hydrazinylimidazo[2,1-b]thiazole (**3b**)*

Yield: 65%; mp 144–146 °C; IR (KBr, ν, cm^{-1}): 3438, 3283 cm^{-1} (NH, NH_2), 3111 (CH aromatic), 1632, 1533 cm^{-1} (C=N); ^{1}H NMR (δ, ppm, DMSO-d_6): 4.33 (s, 2H, NH_2), 7.02 (d, 2H, Ar–H), 7.37 (s, 1H, H–thiazole), 7.76 (s, 1H, CH–imidazole), 8.01 (d, 2H, Ar–H), 8.78 (s, 1H, NH); EI-MS (70 eV) *m*/*z* (Rel. Int.): 266 (M^+ + 2, 0.38), 264 (M^+, 0.09), 210 (100.00), 168 (39.33), 146 (4.86); Anal. for $C_{11}H_9ClN_4S$ (264.73) C, H, N.

*4.3.3. 6-Hydrazinyl-3-p-tolylimidazo[2,1-b]thiazole (**3c**)*

Yield: 56%; mp 120–122 °C; IR (KBr, ν, cm^{-1}): 3453, 3286 cm^{-1} (NH, NH_2), 3180 (CH aromatic), 1612, 1522 cm^{-1} (C=N); EI-MS (70 eV) *m*/*z* (Rel. Int.): 244 (M^+, 72.29), 228 (13.25), 213 (0.19), 168 (85.54), 126 (19.28); Anal. for $C_{12}H_{12}N_4S$ (244.32) C, H, N.

*4.4. General procedure for the synthesis of compounds **4–6**(**a–c**)*

A mixture of 6-hydrazinyl-3-(un)substituted phenylimidazo [2,1-*b*]thiazole **3a–c** (10 mmol) and diethyl malonate, ethyl acetoacetate, or acetylacetone (10 mmol) in glacial acetic acid (10 mL) was refluxed for 6–8 h. After cooling, the formed precipitate was filtered, dried and crystallized from aqueous acetic acid to furnish the entitled compounds.

*4.4.1. 1-(3-Phenylimidazo[2,1-b]thiazol-6-yl)pyrazolidine-3,5-dione (**4a**)*

Yield: 63%; mp 190–192 °C; IR (KBr, ν, cm^{-1}): 3251 (NH), 3167 (CH aromatic), 3064, 2999 (CH aliphatic), 1654, 1645 (C=O), 1599, 1584 cm^{-1} (C=N); ^{1}H NMR (δ, ppm, DMSO-d_6): 3.37 (s, 2H, CH_2), 7.31 (t, 2H, Ar–H), 7.43 (t, 1H, Ar–H), 7.58 (s, 2H, H–thiazole, CH–imidazole), 7.77 (s, 1H, NH), 7.90 (d, 2H, Ar–H); ^{13}C NMR (δ, ppm, DMSO-d_6): 39.98 (C_4–pyrazolidinedione), 107.78 (CH–imidazole, –S–CH–thiazole), 125.62, 127.70, 128.68 (Ar–CH), 134.31 (quaternary Ar–C, thiazole–C), 148.69 (C_6–imidazothiazole), 157.94 (2 C=O), 168.60 (–S–C(N)=*N*-imidazothiazole); EI-MS (70 eV) *m*/*z* (Rel. Int.): 298 (M^+, 3.61), 285 (12.18), 246 (3.49), 199 (3.37), 161 (6.39); Anal. for $C_{14}H_{10}N_4O_2S$ (298.32) C, H, N.

*4.4.2. 1-(3-(4-Chlorophenyl)imidazo[2,1-b]thiazol-6-yl)pyrazolidine-3,5-dione (**4b**)*

Yield: 60%; mp 234–236 °C; ^{1}H NMR (δ, ppm, DMSO-d_6): 3.46 (s, 2H, CH_2), 7.46 (d, 2H, Ar–H), 7.63 (s, 1H, H–thiazole), 7.65 (s, 1H, NH), 7.86 (d, 2H, Ar–H), 7.89 (s, 1H, CH–imidazole); EI-MS (70 eV) *m*/*z* (Rel. Int.): 332 (M^+, 0.58), 320 (0.58), 280 (1.49), 252 (30.02), 210 (100.00), 168 (30.69), 111 (9.82); Anal. for $C_{14}H_9ClN_4O_2S$ (332.76) C, H, N.

*4.4.3. 1-(3-p-Tolylimidazo[2,1-b]thiazol-6-yl)pyrazolidine-3,5-dione (**4c**)*

Yield: 55%; mp 126–128 °C; ^{1}H NMR (δ, ppm, DMSO-d_6): 2.29 (s, 3H, $-CH_3$), 3.50 (s, 2H, CH_2), 7.22 (d, 2H, Ar–H), 7.31 (s, 1H, H–thiazole), 7.47 (s, 1H, NH, D_2O exchangeable), 7.75 (d, 2H, Ar–H), 7.79 (s, 1H, CH–imidazole); EI-MS (70 eV) *m*/*z* (Rel. Int.): 312 (M^+, 5.42), 290 (5.03), 232 (25.08), 190 (100.00), 176 (16.41), 148 (33.13), 97 (10.60); Anal. for $C_{15}H_{12}N_4O_2S$ (312.35) C, H, N.

*4.4.4. 3-Methyl-1-(3-phenylimidazo[2,1-b]thiazol-6-yl)-1H-pyrazol-5(4H)-one (**5a**)*

Yield: 51%; mp 190–192 °C; IR (KBr, ν, cm^{-1}): 3169 (CH aromatic), 3065, 2988 (CH aliphatic), 1654, 1645 (C=O), 1596, 1583 cm^{-1} (C=N); ^{1}H NMR (δ, ppm, DMSO-d_6): 2.18 (s, 3H, pyrazoline–CH_3), 3.36 (s, 2H, CH_2), 7.33 (t, 2H, Ar–H), 7.43 (t, 1H, Ar–H), 7.58 (s, 1H, H–thiazole), 7.62 (s, 1H, CH–imidazole), 7.90 (d, 2H, Ar–H); ^{13}C NMR (δ, ppm, DMSO-d_6): 22.46 (pyrazoline–CH_3), 41.02 (C_4–pyrazoline), 107.78 (CH–imidazole, –S–CH–thiazole), 125.63, 127.70, 128.68 (Ar–CH), 134.33 (quaternary Ar–C, thiazole–C), 148.71 (C_6–imidazothiazole), 157.95 (C_3-pyrazoline,C=O), 168.59 (–S–C(N)=*N*-imidazothiazole); EI-MS (70 eV) *m*/*z* (Rel. Int.): 296 (M^+, 0.01), 255 (0.06), 241 (0.10), 218 (34.91), 176 (100.00), 134 (64.90), 104 (15.39), 77 (12.20); Anal. for $C_{15}H_{12}N_4OS$ (296.35) C, H, N.

*4.4.5. 1-(3-(4-Chlorophenyl)imidazo[2,1-b]thiazol-6-yl)-3-methyl-1H-pyrazol-5(4H)-one (**5b**)*

Yield: 48%; mp 236–238 °C; ^{1}H NMR (δ, ppm, DMSO-d_6): 1.80 (s, 3H, pyrazoline–CH_3), 3.31 (s, 2H, CH_2), 7.46 (d, 2H, Ar–H), 7.63 (s, 1H, H–thiazole), 7.86 (d, 2H, Ar–H), 7.89 (s, 1H, CH–imidazole); EI-MS (70 eV) *m*/*z* (Rel. Int.): 330 (M^+, 0.23), 289 (0.26), 275 (0.40), 252 (30.17), 210 (100.00), 168 (31.68), 138 (10.84), 111 (11.08); Anal. for $C_{15}H_{11}ClN_4OS$ (330.79) C, H, N.

*4.4.6. 3-Methyl-1-(3-p-tolylimidazo[2,1-b]thiazol-6-yl)-1H-pyrazol-5(4H)-one (**5c**)*

Yield: 44%; mp 158–160 °C; ^{1}H NMR (δ, ppm, DMSO-d_6): 1.81 (s, 3H, pyrazoline–CH_3), 2.29 (s, 3H, Ar–CH_3), 3.51 (s, 2H, CH_2), 7.22 (d, 2H, Ar–H), 7.32 (s, 1H, H–thiazole), 7.73 (d, 2H, Ar–H), 7.80 (s, 1H, CH–imidazole); EI-MS (70 eV) *m*/*z* (Rel. Int.): 310 (M^+, 2.44), 269 (17.98), 255 (1.40), 232 (25.31), 190 (100.00), 148 (29.14), 118 (51.48), 91 (65.79); Anal. for $C_{16}H_{14}N_4OS$ (310.37) C, H, N.

*4.4.7. 6-(3,5-Dimethyl-1H-pyrazol-1-yl)-3-phenylimidazo[2,1-b]thiazole (**6a**)*

Yield: 66%; mp 188–190 °C; IR (KBr, ν, cm^{-1}): 3168 (CH aromatic), 3065, 2988 (CH aliphatic), 1596, 1583 cm^{-1} (C=N); ^{1}H NMR

(δ, ppm, DMSO-d_6): 2.18 (s, 3H, CH_3–pyrazole), 2.51 (s, 3H, CH_3–pyrazole), 5.71 (s, 1H, CH–pyrazole), 7.32 (t, 2H, Ar–H), 7.43 (t, 1H, Ar–H), 7.58 (s, 1H, H–thiazole), 7.62 (s, 1H, CH–imidazole), 7.89 (d, 2H, Ar–H); ^{13}C NMR (δ, ppm, DMSO-d_6): 22.46 (2 CH_3–pyrazole), 107.78 (CH–imidazole, –S–CH–thiazole, C_4–pyrazole), 125.62, 127.70, 128.67 (Ar–CH), 134.32 (quaternary Ar–C, thiazole–C), 148.70 (C_6–imidazothiazole, C_5–pyrazole), 157.94 (C_3–pyrazole), 168.59 (–S–C(N)=*N*-imidazothiazole); EI-MS (70 eV) *m*/*z* (Rel. Int.): 294 (M^+, 0.01), 252 (0.01), 218 (40.24), 195 (0.08), 176 (100.00), 134 (46.08), 104 (10.96), 77 (2.63); Anal. for $C_{16}H_{14}N_4S$ (294.37) C, H, N.

4.4.8. 3-(4-Chlorophenyl)-6-(3,5-dimethyl-1H-pyrazol-1-yl) imidazo[2,1-b]thiazole (**6b**)

Yield: 70%; mp 216–218 °C; ^{1}H NMR (δ, ppm, DMSO-d_6): 2.17 (s, 3H, CH_3–pyrazole), 2.46 (s, 3H, CH_3-pyrazole), 5.86 (s, 1H, CH–pyrazole), 7.45 (d, 2H, Ar–H), 7.62 (s, 1H, H–thiazole), 7.86 (d, 2H, Ar–H), 7.90 (s, 1H, CH–imidazole); EI-MS (70 eV) *m*/*z* (Rel. Int.): 328 (M^+, 1.88), 286 (1.40), 252 (22.15), 229 (1.19), 210 (64.84), 168 (22.12), 138 (11.87), 111 (19.36); Anal. for $C_{16}H_{13}ClN_4S$ (328.82) C, H, N.

4.4.9. 6-(3,5-Dimethyl-1H-pyrazol-1-yl)-3-p-tolylimidazo[2,1-b] thiazole (**6c**)

Yield: 67%; mp 196–198 °C; ^{1}H NMR (δ, ppm, DMSO-d_6): 2.12 (s, 3H, pyrazole–CH_3), 2.28 (s, 3H, Ar–CH_3), 2.46 (s, 3H, pyrazole–CH_3), 5.72 (s, 1H, CH–pyrazole), 7.22 (d, 2H, Ar–H), 7.32 (s, 1H, H–thiazole), 7.75 (d, 2H, Ar–H), 7.81 (s, 1H, CH–imidazole); EI-MS (70 eV) *m*/*z* (Rel. Int.): 308 (M^+, 50.68), 266 (43.15), 209 (41.10), 190 (84.93), 148 (36.99), 118 (8.90); Anal. for $C_{17}H_{16}N_4S$ (308.40) C, H, N.

4.5. In vitro anticancer screening

Eleven of the synthesized compounds including **2a**, **b** and **4**–**6**(**a**–**c**) were subjected to the National Cancer Institute (NCI) *in vitro* disease-oriented human cells screening panel assay for *in vitro* antitumor activity [27–30]. The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. Cells are inoculated into 96 well microtiter plates in 100 μL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are 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 are fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (T_z). Experimental drugs are solubilized in dimethylsulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is 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 are made to provide a total of five drug concentrations plus control. Aliquots of 100 μL of these different drug dilutions are added to the appropriate microtiter wells already containing 100 μL of medium, resulting in the required final drug concentrations.

Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are 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 is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is 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, (T_z), control growth, (C), and test growth in the presence of drug at the five concentration levels (T_i)], the percentage growth is calculated at each of the drug concentration levels. Percentage growth inhibition is calculated as:

- $[(T_i - T_z)/(C - T_z)] \times 100$ for concentrations for which $T_i \geq T_z$
- $[(T_i - T_z)/T_z] \times 100$ for concentrations for which $T_i < T_z$

4.6. Target fishing

The target compounds **2a**–**c and 4**–**6**(**a**–**c**) were uploaded in Tripos Mol2 format. PharmMapper adopts semi-rigid pharmacophore mapping protocol. As a result, multiple conformations of the query molecule were required prior to mapping which could be achieved by online service provided by the server. PharmMapper found the best mapping poses of the uploaded molecules against all the targets in PharmTargetDB and top N potential drug targets (default value is 300) as well as respective molecule's aligned poses were outputted [42].

4.7. Docking study

Docking study was performed with the aid of Docking Server. Gasteiger partial charges were added to the ligand atoms after energy minimization using the MMFF94 force field. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools to protein model. Affinity (grid) maps of 20 × 20 × 20 Å grid points and 0.375 Å spacing were generated using the Autogrid program. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method. Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250,000 energy evaluations [43].

Acknowledgment

We are thankful to the National Cancer Institute (NCI), Bethesda, Maryland, USA, for performing the anticancer evaluation over the 60-cancer cell line panel.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2013.12.023. These data include MOL files and InChiKeys of the most important compounds described in this article.

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