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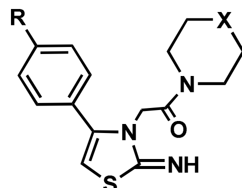
The manuscript deals with the synthesis of a novel series of 2-imino-4-arylthiazoles bearing acetamidomorpholine or acetamidopiperazine moieties to be evaluated for their anticancer activities.



X, 4a-c(X=O), 5a-c(X=NMe), 6a-c(X=NAc), 7a-c(X=NPh)
a, R = H; b, R = Cl; c, R = CH_3

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Novel acetamidothiazole derivatives: Synthesis and *in vitro* anticancer evaluation

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Abstract:

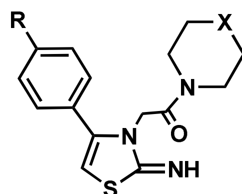
A novel series of acetamide derivatives possessing both 2-imino-4-arylthiazoles and morpholine or different piperazines were synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR, elemental and mass spectral analyses. Twelve compounds were granted NSC codes at National Cancer Institute (NCI), USA for anticancer activity at a single high dose (10^{-5} M) in full NCI 60 cell panel. Among the compounds tested, compounds **5a** and **6b** were found to be the most active candidates of the synthesized series. Assessment of toxicities, druglikeness, and drug score profiles of compounds **5a** and **6b** are promising. Some of the synthesized compounds showed a good docking score with potential anticancer targets, chosen based on pharmacophore mapping of the established derivatives.

Keywords: aminothiazole, acetamidomorpholine, acetamidopiperazine, anticancer evaluation.

1. Introduction

Much attention was given by medicinal chemistry researchers to thiazole ring system as the basis for development of many drugs with a

diversity of biological activities. 2-Amino-4-aryl-1,3-thiazoles displayed a wide range of biological potencies including antimicrobial [1-5], analgesic [6,7], anticonvulsant [8,9], antioxidant [10], hypolipidemic [11], anti-HIV-1 [12,13], adenosine receptor antagonist [14,15], osteoporosis inhibitor [16] and anti-Alzheimer [17-19] activities. Antitumor activity of thiazole scaffold was readily established *via* being incorporated into a variety of therapeutically active agents like bleomycin [20], vosaroxin [21], epothilones [22,23] and dasatinib [24-26]. From literature survey, it was found that aminothiazoles proved to have a broad spectrum of activity against most of the tested subpanel tumor cell lines.[27-31] In addition, 2-aminothiazole analogue of the natural cytotoxic agent, giroline, [32] and cantharidin [33] demonstrated increased apoptotic activity compared to their parent compounds. Furthermore, several reports indicated that series of 2-amino-4-arylthiazoles were synthesized and reported to be active as anticancer agents.[34-37] Moreover, several acetamide derivatives comprising both thiazole ring [38-40] or different fused heterocyclic systems [41,42] and different amines such as morpholine or piperazines were reported to possess good antitumor activity. Thus, it was proposed to prepare a series of 2-imino-4-arylthiazoles bearing acetamidomorpholine or acetamidopiperazine moieties of the general structure **(A)** to be evaluated for their antitumor activities.



(A)
X=O, NMe, NAc, NPh
R=H, Cl, CH_3

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2. Results and discussion

2.1. Chemistry

The synthesis of the starting compounds **3a-c** is outlined in Scheme 1.

Scheme 1. Synthesis of the starting compounds 3a-c

2-Amino-4-arylthiazoles **2a-c** were prepared utilizing phenacyl bromide according to a reported procedure [43] which is considered to be an easy, rapid and purification-free procedure. Thiourea was allowed to react with phenacyl bromide at room temperature for 2-3 minutes to yield the corresponding arylthiazole. The formation of 2-amino-4-arylthiazoles was confirmed by their IR spectra. From literature survey, it was reported that a variety of aminoheterocyclic systems could yield fused ring systems containing keto group through reaction with chloroacyl chloride [44-46] or ethyl chloroacetate [47,48]. Hurd and Hayao [49] reported the fusion between 2-aminothiazole and 3-bromopropionic acid to produce 5,6-dihydrothiazolo[3,2-*a*]pyrimidin-7-one hydrobromide in 30% yield. Recently, it was demonstrated that the reaction between 2-amino-4-phenylthiazole and chloroacetic acid could be furnished in ethanol yielding fused imidazothiazole derivative.[50] In the present study, 2-amino-4-arylthiazoles **2a-c** were reacted with chloroacetic acid in glacial acetic acid in the presence of anhydrous sodium acetate. This was accomplished *via* prolonged heating up to 40 h and the products were obtained in 72-82% yield. The structures of compounds **3a-c** were confirmed on the basis of spectral data. The CH_2 protons appeared as a broad singlet around 3.46-3.87 in the ^{1}H-NMR spectrum.

Mavrova *et al.* [51] demonstrated non-traditional reaction in which the condensed thiazole ring underwent cleavage in a basic medium

and took benefit of this ring opening to prepare many piperazine derivatives by the reaction between 1,3-thiazolo[3,2-*a*]benzimidazol-3(2*H*)-one and different piperazines. In this study, the new target compounds **4-7(a-c)** were prepared adopting the aforementioned procedure through the reaction of 3-(un)substituted phenylimidazo[2,1-*b*]thiazol-6(5*H*)-one **3a-c** with secondary amines; morpholine or piperazine derivatives. The synthesis of the new target compounds **4-7(a-c)** is outlined in Scheme 2.

Scheme 2. Synthesis of the title compounds 4-7(a-c)

The structures of the synthesized compounds **4-7(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. In the ^{1}H NMR spectra of these compounds, a singlet peak for CH_2-protons appeared at 4.10-4.23 ppm with a D_2O exchangeable peak at 7.47-7.70 ppm for the NH proton. The ^{13}C NMR spectra of these compounds showed signals for the morpholine and piperazine ring at the expected regions. In the ^{1}H NMR spectra of compounds **5a-c**, a singlet peak for N-CH_3 protons appeared at 2.12-2.18 ppm. In the ^{1}H NMR spectra of compounds **6a-c**, a singlet peak appeared at 1.95-2.17 ppm for -$COCH_3$ protons, while ^{13}C signals of -CO**CH_3** in compound **6a** were observed around 22.57 ppm. The ^{1}H NMR spectra of compounds **7a-c** is characterized by the appearance of signals for phenyl ring attached to piperazine ring at 6.73-6.79, 6.88-6.93 and 7.17-7.21 ppm. The mass spectral data of these compounds displayed molecular ion peaks which confirmed their molecular weights.

2.2. Biological evaluation

2.2.1. In vitro anticancer screening

The target compounds **4-7(a-c)** were submitted to National Cancer Institute (NCI) [52], Bethesda, Maryland, USA, under the Developmental Therapeutic Program (DTP) which is designed to screen up to 3,000 compounds per year for potential anticancer activity. 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. Compounds with drug-like properties, based on computer-aided design, are to be prioritized in the NCI screening service. Twelve compounds were selected for screening based on their ability to add diversity to the NCI small molecule compound collection. All compounds submitted to the NCI 60 cell screen were tested at a single dose (10^{-5} M) in the full NCI 60 cell panel. The compounds were added at a single concentration and the culture was incubated for 48 h. End point determinations were made with a protein binding dye, Sulforhodamine B.[53-55]

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 target compounds **4-7(a-c)** against the full 60-cell line panel are illustrated in Table 1.

Table 1: Sixty human tumor cell line anticancer screening data at single dose assay (10^{-5} M concentration) as percentage cell growth

The mean percentage growth and the growth percentage with the most sensitive cell lines of all of the tested compounds over the full panel of cell lines are illustrated in Table 2.

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

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

- The tested synthesized compounds exhibited low growth inhibition against colon cancer cell lines.
- Although the majority of the target compounds **4-7(a-c)** were inactive against melanoma cell lines, compounds like **5c** and **6c** showed moderate activity towards *UACC-62* cell line with cell promotion percentage 72.63 and 74.19%, respectively.
- Compounds **6b** and **5a** were the most active members in the synthesized series with mean percentage growth values of 89.88 and 91.67%, respectively.
- Compound **6b** was found to exhibit moderate potency towards ovarian *OVCAR-4* cancer cell line with cell promotion percentage 77.23%. On the other hand, the remaining tested compounds were almost inactive against ovarian cancer.
- Anticancer activity of target compounds **4-7(a-c)** was found to be sensitive to the nature of substituent in phenyl ring when compared to other compounds in the same series. The absence of substituent in compounds **4-7(a)** led to increase in the potency towards *CNS SF-295* (when tested), *CNS SNB-75*, and *Renal UO-31* cancer cell lines.
- The introduction of chloro substituent in compounds **4-6(b)** led to more potent compounds towards *Leukemia SR* and *MOLT-4*. On the other hand, reduced potency towards *Non-Small Cell Lung HOP-92* cancer cell line was probably associated with chloro substituent in compounds **4-7(b)**.
- Methyl group in compounds **4-7(c)** has increased the potency towards *Non-Small Cell Lung HOP-92* and *Prostate PC-3* cancer cell line than other compounds in the same series. In addition, when compared to **7c**, compound **7a**, that has unsubstituted phenyl attached to the thiazole ring with the phenylpiperazine moiety, showed more

enhanced potency towards both these two cell lines with cell promotion percentage of 59.05 and 72.32%, respectively.

We performed COMPARE [56] analyses for compounds **4-7(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.[57,58] 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 the majority of the tested compounds demonstrated considerable to weak correlation levels with rapamycin (NSC S226080) with PCC values illustrated in Table 3. Such similarity in COMPARE results could indicate potential 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.[59-61]

Table 3: PCC values of the tested compounds with rapamycin (NSC S226080)

As shown in Table 3, although compounds **4c**, **7b** and **7c** exhibited the least correlation with rapamycin, they showed considerable correlation with other standard anticancer agents. Compound **4c** exhibited a considerable correlation with spirohydantoin mustard (NSC S172112),

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which is a classical bifunctional alkylating agent synthesized in an effort to develop antitumor agents effective against CNS tumors [62], with PCC value of 0.522. In addition, compound **7b** exhibited a considerable correlation with hexamethylene bisacetamide (NSC S95580) with PCC value of 0.472. Hexamethylene bisacetamide interferes with the morphological and functional differentiation of malignant cells *in vitro*. [63] Compound **7c** showed a moderate correlation with flavone acetic acid (NSC S347512) with PCC value of 0.484. Flavone acetic acid belongs to a class of chemicals having a wide range of biological properties and is considered to be active in solid cancers.[64]

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. Also, in order to attain a reasonable probability of being well absorbed, compounds must have log P value not greater than 5.0. Nearly 40% of drug candidates fail in clinical trials because of poor ADME [65]. From all the previous observations, we evaluated the compliance of the designed compounds to the Lipinski's rule of five, calculated by Canvas [66] and Osiris [67] program. 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 4. The results showed that all the tested compounds comply with these rules suggesting that the synthesized compounds would be possible drug candidates.

The total polar surface area (TPSA) using Canvas [66] program was calculated 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.[68] Since all the synthesized

compounds **4-7(a-c)** have TPSA value ranging from 37.53 to 53.14, they theoretically should present good passive oral absorption, but the differences in their biological activity cannot be attributed to this property.

Table 4: Solubility, molar refractivity, and calculated Lipinski's rule of five for target compounds

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

Osiris program [67] was used for prediction of the overall toxicity of the designed derivatives. The prediction process relies on a substructure search process determining the occurrence frequency of any fragment (core and constructed fragments) within any of toxicity classes. All target compounds **4-7(a-c)** showed low *in silico* possible toxicity risks. Osiris program was also used for calculating the fragment-based druglikeness by summing up score values of those fragments shredded at rotatable bonds. The score value of each fragment was estimated based on their occurrence frequency within the collection of traded drugs and within the supposedly non drug-like collection of Fluka compounds. A positive value states that designed molecule contains predominately fragments which are frequently present in commercial drugs.

The drug score combines druglikeness, cLogP, LogS, molecular weight and toxicity risks in one handy value that may be used to judge the compound's overall potential to qualify for a drug. A value of 0.5 or more makes this compound a promising lead for future development of safe and efficient drug. Predictions of potential toxicity, druglikeness and drug score for the studied compounds are given in Table 5. Almost all of the synthesized compounds possess good values of druglikeness and drug score. Among all the synthesized compounds, compounds **5a** and **6b** with

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high values of both druglikeness and drug score showed the lowest mean percentage growth over 60 cell line panel.

Table 5: Toxicity risks, druglikeness and drug score of the designed compounds

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. *In silico* target fishing is an emerging technology that enables the prediction of biological targets of compounds on the basis of chemical structure by using information from increasingly available biologically annotated chemical databases.[69] PharmMapper server is open-accessed web server. PharmMapper automatically finds the best mapping poses of the query molecule against all the pharmacophore models using reverse pharmacophore mapping approach.[70]

PharmMapper is available at http://59.78.96.61/pharmmapper. PharmMapper demonstrated a variety of putative targets that might exhibit considerable binding affinity to the synthesized compounds. Seven targets, involved in cancer therapy, are common between the synthesized compounds. These targets might explain the observed antiproliferative activity. The fit scores of the synthesized compounds with the top targets are illustrated in Table 6.

Table 6: Fit score of the synthesized compounds against the top seven enzymes

Compound **7b** exhibiting the highest fit score with Vitamin D_3 receptor is illustrated in Figure 1. As with the other remaining compounds, it demonstrated hydrophobic, hydrogen bond acceptor and hydrogen bond donor centers.

Figure 1: Pharmacophore map with the highest fit score derived from compound 7b

Targets proposed by PharmMapper are employed in cancer therapy in a diversity of approaches.[71-89] As a result, we have studied the potential interaction of the synthesized compounds **4-7(a-c)** against these seven targets which are involved in cancer.

2.2.5. Docking study

The seven potential targets proposed by pharmacophore mapping approach were used to investigate their interaction with the designed compounds. The target compounds **4-7(a-c)** were comparatively evaluated in terms of estimated free energy of binding (kcal/mol), and inhibition constant K_i (uM) to the seven proposed enzymes and the results are listed in Table 7. Molecular docking simulations were performed for the synthesized compounds to evaluate their recognition profile at the binding pocket of the proposed enzymes. The binary complex of the enzyme coupled with its natural ligand was used as a reference for docking and modeling in this investigation.

Docking simulations were carried out with the aid of Docking Server [90], a web-based interface that calculates the geometry and electronic properties of the ligands at a given pH.

Table 7: Estimated free energy of binding and inhibition constants of the synthesized compounds with the top seven targets

Compounds **5a** and **6b** exhibiting the lowest mean percentage growth over 60 cell line panel showed reasonable docking scores with the proposed enzymes. The binding mode of compound **7b** showing the least inhibition constant is illustrated in Figure 2. Compound **7b** seem to have hydrogen bond interaction with GLN136, GLY269 and MET270 and GLU296, polar interaction with HIS295, GLU296 and TYR383, and hydrophobic interaction with TYR267, MET270 and VAL292 with K_i equal to 0.093 µM.

Figure 2: Binding mode of compound 7b

2.2.6. Predicting potential side effects

SePreSA is a server for the prediction of adverse drug reactions. The server has comprehensive collection of the structural models of nearly all the well-known serious adverse drug reactions targets and calculates chemical–protein interactome using the DOCK program. The server utilizes a 2-directional *Z*-transformation scoring algorithm, which computes the relative drug–protein interaction strength based on the docking-score matrix, thus achieves greater accuracy in prioritizing targets than simply using dock scoring functions.[91] The server is freely available at http://SePreSA.Bio-X.cn/.

The drug molecule tends to interact with the protein if Z'-score is less than -0.5. The protein id, adverse drug reaction and Z'-score for each compound are recorded in Table 8.

Table 8: Adverse drug reactions and Z'-scores for the designed compounds

From the data obtained, a decrease in the affinity of the synthesized compounds containing phenylpiperazine moiety **7a-c** towards estrogen and androgen receptors was observed when compared with the remaining compounds**.** The absence of substituent in compounds **4**-**7(a)** led to increase in the affinity towards DNA topoisomerase 2-alpha supposing possible risk factor for cytotoxicity. In addition, when compared to **7a**, compound **7b** showed lower Z'-score with the same receptor. Cytotoxicity manifested by interaction with DNA topoisomerase 2-alpha is displayed in the following order: acetylpiperazine series **6a-c** > methylpiperazine series **5a-c** > morpholine series **4a-c** > phenylpiperazine series **7a-c**. Also, it can be assumed from the previous findings that the introduction of chloro substituent in compounds **4-6(b)** resulted in increased affinity towards purine nucleoside phosphorylase

enzyme. The higher affinity of these compounds to the previous enzyme might suggest potential bone marrow toxicity.

3. Conclusion

The synthesized compounds **4-7(a-c)** were tested at a single dose of 10 μM at the NCI over 60 cell line panel to screen for their potential anticancer activity. On the basis of results obtained, it was found that compounds **5a** (NSC 768193) and **6b** (NSC 768174) proved to be the most active members in this study. They showed moderate potency over certain cancer cell lines. From the obtained results, it is clear that substituents affect the activity of compounds in different series. The *in silico* ADME profiling, toxicity, druglikeness, drug score, and the docking scores with enzymes derived from pharmacophore similarity results together with *in vitro* anticancer activities make **5a** and **6b** promising lead compounds for development of more potent anticancer agents. Further modifications of these compounds in order to improve their potency are currently under consideration.

4. Experimental

4.1. General

2-Amino-4-arylthiazoles **2a-c** were prepared following the procedure reported by Dighe [43]. 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 (^{o}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. ^{1}H and ^{13}C NMR for compounds **4-7(a)** were recorded on Bruker 500 MHz FT NMR spectrometer and ^{1}H 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. Synthesis of 3-(un)substituted phenylimidazo[2,1-b]thiazol-6(5H)-one (**3a–c**)*

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

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

Yield: 82%; mp 212-214 ^{o}C [50]; IR (KBr, ν, cm^{-1}): 3169 (CH aromatic), 3023, 2987 (CH aliphatic), 1654, 1644 (C=O), 1599, 1584 cm^{-1}

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(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 (**3b**)*

Yield: 80%; mp 254-256 °C; ^{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), 233 (2.36), 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 (**3c**)*

Yield: 70%; mp 132-134 °C; ^{1}H NMR (δ, ppm, DMSO-d_6): 2.29 (s, 3H, -CH_3), 3.84 (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. Synthesis of 2-(2-imino-4-(un)substituted phenylthiazol-3(2H)-yl)-1-morpholinoethanone (**4a–c**)*

A mixture of 3-(un)substituted phenylimidazo[2,1-*b*]thiazol-6(5*H*)-one **3a-c** (10 mmol) and morpholine (1.03 mL, 12 mmol) was dissolved in absolute ethanol (15 mL) and refluxed for 6 h. The solvent was distilled under reduced pressure and the obtained product was washed with water and crystallized from aqueous ethanol to give the corresponding compounds.

*4.3.1. 2-(2-Imino-4-phenylthiazol-3(2H)-yl)-1-morpholinoethanone (**4a**)*

Yield: 66%; mp 196-198 °C; IR (KBr, ν, cm^{-1}): 3453 (NH), 3169 (CH aromatic), 3064, 2986 (CH aliphatic), 1651 (C=O), 1584 cm^{-1} (C=N); ^{1}H NMR (δ, ppm, DMSO-d_6): 3.38 (t, 4H, $(CH_2)_2$N-morpholine),

3.57 (t, 4H, $(CH_2)_2O$-morpholine), 4.20 (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, NH), 7.90 (d, 2H, Ar-H); ^{13}C NMR (δ, ppm, DMSO-d_6): 40.01 (N**CH_2**CO), 45.16 ($(CH_2)_2N$-morpholine), 68.32 ($(CH_2)_2O$-morpholine), 107.77 (-S-**CH**-thiazole), 125.63, 127.69, 128.67 (Ar-CH), 134.33 (quaternary Ar-C), 148.71 (quaternary thiazole-C), 157.96 (-S-C(N)=NH-thiazole), 168.59 (C=O); EI-MS (70 eV) m/z (Rel. Int.): 303 (M^+, 0.15), 245 (0.40), 234 (0.11), 218 (30.73), 203 (0.50), 189 (0.39), 176 (100.00), 165 (0.27), 134 (61.83), 119 (1.86), 114 (0.58), 104 (16.27), 77 (21.79); Anal. Calcd for $C_{15}H_{17}N_3O_2S$ (303.38) C, H, N.

*4.3.2. 2-(4-(4-Chlorophenyl)-2-iminothiazol-3(2H)-yl)-1-morpholinoethanone (**4b**)*

Yield: 62%; mp 228-230 °C; ^{1}H NMR (δ, ppm, DMSO-d_6): 3.32 (t, 4H, $(CH_2)_2N$-morpholine), 3.57 (t, 4H, $(CH_2)_2O$-morpholine), 4.12 (s, 2H, CH_2), 7.45 (d, 2H, Ar-H), 7.62 (s, 1H, H-thiazole), 7.69 (s, 1H, NH), 7.87 (d, 2H, Ar-H); EI-MS (70 eV) m/z (Rel. Int.): 339 (M^++2, 0.99), 337 (M^+, 1.88), 279 (0.43), 268 (3.46), 252 (23.82), 237 (2.33), 223 (1.25), 210 (100.00), 199 (0.82), 168 (34.48), 138 (10.82), 114 (3.06), 111 (15.12), 77 (7.21); Anal. Calcd for $C_{15}H_{16}ClN_3O_2S$ (337.82) C, H, N.

*4.3.3. 2-(2-Imino-4-p-tolylthiazol-3(2H)-yl)-1-morpholinoethanone (**4c**)*

Yield: 55%; mp 144-148 °C; ^{1}H NMR (δ, ppm, DMSO-d_6): 2.29 (s, 3H, $-CH_3$), 3.50 (t, 4H, $(CH_2)_2N$-morpholine), 3.88 (t, 4H, $(CH_2)_2O$-morpholine), 4.11 (s, 2H, CH_2),7.19 (d, 2H, Ar-H), 7.34 (s, 1H, H-thiazole), 7.47 (s, 1H, NH, D_2O exchangeable), 7.75 (d, 2H, Ar-H); EI-MS (70 eV) m/z (Rel. Int.): 317 (M^+, 5.54), 259 (33.70), 248 (5.12), 232 (28.20), 217 (52.45), 203 (35.38), 190 (100.00), 179 (90.22), 148 (35.76),

118 (71.22), 114 (8.36), 91 (82.85), 77 (16.87); Anal. Calcd for $C_{16}H_{19}N_3O_2S$ (317.41) C, H, N.

*4.4. General procedure for the synthesis of compounds **5-7(a-c)***

A mixture of 3-(un)substituted phenylimidazo[2,1-*b*]thiazol-6(5*H*)-one **3a-c** (10 mmol) and piperazine or *N*-substituted piperazine (12 mmol) was dissolved in absolute ethanol (10 mL) and refluxed for 6 h. The solvent was distilled under reduced pressure and the obtained oily product was triturated with petroleum ether several times in order to remove the excess piperazine. The obtained solid was crystallized from aqueous ethanol to give the corresponding compounds.

*4.4.1. 2-(2-Imino-4-phenylthiazol-3(2H)-yl)-1-(4-methylpiperazin-1-yl)ethanone (**5a**)*

Yield: 52%; mp 190-192 ^{o}C; IR (KBr, ν, cm^{-1}): 3434 (NH), 3169 (CH aromatic), 3063, 2985 (CH aliphatic), 1644 (C=O), 1598 cm^{-1} (C=N); ^{1}H NMR (δ, ppm, DMSO-d_6): 2.18 (s, 3H, N-CH_3), 2.51 (br s, 4H, $(CH_2)_2N^4$ piperazine), 3.51 (br s, 4H, $(CH_2)_2N^1$ piperazine), 4.19 (s, 2H, CH_2), 7.32 (t, 2H, Ar-H), 7.42 (t, 1H, Ar-H), 7.57 (s, 1H, H-thiazole), 7.63 (s, 1H, NH), 7.90 (d, 2H, Ar-H); ^{13}C NMR (δ, ppm, DMSO-d_6): 39.80 ($(CH_2)_2N^1$ piperazine), 39.97 (N**<u>CH₂</u>**CO,N**<u>CH₃</u>**), 61.13 ($(CH_2)_2N^4$ piperazine), 108.00 (-S-**<u>CH</u>**-thiazole), 125.62, 127.69, 128.67 (Ar-CH), 134.32 (quaternary Ar-C), 148.70 (quaternary thiazole-C), 157.44 (-S-C(N)=NH-thiazole), 168.60 (C=O); EI-MS (70 eV) m/z (Rel. Int.): 316 (M^+, 0.01), 245 (0.01), 234 (0.03), 218 (35.10), 203 (0.55), 189 (0.14), 176 (100.00), 165 (0.04), 141 (0.09), 134 (54.02), 127 (0.08), 104 (12.09), 77 (9.39); Anal. Calcd for $C_{16}H_{20}N_4OS$ (316.42) C, H, N.

*4.4.2. 2-(4-(4-Chlorophenyl)-2-iminothiazol-3(2H)-yl)-1-(4-methylpiperazin-1-yl)ethanone (**5b**)*

Yield: 57%; mp 228-230 °C; ^{1}H NMR (δ, ppm, DMSO-d_6): 2.12 (s, 3H, N-CH_3), 2.46 (br s, 4H, $(CH_2)_2N^4$ piperazinyl), 3.44 (br s, 4H, $(CH_2)_2N^1$ piperazinyl), 4.12 (s, 2H, CH_2), 7.45 (d, 2H, Ar-H), 7.63 (s, 1H, H-thiazole), 7.67 (s, 1H, NH), 7.86 (d, 2H, Ar-H); EI-MS (70 eV) m/z (Rel. Int.): 352 (M^++2, 0.18), 350 (M^+, 0.37), 279 (0.17), 268 (2.93), 252 (17.58), 237 (1.12), 223 (0.71), 210 (100.00), 199 (0.35), 168 (35.75), 141 (3.31), 138 (10.59), 127 (1.23), 111 (12.35), 77 (5.85); Anal. Calcd for $C_{16}H_{19}ClN_4OS$ (350.87) C, H, N.

*4.4.3. 2-(2-Imino-4-p-tolylthiazol-3(2H)-yl)-1-(4-methylpiperazin-1-yl)ethanone (**5c**)*

Yield: 51%; mp 142-144 °C; ^{1}H NMR (δ, ppm, DMSO-d_6): 2.15 (s, 3H, N-CH_3), 2.30 (s, 3H, Ar-CH_3), 2.48 (br s, 4H, $(CH_2)_2N^4$ piperazinyl), 3.47 (br s, 4H, $(CH_2)_2N^1$ piperazinyl), 4.10 (s, 2H, CH_2), 7.18 (d, 2H, Ar-H), 7.36 (s, 1H, H-thiazole), 7.56 (s, 1H, NH, D_2O exchangeable), 7.77 (d, 2H, Ar-H); EI-MS (70 eV) m/z (Rel. Int.): 330 (M^+, 0.35), 259 (8.32), 248 (6.25), 232 (24.45), 217 (18.28), 203 (7.23), 190 (83.45), 179 (100.00), 148 (31.26), 141 (1.38), 127 (1.85), 118 (53.36), 91 (46.07), 77 (12.65); Anal. Calcd for $C_{17}H_{22}N_4OS$ (330.45) C, H, N.

*4.4.4. 1-(4-Acetylpiperazin-1-yl)-2-(2-imino-4-phenylthiazol-3(2H)-yl)ethanone (**6a**)*

Yield: 33%; mp 180-182 °C; IR (KBr, ν, cm^{-1}): 3433 (NH), 3169 (CH aromatic), 3064, 2986 (CH aliphatic), 1643 (C=O), 1599 cm^{-1} (C=N); ^{1}H NMR (δ, ppm, DMSO-d_6): 2.17 (s, 3H, -$COCH_3$), 3.35 (br s, 8H, piperazine), 4.10 (s, 2H, CH_2), 7.32 (t, 2H, Ar-H), 7.43 (t, 1H, Ar-H), 7.59 (s, 1H, H-thiazole), 7.70 (s, 1H, NH), 7.90 (d, 2H, Ar-H); ^{13}C NMR

(δ, ppm, DMSO-d_6): 22.57 (-CO**CH$_3$**), 39.76 (N**CH$_2$**CO), 48.53 (C-piperazine), 107.80 (-S-**CH**-thiazole), 125.65, 127.71, 128.72 (Ar-CH), 134.31 (quaternary Ar-C), 148.78 (quaternary thiazole-C), 157.93 (-S-C(N)=NH-thiazole), 168.60 (2 C=O); EI-MS (70 eV) m/z (Rel. Int.): 344 (M^+, 0.44), 245 (0.07), 234 (0.14), 218 (31.73), 203 (1.15), 176 (100.00), 165 (0.88), 155 (0.50), 134 (67.52), 127 (0.18), 104 (17.19), 77 (18.22); Anal. Calcd for $C_{17}H_{20}N_4O_2S$ (344.43) C, H, N.

*4.4.5. 1-(4-Acetylpiperazin-1-yl)-2-(4-(4-chlorophenyl)-2-iminothiazol-3(2H)-yl)ethanone (**6b**)*

Yield: 45%; mp 226-228 °C; ^{1}H NMR (δ, ppm, DMSO-d_6): 1.95 (s, 3H, -$COCH_3$), 3.32 (br s, 8H, piperazine ring), 4.12 (s, 2H, CH_2), 7.45 (d, 2H, Ar-H), 7.63 (s, 1H, H-thiazole), 7.67 (s, 1H, NH), 7.86 (d, 2H, Ar-H); EI-MS (70 eV) m/z (Rel. Int.): 380 (M^++2, 5.17), 378 (M^+, 12.98), 279 (8.56), 252 (3.56), 237 (37.29), 223 (3.95), 210 (3.54), 199 (1.45), 169 (2.05), 155 (1.05), 138 (3.05), 127 (7.99), 111 (19.19); Anal. Calcd for $C_{17}H_{19}ClN_4O_2S$ (378.88) C, H, N.

*4.4.6. 1-(4-Acetylpiperazin-1-yl)-2-(2-imino-4-p-tolylthiazol-3(2H)-yl)ethanone (**6c**)*

Yield: 40%; mp 140-142 °C; ^{1}H NMR (δ, ppm, DMSO-d_6): 1.95 (s, 3H, -$COCH_3$), 2.29 (s, 3H, Ar-CH_3), 3.52 (br s, 8H, piperazine ring), 4.12 (s, 2H, CH_2), 7.19 (d, 2H, Ar-H), 7.35 (s, 1H, H-thiazole), 7.48 (s, 1H, NH, D_2O exchangeable), 7.73 (d, 2H, Ar-H); EI-MS (70 eV) m/z (Rel. Int.): 358 (M^+, 15.81), 259 (24.62), 232 (19.76), 203 (17.33), 190 (27.66), 169 (20.67), 148 (16.41), 118 (38.30), 91 (50.76), 77 (13.98); Anal. Calcd for $C_{18}H_{22}N_4O_2S$ (358.46) C, H, N.

*4.4.7. 2-(2-Imino-4-phenylthiazol-3(2H)-yl)-1-(4-phenylpiperazin-1-yl)ethanone (**7a**)*

Yield: 39%; mp 174-176 °C; IR (KBr, ν, cm^{-1}): 3446 (NH), 3179 (CH aromatic), 3069, 2995 (CH aliphatic), 1651 (C=O), 1582 cm^{-1} (C=N); ^{1}H NMR (δ, ppm, DMSO-d_6): 3.17 (t, 4H, $(CH_2)_2N^4$ piperazine), 3.41 (t, 4H, $(CH_2)_2N^1$ piperazine), 4.23 (s, 2H, CH_2), 6.79 (t, 1H, piperazine-Ar-H), 6.93 (d, 2H, piperazine-Ar-H), 7.21 (t, 2H, piperazine-Ar-H), 7.33 (t, 2H, Ar-H), 7.44 (t, 1H, Ar-H), 7.59 (s, 1H, H-thiazole), 7.64 (s, 1H, NH), 7.92 (d, 2H, Ar-H); ^{13}C NMR (δ, ppm, DMSO-d_6): 39.99 (N**<u>CH_2</u>**CO), 48.20 ($(CH_2)_2N^1$ piperazine), 52.50 ($(CH_2)_2N^4$ piperazine), 107.79 (-S-**<u>CH</u>**-thiazole), 115.42, 118.82 (piperazine-Ar-C), 125.65, 127.71, 128.69, 128.88 (Ar-CH,piperazine-Ar-C), 134.34 (quaternary Ar-C), 148.73, 148.87 (quaternary thiazole-C,piperazine-Ar-C), 157.99 (-S-C(N)=NH-thiazole), 168.63 (C=O); EI-MS (70 eV) m/z (Rel. Int.): 378 (M^+, 0.01), 363 (0.05), 336 (0.04), 308 (0.05), 288 (0.07), 266 (0.06), 245 (0.03), 234 (0.21), 218 (30.65), 203 (0.89), 189 (0.68), 176 (100.00), 165 (0.66), 161 (1.92), 134 (56.19), 77 (17.34); Anal. Calcd for $C_{21}H_{22}N_4OS$ (378.49) C, H, N.

*4.4.8. 2-(4-(4-Chlorophenyl)-2-iminothiazol-3(2H)-yl)-1-(4-phenylpiperazin-1-yl)ethanone (**7b**)*

Yield: 25%; mp 220-222 °C; ^{1}H NMR (δ, ppm, DMSO-d_6): 3.15 (t, 4H, $(CH_2)_2N^4$ piperazine), 3.47 (t, 4H, $(CH_2)_2N^1$ piperazine), 4.12 (s, 2H, CH_2), 6.73 (t, 1H, piperazine-Ar-H), 6.88 (d, 2H, piperazine-Ar-H), 7.17 (t, 2H, piperazine-Ar-H), 7.45 (d, 2H, Ar-H), 7.63 (s, 1H, H-thiazole), 7.67 (s, 1H, NH), 7.86 (d, 2H, Ar-H); EI-MS (70 eV) m/z (Rel. Int.): 414 (M^++2, 6.88), 412 (M^+, 15.95), 397 (1.35), 370 (0.87), 342 (5.01), 322 (0.53), 300 (4.14), 279 (4.23), 268 (4.45), 252 (6.90), 237 (30.01), 223 (8.23), 210 (32.62), 203 (5.11), 199 (6.59), 189 (3.36), 168 (21.60), 161 (28.25), 111 (22.54), 77 (72.85); Anal. Calcd for $C_{21}H_{21}ClN_4OS$ (412.94) C, H, N.

*4.4.9. 2-(2-Imino-4-p-tolylthiazol-3(2H)-yl)-1-(4-phenylpiperazin-1-yl)ethanone (**7c**)*

Yield: 20%; mp 126-128 °C; ^{1}H NMR (δ, ppm, DMSO-d$_6$): 2.28 (s, 3H, Ar-CH_3), 3.15 (t, 4H, $(CH_2)_2N^4$ piperazine), 3.53 (t, 4H, $(CH_2)_2N^1$ piperazine), 4.10 (s, 2H, CH_2), 6.78 (t, 1H, piperazine-Ar-H), 6.91 (d, 2H, piperazine-Ar-H), 7.19 (m, 4H, piperazine-Ar-H, thiazole-Ar-H), 7.35 (s, 1H, H-thiazole), 7.48 (s, 1H, NH, D_2O exchangeable), 7.78 (d, 2H, Ar-H); EI-MS (70 eV) m/z (Rel. Int.): 392 (M^+, 2.51), 377 (2.30), 350 (4.21), 322 (54.25), 302 (1.52), 280 (30.74), 259 (12.44), 248 (3.02), 232 (12.23), 217 (26.50), 203 (27.12), 190 (53.60), 179 (100.00), 161 (20.12), 148 (20.01), 91 (93.39), 77 (72.45); Anal. Calcd for $C_{22}H_{24}N_4OS$ (392.52) C, H, N.

4.5. In vitro anticancer screening

Twelve of the synthesized compounds including **4-7(a-c)** were subjected to the National Cancer Institute (NCI) *in vitro* disease-oriented human cells screening panel assay for *in vitro* antitumor activity.[52-55] 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. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates 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

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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 minutes 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 minutes 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

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

During the shredding, any molecule was first cut at every rotatable bond leading to a set of core fragments. These in turn were used to reconstruct all possible bigger fragments being a substructure of the original molecule. Afterwards, a substructure search process determined the occurrence frequency of any fragment (core and constructed fragments) within all compounds of 3300 traded drugs as well as 15000 commercially available chemicals (Fluka).[67]

4.7. *Target* fishing

The newly synthesized compounds **4-7(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.[70]

4.8. *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 A° grid points and 0.375 A° 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 250000 energy evaluations.[90]

4.9. Predicting potential side effects

The key residue ionization state was assigned considering the most probable one at the physiological pH using Chimera. The interactome of a drug molecule towards all SADR targets was calculated using DOCK.

For each adverse drug reaction (ADR) target, residues within a 10 A° distance from the ligand were defined as the bioactive pocket of the protein. The server computes a relative drug-protein interaction score from a scoring matrix to prioritize SADR targets which might be affinitive with the user's compound. The prediction mechanism was based on the user-oriented interactome, which calculates the Z'-score of the current molecule from the interactome formed by all molecules submitted by the user, no matter when and where these previous molecules are submitted.[91]

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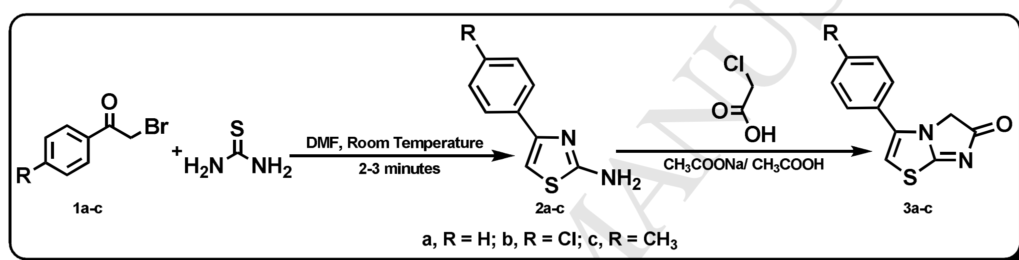
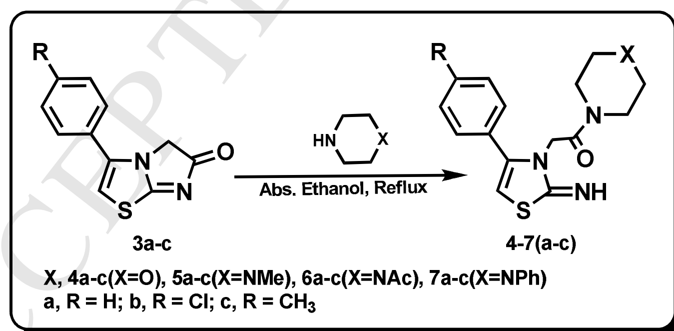
Table 1: Sixty human tumor cell line anticancer screening data at single dose assay (10^{-5} M concentration) as percentage cell growth

Subpanel tumor cell lines	Percentage cell growth											
	4a	**4b**	**4c**	**5a**	**5b**	**5c**	**6a**	**6b**	**6c**	**7a**	**7b**	**7c**
Leukemia												
CCRF-CEM	98.57	91.34	100.64	97.61	89.08	87.14	103.36	92.31	88.15	95.75	96.22	103.08
HL-60(TB)	118.21	96.61	93.23	100.10	102.18	108.81	111.48	97.98	107.97	99.01	89.85	126.88
K-562	89.20	**70.94**[a]	100.04	98.18	**73.08**[a]	**82.88**[a]	86.77	**72.04**[a]	**84.35**[a]	103.26	**84.80**[a]	100.36
MOLT-4	95.19	**69.25**[a]	99.74	95.16	**75.11**[a]	**76.27**[a]	96.22	**76.95**[a]	**72.99**[a]	99.62	87.02	94.55
RPMI-8226	95.40	**78.71**[a]	92.77	92.45	**79.13**[a]	83.54	97.35	**73.27**[a]	**82.35**[a]	92.09	83.40	92.36
SR	86.67	**72.74**[a]	96.22	**74.10**[a]	**78.50**[a]	86.49	88.67	**74.71**[a]	**81.29**[a]	89.94	87.98	98.76
Non-Small Cell Lung Cancer												
A549/ATCC	101.94	**84.56**[a]	94.14	91.95	90.06	86.84	95.96	**75.90**[a]	**83.37**[a]	90.35	**84.76**[a]	97.74
HOP-62	90.05	98.51	111.71	88.88	100.76	99.52	85.29	93.44	101.01	96.59	109.37	110.89
HOP-92	**69.98**[a]	**81.85**[a]	**68.26**[a]	**73.83**[a]	**77.59**[a]	**62.76**[a]	**75.03**[a]	**81.97**[a]	**60.86**[a]	**59.05**[a]	**76.13**[a]	**69.24**[a]
NCI-H226	86.71	94.43	94.35	85.23	95.94	93.14	88.80	96.32	96.04	93.66	92.23	97.23
NCI-H23	95.77	95.63	97.27	90.00	100.00	93.32	98.45	96.94	95.69	91.67	98.31	100.86
NCI-H322M	97.93	103.80	111.25	90.25	95.90	103.86	94.44	90.43	108.10	92.56	99.83	99.69
NCI-H460	105.47	99.34	108.14	99.12	101.13	102.40	110.22	101.30	103.49	96.44	101.06	104.55
NCI-H522	94.21	**79.52**[a]	92.96	**82.32**[a]	86.68	**80.59**[a]	**81.29**[a]	**62.11**[a]	**76.94**[a]	85.09	**76.94**[a]	95.47
Colon Cancer												
COLO 205	107.46	101.41	112.86	102.75	100.75	105.54	99.84	97.00	105.79	102.11	105.09	109.03
HCC-2998	103.94	99.89	98.94	101.00	97.49	102.67	106.64	98.91	101.40	100.46	95.58	104.38
HCT-116	98.56	87.44	100.64	91.58	94.42	103.65	96.06	92.99	85.92	106.88	100.53	103.40
HCT-15	99.16	89.88	98.08	87.60	93.06	91.56	94.47	86.45	90.60	96.98	96.84	103.55
HT29	105.58	94.23	109.06	**83.82**[a]	96.01	110.00	100.99	85.75	105.06	106.05	92.88	111.48
KM12	102.97	105.62	109.79	102.37	98.73	103.81	101.97	95.24	99.37	104.05	108.39	103.80
SW-620	98.03	103.03	113.69	96.06	102.31	98.41	109.29	106.14	97.57	100.60	103.56	104.71
CNS Cancer												
SF-268	104.49	102.17	101.08	96.17	98.43	96.65	100.38	95.12	101.28	100.70	104.53	105.07
SF-295	NT[b]	NT[b]	**83.56**[a]	**65.94**[a]	NT[b]	**83.40**[a]	NT[b]	NT[b]	NT[b]	**72.40**[a]	89.72	NT[b]
SF-539	98.78	95.75	100.30	98.68	100.06	101.14	91.60	90.17	99.88	97.20	100.01	100.14
SNB-19	98.35	91.16	105.86	93.71	92.69	99.82	102.76	**84.16**[a]	99.95	98.57	99.99	96.61
SNB-75	**59.43**[a]	**75.30**[a]	90.60	**56.53**[a]	**79.49**[a]	**73.49**[a]	**67.70**[a]	**74.40**[a]	**82.27**[a]	**74.58**[a]	**78.43**[a]	95.80
U251	95.09	88.31	91.85	88.21	91.16	**83.88**[a]	96.90	85.86	85.03	91.15	89.62	95.26
Melanoma												
LOX IMVI	92.03	97.91	92.77	87.31	96.83	89.11	94.34	93.75	85.50	90.70	102.79	96.75
MALME-3M	102.43	104.79	131.16	89.06	99.64	127.93	141.34	133.08	120.84	94.23	106.87	117.77
M14	100.43	92.15	110.20	94.24	98.87	104.52	97.05	95.06	97.91	101.40	99.68	107.93
MDA-MB-435	100.99	84.56	87.24	104.28	92.23	**83.43**[a]	104.77	85.35	**80.64**[a]	96.53	92.22	92.22
SK-MEL-2	106.04	107.40	110.94	118.31	102.46	107.89	108.32	95.66	112.65	104.23	**78.96**[a]	116.60
SK-MEL-28	112.05	97.11	108.16	100.06	106.54	97.45	101.45	90.79	98.93	98.76	98.61	100.06
SK-MEL-5	95.52	96.48	98.21	88.25	100.33	87.19	90.18	86.37	92.14	94.84	91.87	88.25
UACC-257	111.11	94.66	102.88	106.17	103.61	101.86	98.52	91.01	102.13	107.98	104.79	106.17
UACC-62	92.50	**83.20**[a]	98.13	85.88	90.25	**72.63**[a]	89.57	**81.93**[a]	**74.19**[a]	93.51	98.07	85.88



Scheme 1. Synthesis of the starting compounds 3a-c



Scheme 2. Synthesis of the title compounds 4-7(a-c)

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Highlights:

► A novel series of acetamide derivatives of 2-imino-4-arylthiazoles was prepared. ► Twelve compounds were selected and tested for their anticancer activities by NCI. ► Compounds **5a** and **6b** proved promising for further development.

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