Design, Synthesis, Cytoselective Toxicity, Structure–Activity Relationships, and Pharmacophore of Thiazolidinone Derivatives Targeting Drug-Resistant Lung Cancer Cells

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Received September 24, 2007

Ten cytoselective compounds have been identified from 372 thiazolidinone analogues by applying iterative library approaches. These compounds selectively killed both non-small cell lung cancer cell line H460 and its paclitaxel-resistant variant $H460_{taxR}$ at an IC_{50} between 0.21 and 2.93 μM while showing much less toxicity to normal human fibroblasts at concentrations up to 195 μM. Structure–activity relationship studies revealed that (1) the nitrogen atom on the 4-thiazolidinone ring (ring B in Figure 1) cannot be substituted, (2) several substitutions on ring A are tolerated at various positions, and (3) the substitution on ring C is restricted to the $-NMe_2$ group at the 4-position. A pharmacophore derived from active molecules suggested that two hydrogen bond acceptors and three hydrophobic regions were common features. Activities against P-gp-overexpressing and paclitaxel-resistant cell line $H460_{taxR}$ and modeling using a previously validated P-gp substrate pharmacophore suggested that active compounds were not likely P-gp substrates.

Introduction

Lung cancer is the number one cause of cancer-related deaths worldwide and in the United States (~160,000 deaths annually). Despite advances in cancer research, the overall five-year survival of lung cancer patients remains a dismal 15% in contrast to most other solid organ tumors according to the American Association for Cancer Research (http://www.aacr.org/home/public--media/for-the-media/fact-sheets/organ-site-fact-sheets/lung-cancer.aspx). More than 80% of bronchogenic malignancy is from non-small cell lung cancer (NSCLC).[a] Among other factors, inherent and acquired resistance to treatment[1] and the dose-limiting toxicity caused by the narrow therapeutic window of many cancer drugs[2] are recognized as major obstacles for effective cancer therapy. Multidrug resistance (MDR) is a phenotype of cross-resistance to multiple drugs with diverse chemical structures. One of the well-documented MDR mechanisms is the overexpression of the MDR-1 gene that encodes the transmembrane, ATP-dependent drug efflux transporter P-glycoprotein (P-gp) in response to chemotherapy.[3–5] P-gp prevents the intracellular accumulation of many cancer drugs by increasing their efflux out of cancer cells as well as through hepatic, renal, or intestinal clearance pathways.[4] Attempts to coadminister P-gp modulators or inhibitors to increase cellular availability by blocking the actions of P-gp have met with limited success.[4,6–8] Therefore, a more promising approach lies in the design and discovery of novel compounds that are not substrates of P-gp and are effective against drug-resistant cancer while at the same time exhibiting minimal toxicity to normal cellular functions.

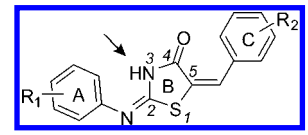

Figure 1. Early hit: MMPT, $R_1 = H$, $R_2 = 4$-Me [5-[(4-methylphenyl)methylene-2-(phenylamino)-4(5*H*)-thiazolone].

Thiazolidinone derivatives have been investigated for a range of pharmacologic indications such as anti-inflammatory,[9] antimicrobial,[10] antiproliferative,[11,12] antiviral,[13] anticonvulsant,[14,15] antifungal,[16] and antibacterial[17] activities but their anticancer effects have been less widely documented.[18] In an early random screening of commercial compounds, a compound with a 4-thiazolidinone core structure was found to selectively kill drug-resistant cancer cells and induce apoptosis[19] (Figure 1). However, the optimal pharmacophore structure and the structure–activity relationship for the cytoselective toxicity have not to date been explored, and the expected therapeutic window is small. To search for more selective and novel thiazolidinone compounds with a wider therapeutic window that could reveal the structure–activity relationship for the cytoselective anticancer activity, we designed and synthesized iterative focused thiazolidinone libraries. These consecutive libraries were screened against paclitaxel-sensitive and -resistant NSCLC cell lines H460 and $H460_{taxR}$, respectively, using a fast-dividing normal human fibroblast (NHFB) line as a general cell line for measuring cytotoxicity against normal cells. Here we report our hit-follow-up approaches that led to the discovery of novel thiazolidinone-related compounds that were highly toxic to NSCLC H460 cells

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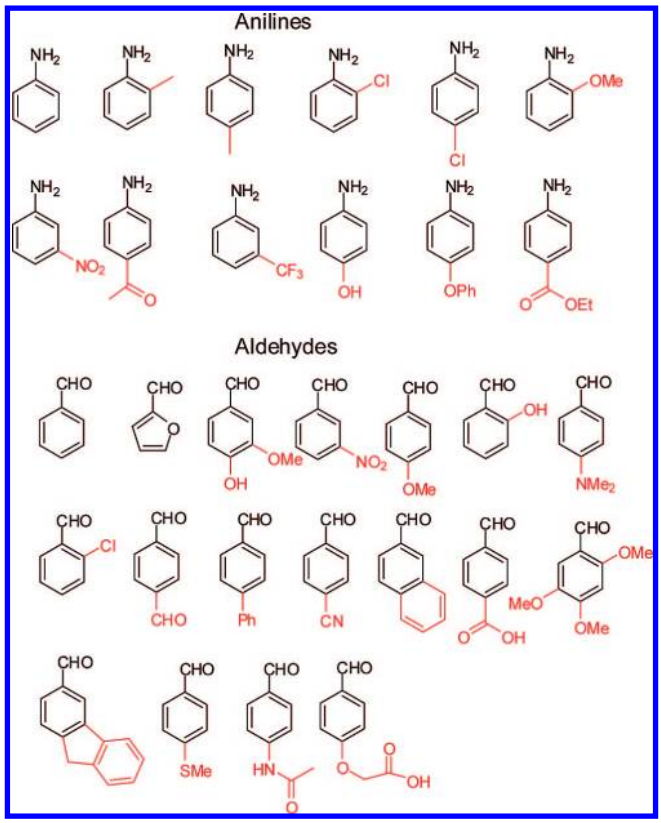
[a] NSCLC, non-small cell lung cancer; MDR, multidrug resistance; P-gp, P-glycoprotein; NHFB, normal human fibroblast; LC/MS, liquid chromatograpy/mass spectrometry; ESI-MS, electrospray ionization mass spectrometry; HRMS, high-resolution mass spectrometry; NMR, nuclear magnetic resonance; FTIR, Fourier transform infrared; TLC, thin layer chromatography.

10.1021/jm7012024 CCC: $40.75
Published on Web 02/08/2008

Chart 1. Building Blocks for Library I



and their paclitaxel-resistant variant $H460_{taxR}$, yet with much less toxicity to NHFBs. Furthermore, pharmacophore modeling revealed unique features and structural requirements for active compounds.

Results and Discussion

Early cytotoxicity studies of commercial compounds[19,20] identified a hit that contained two substituted phenyl moieties on the 4-thiazolidinone core, suggesting that the three-ring system might be required for the anticancer activity. The discovery of novel and more potent lead compounds with a wider therapeutic window would require a thorough exploration of all the combinations of diverse functional groups on each of the three rings. Therefore, we kept the three-ring feature and designed focused combinatorial libraries with maximal diversity on substitution groups on the three rings.

Design of Focused Combinatorial Libraries. The diversity in building blocks eventually determines the chemical space coverage of a library. We selected aniline and aromatic aldehyde building blocks using the following criteria: they must (1) have structural diversity as determined by calculated physicochemical properties of the virtual product, (2) form products that obey Lipinski's "rule of five",[21] and (3) generate products with synthetic feasibility. A total of 18 anilines and 20 aldehydes were first used to generate a virtual library of 360 compounds. Using Lipinski's rule of five selection criteria, 120 compounds were eliminated. Chemical feasibility and building block validation studies eliminated another 24 compounds. The final library consisted of 216 compounds (Chart 1). The diverse molecular properties of the virtual products (Figure 2) indicated that they were theoretically "drug-like" albeit on the basis of the limited criteria above.

Synthesis, Purification, and Characterization of Libraries. Syntheses of thiazolidinones are well documented in the literature.[9,10,16,22–25] Although combinatorial syntheses of thiazolidinones[26–28] and their analogues, such as thiazolines,[29,30] thiohydantoins,[31,32] and thiazolidinediones,[33,34] were reported using both solid- and solution-phase strategies, there has been, to our knowledge, no report on the synthesis of thiazolidinones with an imino group at the 3-position. Our synthetic route to the thiazolidinone libraries is shown in Scheme 1. Due to the shortage of commercial isothiocyanates, the synthesis route starting from **3** was not used in library synthesis. The construction of primary thioureas **5** was achieved by the reaction of aniline **1** with ammonium thiocyanate, in the presence of acid. Thioureas **5** reacted with ethyl 2-chloroacetate to produce thiazolidinones **7** as precipitate, which was filtered and washed with absolute ethanol to give the product with a purity of ~90% and a yield of ~75%.

To prepare library I (Scheme 1), the final step of the reaction was carried out in piperidine and absolute ethanol at 60 °C. About 95% of the products formed as precipitate, and they were purified by simple filtration and washing with ethanol. About 5% of the products that did not precipitate were purified by normal phase column chromatography. A total of 185 of 216 designed compounds were successfully prepared and characterized by $LC/MS/UV_{214}$ with an average purity of 91% with the unreacted starting materials as the major impurities. Selected compounds were further characterized by HRMS, ^1H NMR, and FTIR (see chromatograms and spectra in Supporting Information). The compound structure, purity, and yield are summarized in Supporting Information Table 1.

To explore the effect of nitrogen substitution on anticancer activity, we designed library II, in which the nitrogen in the 4-thaizolidinone ring was substituted (Scheme 2). Isothiocyanates **2** reacted with amines to yield N-substituted thioureas **11**. They then reacted with ethyl 2-chloroacetate **6** to give N-substituted thiazolidinones **12** with a purity of ~90% and a yield of ~70%. After the coupling reaction with aromatic aldehydes, a total of 17 compounds were synthesized and purified. The average purity of this library was 92%, as determined by $LC/MS/UV_{214}$ with the unreacted starting materials as the major impurities. The structure, yield, and purity of these compounds are summarized in Supporting Information Table 2.

Cytoselective Anticancer Toxicity Assays. We used a panel of drug-sensitive (H460) and drug-resistant ($H460_{taxR}$) cells and NHFBs to screen the libraries I and II. Screening was carried out in three stages: the primary screening, the secondary confirmation, and the dose–response determinations. In the primary screening of library I, compounds at a concentration of 10 μM were tested against the cell panel. Compound effects were monitored by observing cell numbers and cell morphology changes in 96-well plates at 24 and 48 h after compound addition. Compounds that exhibited toxicity to both cancer cell lines but not to normal cells were selected for the secondary confirmation assays. In the secondary screening, compounds at the same concentration as in the primary screening were screened in triplicate. As a result, eleven compounds were identified as potent agents for inducing cytoselective toxicity. Dose–response studies showed that these compounds selectively killed or inhibited H460 (IC_{50} at 0.28−14.79 μM) and $H460_{taxR}$ (IC_{50} at 0.42−12.88 μM) cells in a dose-dependent manner and showed less toxicity to NHFBs (35−>100 μM; Figure 3). Structural analysis of these compounds revealed that R_1 could tolerate functional groups such as Cl− and Me− at the 2- or 4-position or CF_3− at the 3-position. However, R_2 tended to accept only functional groups such as $-NMe_2$ and −benzene at the 4-position or −OH group at the 2-position.

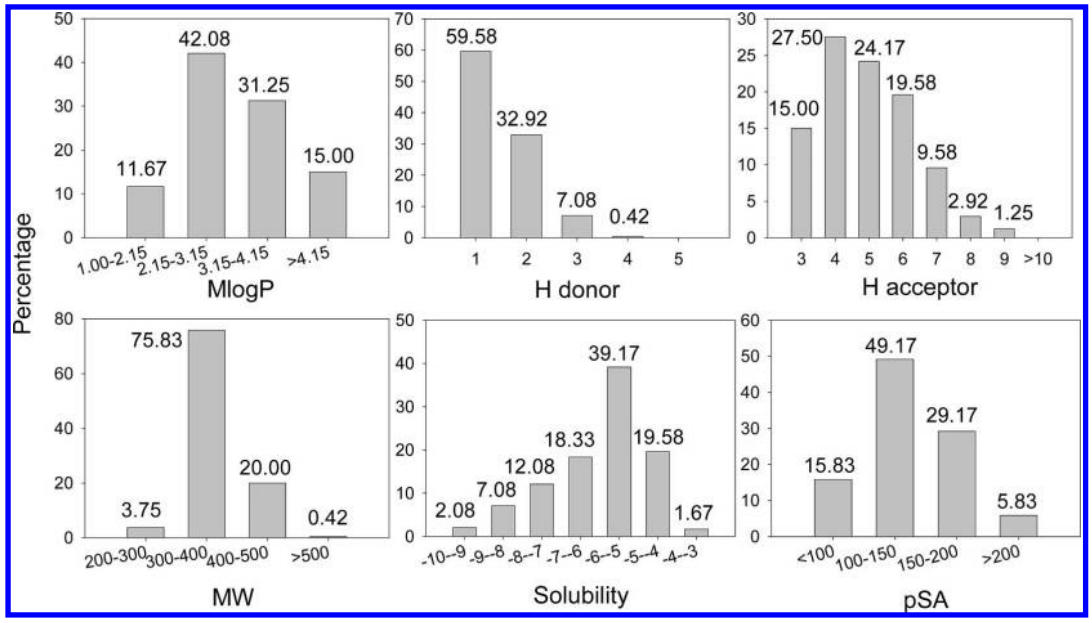
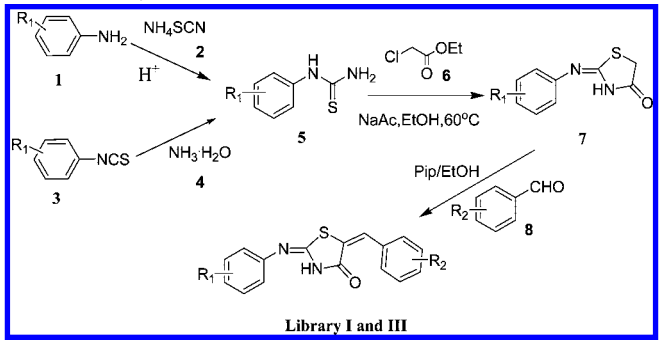


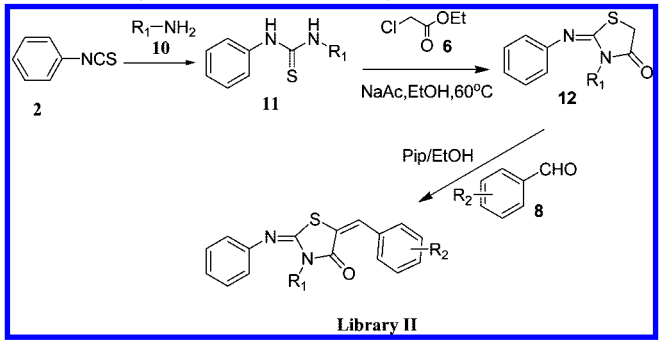
Figure 2. Calculated molecular properties for the focused thiazolidinone library I.

Scheme 1. Synthesis of Route for Libraries I and III[a]



[a] Reagents and conditions: (i) NH_4SCN (**2**), 6 N HCl, 80 °; (ii) $NH_3 \cdot H_2O$ (**4**), rt; (iii) ethyl chloroacetate (**6**); NaOAc, EtOH, 60 °C; (iv) aldehydes (**8**), piperidine, EtOH, 60 °C.

Scheme 2. Synthetic Route for Library II[a]



[a] Reagents and conditions: (i) R_1, $-NH_2$, (**10**), rt; (ii) ethyl chloroacetate (**6**), NaOAc, EtOH, 60 °C; (iii) aldehydes (**8**), piperideine, EtOH, 60 °C.

To test the effect of nitrogen substitution on the cytoselective toxicity of the compounds, we screened library II, which did not yield any compounds with cytoselective toxicity. Compounds in this library fell into two categories: those that showed no toxicity, and those that were toxic to all cell lines tested. Several compounds in this group, when the nitrogen was not substituted, were highly active in selectively killing cancer cells (see **I-7** in Chart 2, for example). Therefore, nitrogen substitution blocked the cytoselective toxicity of the compounds. From this finding, we decided to explore the optimal combination of the selected functional groups from screening library I and leave the nitrogen unsubstituted in the design of the early lead optimization library.

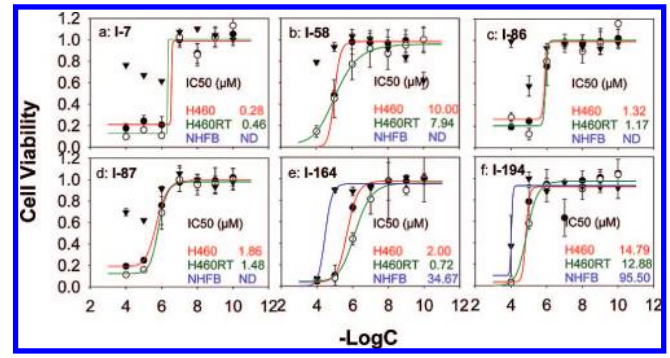


Figure 3. Dose-dependent cytotoxicity assays for compounds from primary screening in cell lines H460, $H460_{taxR}$, and NHFB.

Preparation and Screening of the Early Lead Optimization Library. The early lead optimization library III was designed to have R_1 groups such as $-Cl$, $-Me$, $-OH$, and $-CF_3$ at all possible positions and have R_2 groups such as $-NMe_2$, $-$benzene, and others at the 4-position or $-OH$ at the 2-position (Chart 3). Other functional groups were also incorporated to test for the possible enhancement of activity or solubility. Important goals for this library were to select better lead compounds and to explore the structure–activity relationship of the active compounds. The library was synthesized according to Scheme 1, and all compounds were purified by either recrystallization or chromatography. We enforced two stringent requirements for the early lead optimization library III: (1) all compounds in the library must be obtained to allow exploration of the full chemical space and all substituent combinations and (2) all compounds must have a high purity to ensure unambiguous biological data. We synthesized, purified, and obtained all 170 designed compounds, and the average purity of the library was 95%, shown by $LC/MS/UV_{214}$. The single-concentration (10 μM) primary screening identified 40 hits. Using a lower concentration (5 μM), 10 compounds were confirmed and selected for dose–response studies, which showed that the 50% inhibitory concentration (IC_{50}) for H460 and $H460_{taxR}$ cells was

Chart 2. Active Compounds from the Screening of Libraries I and II

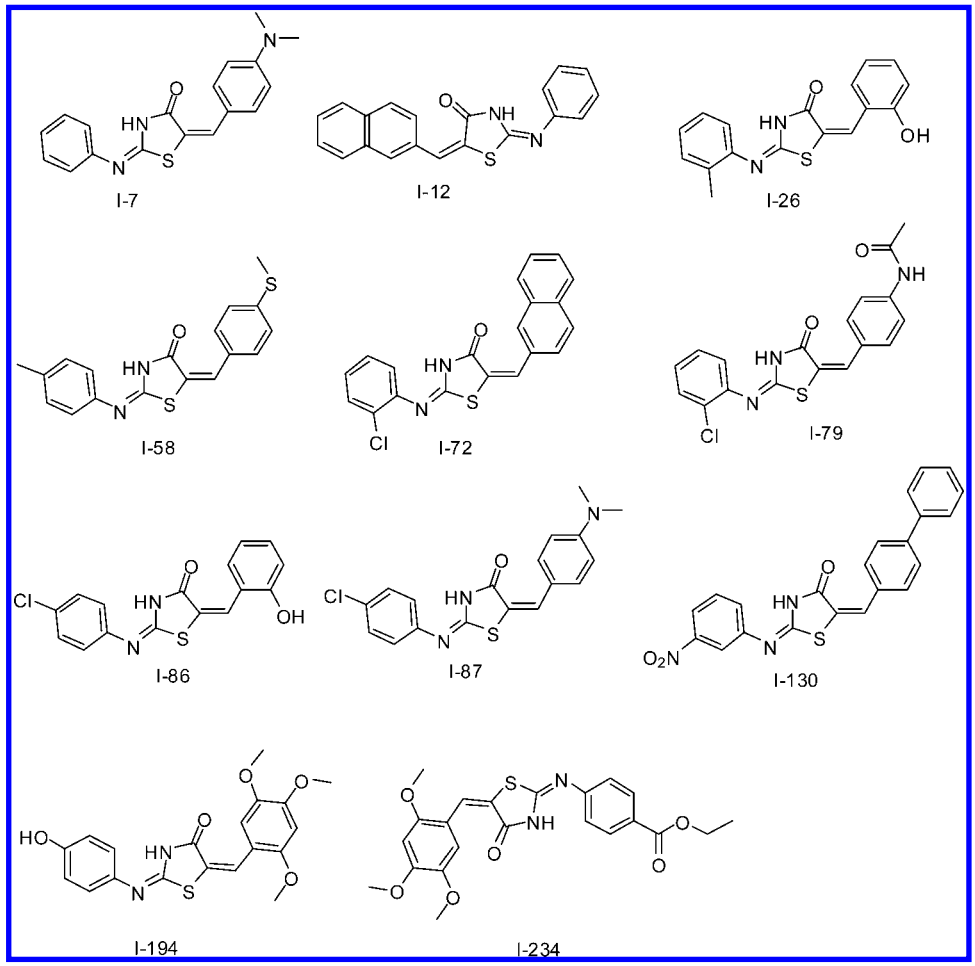
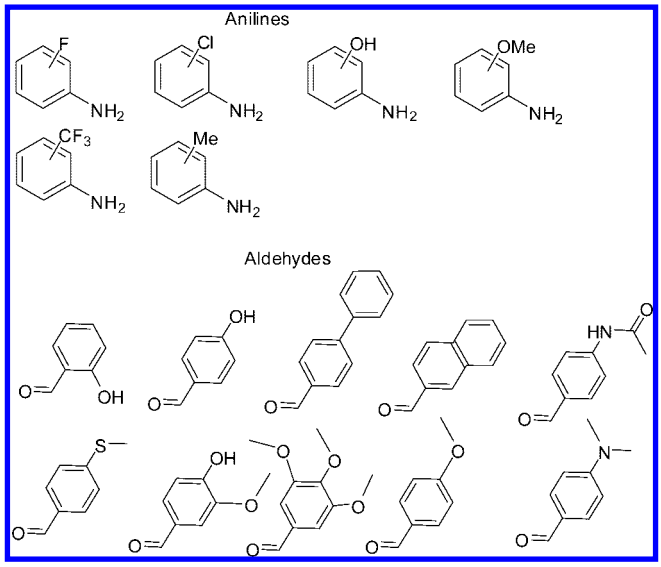


Chart 3. Building Blocks for Library III



as low as 300 nM. Except for compound **I-25** (IC_{50} for NHFB is 195 μM), NHFBs did not reach 50% cell inhibition at the highest compound concentration used (100 μM) for active compounds. Higher compound concentrations could not be used due to the solubility limitation and the limited allowable DMSO (1% for cells used here) in cell cultures. We tentatively took >100 μM as the IC_{50} of the compounds tested against NHFB cells. It was noted that compounds such as **I-27**-, **I-47**-, **III-289**, and **III-324**- showed no sign of 50% cell inhibition at concentrations much higher than 100 μM (Figure 4).

Time-Dependent Compound Effects on Cell Proliferation and Morphology. To examine the time-dependent response of the cells to these compounds, we studied H460 and $H460_{taxR}$ cells and NHFBs for their time-dependent morphology changes and cell proliferation in the presence of compounds **I-7**-, **I-27**, **I-47**, and **I-67** at concentrations of 1 and 2 μM for up to 48 h. Changes in cell morphology were observed using a phase-contrast microscope. Figure 5 shows that compound **I-7** selectively killed both H460 and $H460_{taxR}$ cells in a time-dependent manner while having a much less toxic effect on NHFBs. Results for compounds **I-27**, **I-47**, and **I-67** are in the

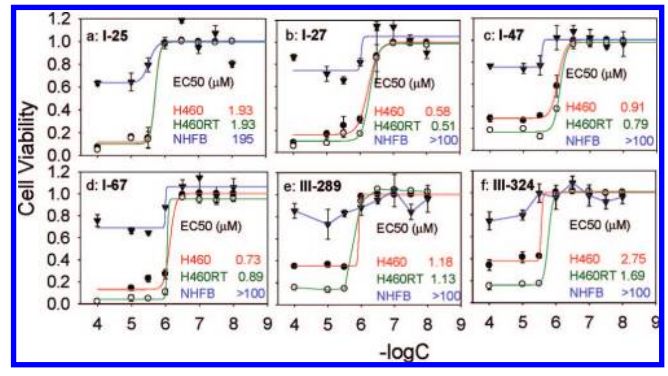


Figure 4. Dose-dependent cell viability for selected compounds from early lead optimization screening in cell lines H460, $H460_{taxR}$, and NHFB.

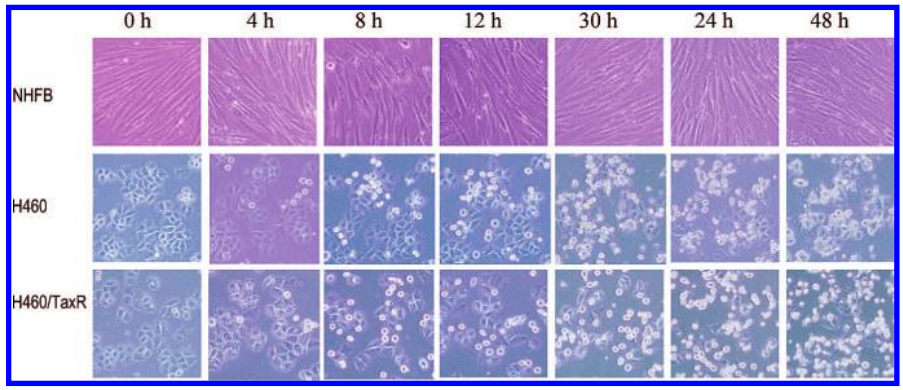


Figure 5. Time-dependent morphology changes of H460, $H460_{taxR}$, and NHFB cells in the presence of compound **I-7** at 1 μM. The magnification is 200×.

Table 1. P-gp Pharmacophore Modeling for Group 1

compd no.	R_1	R_2	P-gp substrate fit	features mapped
I-18	H	4-SMe	no map[a]	
I-36	2-Me	9*H*-fluoren-2-yl	2.64[b]	4H, 1HBA[c]
I-48	4-Me	2-Cl	no map	
I-69	2-Cl	4-formyl	no map	
I-94	4-Cl	2,4,5-trimethoxy	3.86	4H, 1HBA
I-98	4-Cl	4-SMe	1.75	3H, 2HBA
I-114	2-OMe	2,4,5-trimethoxy	3.62	4H, 1HBA
I-118	2-OMe	4-SMe	0.716	3H, 2HBA
I-126	3-NO_2	2-OH	no map	
I-132	3-NO_2	4-naphthalen-2-yl	3.21	3H, 2HBA
I-196	4-OH	9*H*-fluoren-2-yl	0.702	4H, 2HBA
I-213	4-phenoxy	4-COOH	2.6	3H, 2HBA
I-233	4-ethoxycarbonyl	4-COOH	2.61	3H, 2HBA

[a] If two or more features were missed, the compound was designated "no map". [b] Higher values suggested better map. [c] H, hydrophobe; HBA, hydrogen bond acceptor.

Table 2. P-gp Pharmacophore Modeling Results for Group 2

compd no.	R_1	R_2	P-gp substrate fit	features mapped
I-7	H	4-NMe_2	no map[a]	
I-25	2-Me	4-OMe	no map	
I-27	2-Me	4-NMe_2	no map	
I-47	4-Me	4-NMe_2	no map	
I-67	2-Cl	4-NMe_2	no map	
I-86	4-Cl	2-OH	1.88[b]	3H, 2HBA[c]
I-87	4-Cl	4-NMe_2	1	3H, 2HBA
I-166	3-CF_3	2-OH	no map	
I-187	4-OH	4-NMe_2	no map	
III-289	3-Cl	4-NHAc	3.24	3H, 2HBA
III-324	4-OMe	4-NMe_2	1.4	3H, 2HBA

[a] If two or more features were missed, the compound was designated "no map". [b] Higher values suggested better map. [c] H, hydrophobe; HBA, hydrogen bond acceptor.

Supporting Information. These findings confirmed the lack of toxicity to normal cells and the unique cytoselective anticancer effect of this class of compounds.

P-gp Substrate and Inhibitor Pharmacophore Modeling. We selected structures of 13 compounds that killed H460, but not drug-resistant $H460_{taxR}$ (group 1, see Table 1) and 11 compounds that killed both cancer cells, but not normal cells (group 2, see Table 2) for P-gp pharmacophore modeling studies. P-gp pharmacophore models that had been previously generated were used to assess the fit of molecules.[35,36] From group 1, 9 of 13 molecules (70%) were found to map to the P-gp substrate pharmacophore (Table 1 and Figure 6). In contrast, only 4 of 11 molecules (36%) in group 2 mapped to the P-gp substrate pharmacophore (Table 2). This suggests that the molecules in group 2 are less likely to be P-gp substrates than those in group 1. It may be worth noting that the molecules evaluated bear very little 2D structural similarity to the two molecules used in P-gp substrate pharmacophore model building and generally seem to be smaller than molecules previously assessed.

Structure–Activity Relationship and Pharmacophore Modeling. The cytoselective anticancer activities of compounds are summarized in Table 3. In general, these compounds were potent cytoselective anticancer agents that selectively kill drug-resistant cancer cells but not normal cells. IC_{50} values for cancer cell killing were all <2.93 μM, and the IC_{50} for normal NHFB cells were all >100 μM (195 μM for compound **I-25**). The use of a compound concentration >100 μM was effectively prohibited for most compounds due to solubility limitation and the allowable amount of DMSO in cell cultures.

Substitution groups were dominantly electron-donating groups at the 2- or 4-position for both rings A and C. R_1 allowed groups such as −Me and −Cl at both the 2- and 4-positions. However, R_2 was restricted to only the $-NMe_2$ group at the 4-position. Compounds with nitrogen substitutions on the 4-thiazolidinone ring B did not show any cytoselective toxicity. We, therefore, tentatively concluded that nitrogen substitution blocked such activity. This nitrogen and the R_2 on rings B and C are stereochemically confined and are likely to be close to the target binding site. In contrast, R_1 may be remote from the active site (Figure 7). Because R_1 is much more tolerant to structural variations, the future optimization of solubility, absorption, distribution, metabolism, excretion, toxicity, and pharmacokinetic properties can be tested through the modifications of this site. Using all of the active compounds from Table 3, a common feature pharmacophore was developed that indicated that two hydrogen bond acceptors and three hydrophobic regions were common across all of these compounds (Figure 7).

Anticancer Compounds Are Not Michael Acceptors. The stability of anticancer compounds in vivo is important for the potential clinical applications of these compounds. However, the α,β-unsaturated-ketone-like structure of the active compounds raised a concern that they might undergo Michael reactions *in vivo* with nucleophiles. To test this possibility, we carried out aqueous reactions between two nucleophiles, L-glutathione reduced and L-glutathione reduced ethyl ester, and several thiazolidinone analogues (Scheme 3) under basic conditions. The starting materials, products, and reaction progression were monitored by LC/MS. The lack of reaction products after 12 h indicated that these anticancer compounds were not likely to be deactivated by nucleophiles *in vivo* through Michael addition.

Conclusion. To search for effective and selective chemotherapeutic agents against drug-resistant lung cancer, we prepared iterative focused thiazolidinone libraries containing 372

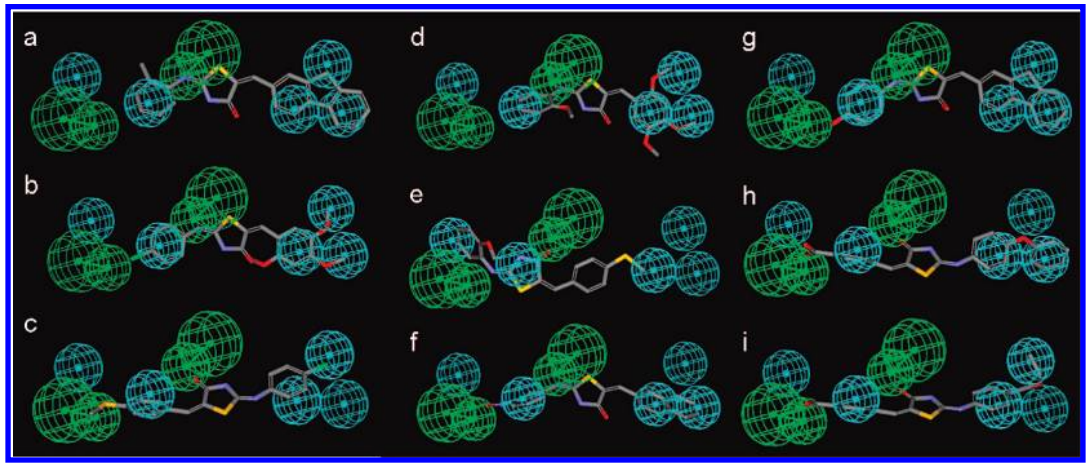
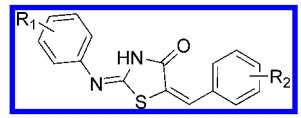


Figure 6. P-gp substrates mapped to the substrate pharmacophore.[36] Green spheres represent hydrogen bond acceptors, and cyan features represent hydrophobic features.

Table 3. Cytoselective Anticancer Activity



compd no. (purity LC/MS/UV_{214})	R_1	R_2	$IC_{50}(\mu M)$ H460	$IC_{50}(\mu M)$ $H460_{taxR}$	$IC_{50}(\mu M)$ NHFB
I-7(95%)	H	4-NMe_2	0.50 ± 0.15	0.21 ± 0.03	>100
I-25(95%)	2-Me	4-OMe	1.78 ± 0.60	1.70 ± 0.50	195
I-27(94%)	2-Me	4-NMe_2	0.65 ± 0.25	0.54 ± 0.10	>100
I-47(96%)	4-Me	4-NMe_2	0.94 ± 0.20	0.82 ± 0.07	>100
I-67(93%)	2-Cl	4-NMe_2	0.73 ± 0.02	0.88 ± 0.01	>100
I-86(90%)	4-Cl	2-OH	1.85 ± 0.65	1.18 ± 0.03	>100
I-87(85%)	4-Cl	4-NMe_2	2.89 ± 0.03	2.93 ± 0.12	>100
I-187(93%)	4-OH	4-NMe_2	1.32 ± 0.25	1.08 ± 0.02	>100
III-289(95%)	3-Cl	4-NHAc	1.19 ± 0.02	1.25 ± 0.10	>100
III-324(93%)	4-OMe	4-NMe_2	2.52 ± 0.12	1.60 ± 0.10	>100

compounds with an average purity of 90–95%. By screening the compounds against drug-sensitive and drug-resistant NSCLC cell lines H460 and $H460_{taxR}$ using NHFBs as a toxicity control, a series of potent compounds that had a much wider expected therapeutic window for their cytoselective toxicity for drug-resistant cancer cells has been identified. We found a unique structure–activity relationship for the cytoselective anticancer activity of the potent compounds. The nitrogen substitution blocked cytoselective anticancer activity. Although the R_1 site was lenient to substitution, the R_2 site required an $-NMe_2$ group at the 4-position for optimal activity. Because $H460_{taxR}$ expressed excessive amounts of P-gp proteins, these anticancer compounds discovered were evidently not P-gp substrates on the basis of their cytotoxicity, and this conclusion was supported by a P-gp substrate pharmacophore assessment. A separate pharmacophore for the most active compounds showed a common arrangement of two hydrogen bond acceptors and three hydrophobic regions (Figure 7). This anticancer compound pharmacophore may be useful in further database searching[35] to scaffold-hop in finding novel molecules that conform to the overall shape, volume, and molecular feature distribution outlined in this study and with potential for fewer interactions with P-gp. Further studies to determine the mechanism of these anticancer compounds will be essential. It is interesting to note the pharmacophore discovered in this study was shared by some nonpeptidic inhibitors of a protease, ubiquitin isopeptidase that were discovered using a simple pharmacophore-based search of the NCI database.[37,38] These inhibitors with IC_{50} values in the low tens of micromolar caused cell death independent of the tumor suppressor p53. The ubiquitin isopeptidase inhibitors shikoccin, dibenzylideneacetone, and curcumin as well as the more recently described punaglandins from coral indicate that an accessible α,β-unsaturated ketone is essential for ubiquitin isopeptidase activity.[39] This raised an interesting possibility that

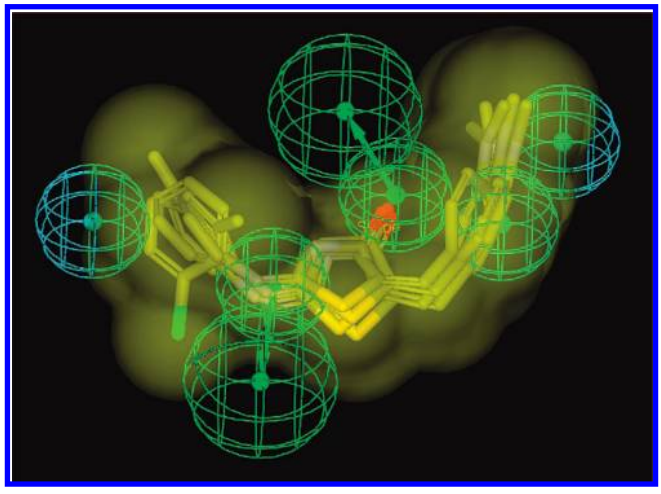
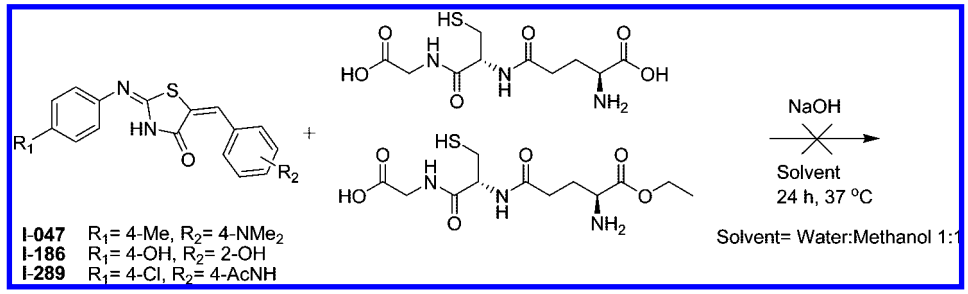


Figure 7. Anticancer molecule pharmacophore generated with the 10 molecules from Table 3 showing the four most active (**I-7**, **I-27**, **I-47**, **I-67**) aligned. Green spheres represent hydrogen bond acceptors, cyan features represent hydrophobic features. The outer translucent shape is the van der Waals surface of all the molecules.

Scheme 3. Test for Possible Michael Reaction *in Vivo*



molecules in this study could be targeting ubiquitin isopeptidase or other known targets.

Experimental Section

The chemical reagents were purchased from Acros Organics (Geel, Belgium) and used without further purification. IR spectra were recorded on a Nicolet 380 FTIR spectrophotometer. LC/MS was performed on a Waters system equipped with a Waters 2795 separation module, a Waters 2996 PDA detector, and a Micromass ZQ detector. A C18 column (2.0 μM, 2.0 × 50 mm) was used for the separation. The eluent was a mixture of methanol and water containing 0.05% trifluoroacetic acid with a linear gradient from 50:50 v/v methanol/H_2O to 100% methanol over 6.5 min at a 1.0 mL/min flow rate. UV detection was at 214 nm. Mass spectra were recorded in positive ion mode using electrospray ionization. NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer using MeOD as solvent. Parallel synthesis was done on a Zhicheng ZHMY-113H parallel synthesizer (Shanghai, China). Molecular properties were calculated using TSAR software from Accelrys (San Diego, CA) and Pipeline Pilot from SciTegic (San Diego, CA). High-resolution mass spectrometry analysis was performed by the Mass Spectroscopy Laboratory of the University of Illinois at Urbana–Champaign.

Arylthioureas (5). General Procedure. To a magnetically stirred solution of primary anilines **1** (10.0 mmol) in 10 mL of 6 N HCl was added NH_4SCN **2** (1.14 g, 15.0 mmol). After being heated to 80 °C, the mixture became clear. The mixture was heated for another 12 h until abundant precipitate appeared. The mixture was cooled to room temperature, filtered, washed with water and anhydrous petroleum ether, and dried under vacuum to produce the corresponding thiourea **3** at high purity and in high yield. This compound was used directly without further purification. For a typical compound, 2-chlorophenyl thiourea, 1.77 g of white powder was obtained in a 95.4% yield with an HPLC purity of >95%. ESI-MS, *m/z* 187.0.

2-Arylimino-thiazolidin-4-ones (7). General Procedure. To a stirred suspension of primary thiourea **5** (6.0 mmol) and anhydrous sodium acetate (2.479 g, 30 mmol) in 20 mL of absolute ethanol was added 1.28 mL of ethyl chloroacetate **6** (12.0 mmol). The mixture was heated at 60 °C for 6 h. After being cooled to room temperature, a precipitate formed. The precipitate was filtered and washed with ethanol to produce the crude product. The filtrate was concentrated under reduced pressure and extracted between water and EtOAc. The organic layer was concentrated to yield more product. The combined crude product was recrystallized from EtOAc to produce 2-arylimino-thiazolidin-4-ones **7**, which was used directly for subsequent reactions without further purification.

2-*p*-Tolylimino-thiazolidin-4-one. 2-*p*-Tolylimino-thiazolidin-4-one was prepared from 1-*p*-tolylthiourea and ethyl chloroacetate according to the procedure described above: yield, 91.7%, green powder; ^{1}H NMR (400 MHz, MeOD), δ 7.56 (d, 1H, J = 8.2 Hz), 7.22 (dd, 2H, J = 8.1 Hz), 7.10 (d, 1H, J = 8.0 Hz), 4.00 (d, 2H, J = 35.6 Hz), 2.35 (d, 3H, J = 11.4 Hz); ESI-MS, *m/z* 208.3 (M + 1).

2-*p*-Fluorophenylimino-thiazolidin-4-one. 2-*p*-Fluorophenylimino-thiazolidin-4-one was prepared from 1-(4-fluorophenyl)-thiourea and ethyl chloroacetate according to the procedure described above: yield, 72.3%, yellow powder; ^{1}H NMR (400 MHz, MeOD), δ 7.69 (s, 1H), 7.13 (m, 3H), 4.02 (d, 2H, J = 39.1 Hz); ESI-MS, *m/z* 211.5 (M + 1).

2-(2-Hydroxyphenyl)imino-thiazolidin-4-one. 2-(2-Hydroxyphenyl)imino-thiazolidin-4-one was prepared from 1-(2-hydroxyphenyl)thiourea and ethyl chloroacetate as described above: yield, 62.5%, black powder; ^{1}H NMR (400 MHz, MeOD), δ 7.35, 7.04 (dd, 1H, J = 7.7 Hz), 7.19 (m, 1H), 6.66 (m, 2H), 4.00 (d, 2H, J = 32.4 Hz); ESI-MS, *m/z* 209.5 (M + 1).

2-Arylimino-5-arylidene-thiazolidin-4-ones (Libraries I and III). General Procedure. 2-Arylimino-thiazolidin-4-ones **7** (0.5 mmol) and benzaldehyde **8** (0.6 mmol) were dissolved in 3 mL of absolute ethanol. Next, 50 μL of piperidine (0.5 mmol) was added to the mixture, and the mixture was stirred for 12 h at 60 °C until a precipitate formed. The mixture was cooled to room temperature, and the precipitate was filtered and washed with petroleum ether and absolute ethanol to yield 2-arylimino-5-arylidene-thiazolidin-4-one at purity of >90%.

Reaction mixtures that did not form precipitates were extracted with EtOAc. The organic layer was washed with brine, dried by anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography and eluted with 20% EtOAc in petroleum ether.

2-(2-Chlorophenylimino)-5-(4-hydroxy-3-methoxybenzylidene)thiazolidin-4-one (I-063). I-063 was prepared from 2-(2-chlorophenyl)imino-thiazolidin-4-one and 4-hydroxy-3-methoxybenzaldehyde (AL003) according to the procedure described above: yield, 33.26%, yellow powder; ^{1}H NMR (400 MHz, MeOD), δ 7.51 (s, 1H), 7.37 (d, 1H, J = 8.0 Hz), 7.21 (d, 1H, J = 7.5 Hz), 7.08 (s, 1H), 6.92 (t, 3H, J = 29.2 Hz), 6.74 (d, 1H, J = 8.2 Hz), 3.77 (d, 3H, J = 38.2 Hz), 1.91, 1.18 (ss, 1H); ESI-MS, *m/z* 361.7 (M + 1). HR-MS calculated for $C_{17}H_{13}ClN_2O_3S$ (M + H)$^+$: 361.0414; found 361.3403.

2-(2-Chlorophenylimino)-5-(4-methoxybenzylidene)thiazolidin-4-one (I-065). I-065 was prepared from 2-(2-chlorophenyl)imino-thiazolidin-4-one and 4-methoxybenzaldehyde (AL005) according to the procedure described above: yield, 73.9%, yellow powder; ^{1}H NMR (400 MHz, MeOD), δ 7.53 (s, 1H), 7.38 (d, 1H, J = 8.0 Hz), 7.32 (d, 2H, J = 8.4 Hz), 7.23 (t, 1H, J = 7.6 Hz), 7.09 (t, 1H, J = 7.6 Hz), 6.98 (d, 1H, J = 7.4 Hz), 6.89 (d, 2H, J = 8.4 Hz), 3.72 (s, 3H), 1.91, 1.19 (ss, 1H); ESI-MS, *m/z* 345.6 (M + 1).

2-(2-Chlorophenylimino)-5-(2-naphthalenyl)methylene-thiazolidin-4-one (I-072). I-072 was prepared from 2-(2-chlorophenyl)imino-thiazolidin-4-one and 2-naphthaldehyde (AL012) according to the procedure described above: yield, 99.0%, orange powder; ^{1}H NMR (400 MHz, MeOD), δ 8.30 (s, 1H), 8.04 (d, 1H, J = 8.1 Hz), 7.82 (d, 2H, J = 4.1 Hz), 7.49 (m, 3H), 7.37 (dd, 2H, J = 7.8 Hz), 7.19 (t, 1H, J = 7.4 Hz), 7.06 (t, 1H, J = 7.6 Hz), 6.96 (d, 1H, J = 7.9 Hz), 1.91, 1.18 (ss, 1H); ESI-MS, *m/z* 365.6 (M + 1). HR-MS calculated for $C_{20}H_{13}ClN_2OS$ (M + H)$^+$: 365.0515; found 365.0516.

2-(3-Trifluoromethyl-phenylimino)-5-(2-hydroxy-benzylidene)thiazolidin-4-one (I-166). I-166 was prepared from 2-(3-trifluoromethyl-phenyl)imino-thiazolidin-4-one and 2-hydroxybenzaldehyde (AL006) according to the procedure described above:

yield, 74.1%, yellow powder; ^{1}H NMR (400 MHz, MeOD), δ 8.12 (d, 1H, J = 20.3 Hz), 7.93 (d, 1H, J = 44.3 Hz), 7.48 (s, 1H), 7.38 (d, 1H, J = 7.5 Hz), 7.16 (d, 3H, J = 32.1 Hz), 6.81 (d, 2H, J = 33.4 Hz), 1.91, 1.19 (ss, 1H); ESI-MS, m/z 365.7 (M + 1).

2-(3-Trifluoromethyl-phenylimino)-5-(2-naphthalenyl)methylene-thiazolidin-4-one (I-172). I-172 was prepared from 2-(3-trifluoromethyl-phenyl)imino-thiazolidin-4-one and 2-naphthaldehyde (AL012) according to the procedure described above: yield, 32.3%, yellow powder; ^{1}H NMR (400 MHz, DMSO), δ 12.61, 11.83 (ss, 1H), 8.21 (m, 2H), 7.99 (m, 3H), 7.58 (m, 5H), 7.26 (s, 1H); ESI-MS, m/z 399.7 (M + 1). HR-MS calculated for $C_{21}H_{13}F_3N_2OS$ (M + H)$^+$: 399.0779; found 399.0776.

2-(4-Ethoxycarbonyl-phenylimino)-5-(4-methoxy-benzylidene)thiazolidin-4-one (I-225). I-225 was prepared from 2-(4-ethoxycarbonyl-phenyl)imino-thiazolidin-4-one and 4-methoxy-benzaldehyde (AL005) according to the procedure described above: yield, 95.7%, yellow powder; ^{1}H NMR (600 M Hz, DMSO), δ 12.47, 11.82 (ss, 1H), 8.02–7.74 (m, 3H), 7.61 (M, 1H), 7.47 (d, 1H, J = 7.78 Hz), 7.16 (s, 2H), 6.98 (d, 1H, J = 7.71 Hz), 4.32(q, 2H, J = 7.08 Hz), 3.81 (t, 3H), 1.32 (t, 3H, J = 7.10 Hz); ESI-MS, m/z 383.5 (M + 1).

2-(3-Hydroxy-phenylimino)-5-(4-hydroxy-3-methoxybenzylidene)thiazolidin-4-one (III-272). III-272 was prepared from 2-(3-hydroxy-phenyl)imino-thiazolidin-4-one and 4-hydroxy-3-methoxybenzaldehyde (AL003) according to the procedure described above: yield, 72.7%, yellow powder; ^{1}H NMR (400 MHz, MeOD), δ 7.61 (d, 1H, J = 36.4 Hz), 7.48 (s, 2H), 7.37 (d, 1H, J = 8.1 Hz), 7.20 (t, 1H, J = 7.8 Hz), 6.97 (m, 3H), 6.81 (s, 1H), 3.76 (d, 3H, J = 18.4 Hz), 2.28 (s, 3H); ESI-MS, m/z 325.7 (M + 1).

2-(3-Hydroxy-phenylimino)-5-(2-hydroxy-benzylidene)thiazolidin-4-one (III-273). III-273 was prepared from 2-(3-hydroxy-phenyl)imino-thiazolidin-4-one and 2-hydroxy-benzaldehyde (AL006) according to the procedure described above: yield, 65.2%, yellow powder; ^{1}H NMR (400 MHz, MeOD), δ 7.34 (d, 1H, J = 55.8 Hz), 6.76 (m, 1H), 6.50 (m, 3H), 6.24 (t, 1H, J = 7.8 Hz), 6.10 (m, 3H), 1.56 (s, 3H); ESI-MS, m/z 311.7 (M + 1). HR-MS calculated for $C_{17}H_{14}N_2O_2S$ (M + H)$^+$: 311.0854; found 311.0857.

2-(3-Hydroxy-phenylimino)-5-(4-dimethylamino-benzylidene)-thiazolidin-4-one (III-274). III-274 was prepared from 2-(3-hydroxy-phenyl)imino-thiazolidin-4-one and 4-dimethylamino-benzaldehyde (AL007) according to the procedure described above: yield, 94.1%, yellow powder; ^{1}H NMR (400 MHz, MeOD) δ, 8.02 (s, 1H), 7.60 (s, 1H), 7.46 (s, 1H), 7.38 (s, 1H), 7.27 (s, 1H), 7.20 (t, 1H, J = 7.8 Hz), 6.95 (d, 1H, J = 7.3 Hz), 6.70 (m, 3H), 2.94 (d, 6H, J = 8.2 Hz), 2.27 (d, 3H, J = 7.8 Hz); ESI-MS, m/z 338.7 (M + 1).

2-(3-Hydroxy-phenylimino)-5-(4-methylthio-benzylidene)thiazolidin-4-one (III-278). III-278 was prepared from 2-(3-hydroxy-phenyl)imino-thiazolidin-4-one and 4-methylthio-benzaldehyde (AL018) according to the procedure described above: yield, 89.4%, yellow powder; ^{1}H NMR (400 MHz, MeOD), δ 7.65 (s, 1H), 7.52 (d, 1H, J = 15.3 Hz), 7.44 (d, 1H, J = 8.6 Hz), 7.30 (dd, 2H, J = 8.0 Hz), 7.19 (dd, 2H, J = 5.1 Hz), 6.95 (m, 1H), 6.82 (s, 1H), 2.42 (m, 3H), 2.27 (d, 2H, J = 6.3 Hz); ESI-MS, m/z 341.6 (M + 1). HR-MS calculated for $C_{18}H_{16}N_2OS_2$ (M + H)$^+$: 341.0782; found 341.0783.

2-(3-Methoxy-phenylimino)-5-(4-hydroxy-3-methoxybenzylidene)thiazolidin-4-one (III-311). III-311 was prepared from 2-(3-methoxy-phenyl)imino-thiazolidin-4-one and 4-hydroxy-3-methoxybenzaldehyde (AL003) according to the procedure described above: yield, 34.8%, yellow powder; ^{1}H NMR (400 MHz, MeOD), δ 7.62 (s, 1H), 7.46 (d, 1H, J = 23.8 Hz), 7.18 (s, 2H), 7.04 (s, 1H), 6.93 (s, 1H), 6.79 (d, 1H, J = 21.4 Hz), 6.62 (d, 2H, J = 45.0 Hz), 3.76 (m, 6H), 1.91, 1.18 (ss, 1H); ESI-MS, m/z 357.7 (M + 1). HR-MS calculated for $C_{18}H_{16}N_2O_4S$ (M + H)$^+$: 357.0909; found 357.0907.

2-(3-Methoxy-phenylimino)-5-(2-naphthalenyl)methylene-thiazolidin-4-one (III-316). III-316 was prepared from 2-(3-methoxy-phenyl)imino-thiazolidin-4-one and 2-naphthaldehyde (AL012) according to the procedure described above: yield, 34.8%, yellow powder; ^{1}H NMR (400 MHz, MeOD), δ 8.33 (d, 1H, J = 54.2 Hz), 8.08 (dd, 1H, J = 8.5, 24.5 Hz), 7.89 (m, 2H), 7.54 (m, 4H), 7.20 (m, 1H), 6.69 (dd, 1H, J = 7.8, 16.5 Hz), 6.53 (d, 1H, J = 11.5 Hz), 3.71 (d, 3H, J = 22.5 Hz); ESI-MS, m/z 361.7 (M + 1).

2-(2-Methoxy-phenylimino)-5-(4-hydroxy-benzylidene)thiazolidin-4-one (III-375). III-375 was prepared from 2-(2-methoxy-phenyl)imino-thiazolidin-4-one and 4-hydroxy-benzaldehyde (AL021) according to the procedure described above: yield, 17.7%, yellow powder; ^{1}H NMR (400 MHz, MeOD), δ 9.66 (m, 1H), 8.08 (s, 1H), 7.65 (m, 1H), 7.50 (s, 1H), 7.37 (s, 1H), 7.16 (d, 2H, J = 40.9 Hz), 7.01 (s, 1H), 6.90 (s, 1H), 6.77 (d, 2H, J = 26.4 Hz), 3.79 (d, 3H, J = 30.4 Hz), 1.91, 1.18 (ss, 1H); ESI-MS, m/z 327.7 (M + 1).

2-(3-Trifluoromethyl-phenylimino)-5-(4-hydroxy-benzylidene)thiazolidin-4-one (III-376). III-376 was prepared from 2-(3-trifluoromethyl-phenyl)imino-thiazolidin-4-one and 4-hydroxy-benzaldehyde (AL021) according to the procedure described above: yield, 21.9%, yellow powder; ^{1}H NMR (400 MHz, MeOD), δ 7.99 (m, 1H), 7.66 (s, 1H), 7.49 (t, 2H, J = 7.8 Hz), 7.38 (d, 2H, J = 7.7 Hz), 7.22 (m, 2H), 6.78 (d, 2H, J = 19.7 Hz), 2.05, 1.18 (ss, 1H); ESI-MS, m/z 375.6 (M + 1). HR-MS calculated for $C_{17}H_{11}F_3N_2O_2S$ (M + H)$^+$: 365.0572; found 365.0573.

N-Substituted Arylthioureas (11). General Procedure. To a stirred solution of phenyl isothiocyanate **2** (1.0 mmol) in 5 mL of anhydrous THF was added dropwise a solution of the substituted amine **10** (2.0 mmol) in 2 mL of THF. After being stirred at room temperature for 10 min, the reaction was heated to reflux in an oil bath for 30 min. The solvent was removed under reduced pressure. The residue was dissolved in EtOAc and extracted with 1 N HCl. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product, which had a purity of >90%, was used directly for the next step without further purification. For a typical compound, phenylthiourea was obtained at a yield of 85%, with an HPLC purity of >94%. ESI-MS, m/z 297.5 (M + 1).

N-Substituted 2-Arylimino-thiazolidin-4-ones (12). General Procedure. N-Substituted primary thiourea **11** (6.0 mmol), anhydrous sodium acetate (2.479 g, 30 mmol), and ethyl chloroacetate **6** (1.28 mL, 12.0 mmol) were dissolved in 20 mL of absolute ethanol. The reaction mixture was heated at 60 °C for 6 h until thin layer chromatography (TLC) showed the disappearance of the N-substituted thiourea. The mixture was concentrated in a vacuum, and the residue was dissolved in EtOAc. The solution was extracted with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in a vacuum to produce crude product. The crude product was crystallized from EtOAc to yield N-substituted 2-arylimino-thiazolidin-4-one **12**, which was used directly for the next step reactions without further purification.

3-Phenethyl-2-phenylimino-thiazolidin-4-one. 3-Phenethyl-2-phenylimino-thiazolidin-4-one was prepared from isothiocyanato-benzene, 2-phenylethanamine, and ethyl 2-chloroacetate according to the procedure described above: white powder; ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.37–7.23 (m, 7H), 7.14 (t, 2H, J = 7.5 Hz), 6.91 (d, 2H, J = 5.4 Hz), 4.11 (t, 2H, J = 7.6 Hz), 3.75 (s, 2H), 3.04 (t, 2H, J = 7.6 Hz); ESI-MS, m/z 297.5 (M + 1).

N-Substituted 2-Arylimino-5-arylidene-thiazolidin-4-ones (II). General Procedure. N-Substituted 2-arylimino-thiazolidin-4-ones **12** (0.5 mmol) and benzaldehyde **8** (0.6 mmol) were dissolved in 3 mL of absolute ethanol. Next, 50 μL of piperidine (0.5 mmol) was added to this mixture, and the mixture was stirred for 12 h at 60 °C until precipitation formation. After the mixture was cooled to room temperature, the precipitation was filtered and washed with petroleum ether and absolute ethanol to yield N-subsituted 2-arylimino-5-arylidene-thiazolidin-4-one at a purity of >90%.

Reaction mixtures that did not yield precipitates were concentrated under reduced pressure to remove ethanol solvent. The residue was dissolved in EtOAc and extracted with 1 N HCl and brine. After being dried by anhydrous Na_2SO_4 and concentrated in a vacuum, the crude compound was purified by silica gel column chromatography and eluted with EtOAc and petroleum ether.

5-(4-Dimethamino-benzylidene)-2-phenylimino-3-(2-hydroethyl)-thiazolidin-4-one (II-18). II-18 was prepared from 3-(2-hydroxyethyl)-2-phenylimino-thiazolidin-4-one and 4-dimethamino-benzaldehyde (AL007) according to the procedure described above: yield, 71.0%, yellow powder; ^{1}H NMR (400 MHz, MeOD), δ 7.52 (s, 1H), 7.26 (m, 4H), 7.08 (t, 1H, J = 7.5 Hz), 6.91 (t, 2H, J = 9.6 Hz), 6.64 (d, 2H, J = 9.0 Hz), 4.02 (t, 2H, J = 6.0 Hz), 3.78 (t, 2H, J = 5.9 Hz), 2.92 (d, 6H, J = 10.0 Hz); ESI-MS, m/z 368.7 (M + 1). HR-MS calculated for $C_{20}H_{21}N_3O_2S$ $(M + H)^+$: 368.1433; found 368.1447.

5-(4-Hydroxy-3-methoxybenzylidene)-2-phenylimino-3-(2-hydroethyl)-thiazolidin-4-one (II-20). II-18 was prepared from 3-(2-hydroxyethyl)-2-phenylimino-thiazolidin-4-one and 4-hydroxy-3-methoxybenzaldehyde (AL003) according to the procedure described above: yield, 70.0%, yellow powder; ^{1}H NMR (400 MHz, MeOD), δ 8.05 (s, 1H), 7.40 (t, 2H, J = 7.9 Hz), 7.19 (t, 1H, J = 7.5 Hz), 7.05 (d, 2H, J = 8.4 Hz), 6.93 (s, 1H), 6.73 (s, 1H), 4.15 (t, 2H, J = 5.9 Hz), 3.89 (m, 8H), 3.71 (s, 3H); ESI-MS, m/z 415.8 (M + 1). HR-MS calculated for $C_{21}H_{22}N_2O_5S$ $(M + H)^+$: 415.1328: found 415.1324.

5-(2,4,5-Trimethoxy-benzylidene)-2-phenylimino-3-(3-methoxy-propyl-thiazolidin-4-one (II-25). II-25 was prepared from 3-(3-methoxy-propyl)-2-phenylimino-thiazolidin-4-one and 2,4,5-trimethoxy-benzaldehyde (AL014) according to the procedure described above: yield, 77.0%, yellow powder; ^{1}H NMR (400 MHz, MeOD), δ 7.91 (s, 1H), 7.27 (t, 2H, J = 7.7 Hz), 7.07 (t, 1H, J = 7.5 Hz), 6.92 (d, 2H, J = 8.4 Hz), 6.78 (s, 1H), 6.61 (d, 1H, J = 13.2 Hz), 3.96 (t, 2H, J = 6.9 Hz), 3.79 (d, 6H, J = 4.5 Hz), 3.58 (s, 3H), 3.41 (t, 2H, J = 6.0 Hz), 3.23 (s, 3H), 1.94 (p, 2H, J = 6.5 Hz); ESI-MS, m/z 443.7 (M + 1). HR-MS calculated for $C_{23}H_{26}N_2O_5S$ $(M + H)^+$: 443.1641; found 443.1648.

General Procedure for the Library Synthesis. Using a 10 × 10 library as an example, 10 intermediates **7** or **12** (5 mmol) were dissolved in 10 mL of absolute ethanol. Ultrasonic and heating baths were used to make sure they were all dissolved. Each solution was divided into 10 parts. Next, 10 different benzylaldehydes (6 mmol) were dissolved in 10 mL of absolute ethanol and divided into 10 parts following the same procedure. Each of the 100 reaction vessels contained a solution of intermediate **7** or **12** (0.5 mmol) and different benzylaldehydes (0.6 mmol) in 2 mL of absolute ethanol. After piperidine was added (50 μL, 0.5 mmol), all reaction vessels were put into a Zhicheng ZHMY-113H parallel synthesizer and shaken overnight. The conversion of the reactions was monitored by HPLC/MS. After reaction completion, the reaction vessels were cooled to room temperature, and the solutions were simultaneously filtered and washed with absolute ethanol repeatedly to yield the target products. All of the residues were dried in a vacuum at 60 °C overnight and analyzed by an HPLC/MS system.

Cells and Culture Conditions. The human NSCLC H460 cell line and paclitaxel-resistant and P-gp-overexpressing $H460_{taxR}$ cell lines were routinely propagated in monolayer culture in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 100 units/mL penicillin, and 100 mg/mL streptomycin. NHFBs were maintained in Dulbecco's modified Eagle's medium with the same supplements. All cells were maintained in the presence of 5% CO_2 at 37 °C.

Cytotoxicity Assays. The inhibitory effects of thiazolidinone analogues on cell growth were determined by the sulforhodamine B assay. Cells [(2–8) × 10^3 cells in 100 μL of culture medium/well] were seeded in 96-well flat-bottom plates and treated the next day with the drugs at the indicated concentrations. The IC_{50} was determined by sulforhodamine B assay 72 h after treatment, as described previously.[40,41] DMSO solvent was used to dissolve compounds, and its volume was <1% in the assay medium. An equal volume of DMSO was used as a control. The experiments were performed at least three times for each cell line. Cell viability was calculated using the following formula: cell viability = 100 × $A_{treatment}/A_{control}$ (percentage). The IC_{50} was determined by the SigmaPlot 10.0 program (Systat Software, Inc., San Jose, CA) using a four-parameter logistic function for the sigmoid dose–response curves.

Time-Dependent Compound Effects on Cell Proliferation and Cell Morphology. To examine the time-dependent response of the cells to these compounds, compounds **I-7**, **I-27**, **I-47**, and **I-67** were dissolved in DMSO to a concentration of 15 mM and stored at 4 °C. The cytotoxicity of compounds was studied in 24-well plates. H460, $H460_{taxR}$, and NHFB cells (3×10^4 cells in 1000 μL of culture medium/well) were seeded in 24-well flat-bottom plates and treated the next day with the compounds at 1 and 2 μM, respectively, for up to 48 h. An equal volume of DMSO (0.1%) had no effect on cell viability and was used as a control. The cell morphology changes were observed under an Olympus I X 71 phase-contrast microscope (Center Valley, PA).

P-gp Substrate Pharmacophore Modeling. Briefly, a P-gp substrate pharmacophore[36] derived from a common feature HIPHOP alignment of verapamil and digoxin was used as described previously.[35,36] All molecules evaluated in this study were imported as sdf files, and up to 255 conformations were generated with the Best conformer generation method, allowing a maximum energy difference of 20 kcal/mol with Discovery Studio 1.7 Catalyst (Accelrys, San Diego, CA). Molecules were mapped to the P-gp substrate pharmacophore using rigid fit and allowing a maximum of two feature misses. The larger the fit value, the closer the molecule maps to the feature centroids.

Anticancer Compound Pharmacophore Modeling. All of the active molecules in Table 3 were imported as sdf files, and up to 255 conformations were generated with the Best conformer generation method, allowing a maximum energy difference of 20 kcal/mol. Molecules **I-7**, **I-27**, **I-47**, and **I-67** were annotated as more active than the others, and a HIPHOP alignment was performed[36] after the selection of hydrogen bond acceptor, donor, and hydrophobic features. The most active molecules were then aligned on the final pharmacophore, and a translucent van der Waals surface shape was added.

Acknowledgment. We thank Hong Ruan and Anang Shelat for cheminformatics assistance and Ms. Sulian Gao for technical assistance. This work was supported by the American Lebanese Syrian Associated Charities (ALSAC), St. Jude Children's Research Hospital, and Shandong University.

Supporting Information Available: Details of library characterizations, including purity, yield, LC/MS, and NMR data, and cell morphology changes. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM7012024