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Modification and Biological Evaluation of Thiazole Derivatives as Novel Inhibitors of Metastatic Cancer Cell Migration and Invasion

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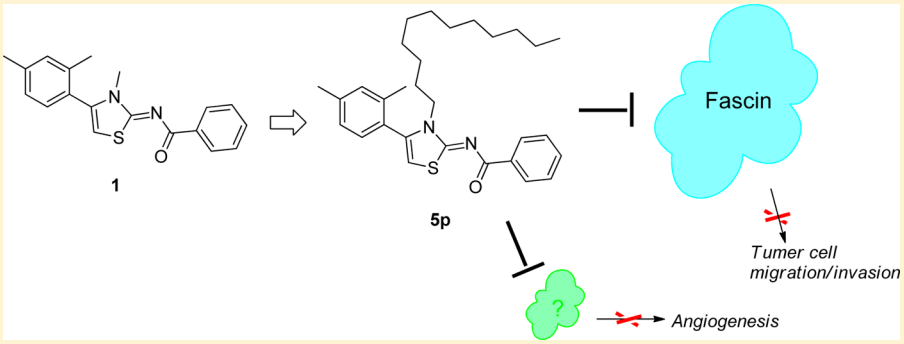

ABSTRACT: Fascin has recently emerged as a potential therapeutic target, as its expression in cancer cells is closely associated with tumor progression and metastasis. Following the initial discovery of a series of thiazole derivatives that demonstrated potent antimigration and antiinvasion activities via possible inhibition of fascin function, we report here the design and synthesis of 63 new thiazole derivatives by further structural modifications in search of more potent fascin inhibitors. The **5** series of analogues with longer alkyl chain substitutions on the thiazole nitrogen exhibited greater antimigration activities than those with other structural motifs. The most potent analogue, **5p**, inhibited 50% of cell migration at 24 nM. Moreover, the thiazole analogues showed strong antiangiogenesis activity, blocking new blood vessel formation in a chicken embryo membrane assay. Finally, a functional study was conducted to investigate the mechanism of action via interaction with the F-actin bundling protein fascin.

INTRODUCTION

The actin-bundling protein fascin has been linked to tumor progression, invasion, and metastasis, a fatal development of the disease.[1−5] Fascin has recently emerged as a novel therapeutic target for treatment of cancer metastasis.[6−8] A cell-based fascin bioassay identified compounds with potential antimetastasis functions, with diverse chemical structural features.[9] However, no follow-up studies of biological activities were reported to confirm that the compounds indeed target fascin to inhibit migration, invasion, and metastasis.[9]

From shape-based molecular modeling and subsequent rational design and synthesis, we have recently identified a thiazole lead compound **1** (Figure 1) that effectively blocked cell migration and invasion via interactions with the protein fascin.[10] With potent activities (IC_{50} in the 100 nM range) in inhibiting migration and invasion of several metastatic human breast cancer cell lines such as MDA-MB-231, HeLa, and A549, this compound exhibited no cytotoxicity at concentrations exceeding 100 μM. The finding of thiazole compounds as antimigration and antiinvasion agents opened up new possibilities of fascin-targeting molecules that can be further optimized for greater potency and bioavailability while maintaining minimal cytotoxicity. To further explore and optimize the structure−activity relationships, we have designed and synthesized 63 additional thiazole derivatives where we sought to (1) homologize the two lead structures by varying the substitution on the thiazole nitrogen, (2) change the linker structure between thiazole and phenyl groups, and (3) modify other substitution groups on both the thiazole ring and the phenyl rings (Figure 1). These thiazole analogues were then biologically evaluated to determine their cytotoxicity, antimigration, and antiinvasion activities. Further, molecular modeling was performed to assist in the elucidation of observed structure−activity-relationships. For the most potent thiazole derivatives, an in vivo assay utilizing chick embryo chorioallantoic membrane (CAM) was performed to assess their in vivo antimetastasis potential by inhibiting angiogenesis. Finally we investigated their mode of action by overexpressing the protein fascin in cancer cell lines to determine if the activities of the compounds can abrogate the enhanced migration and invasion of the transfected cell lines.

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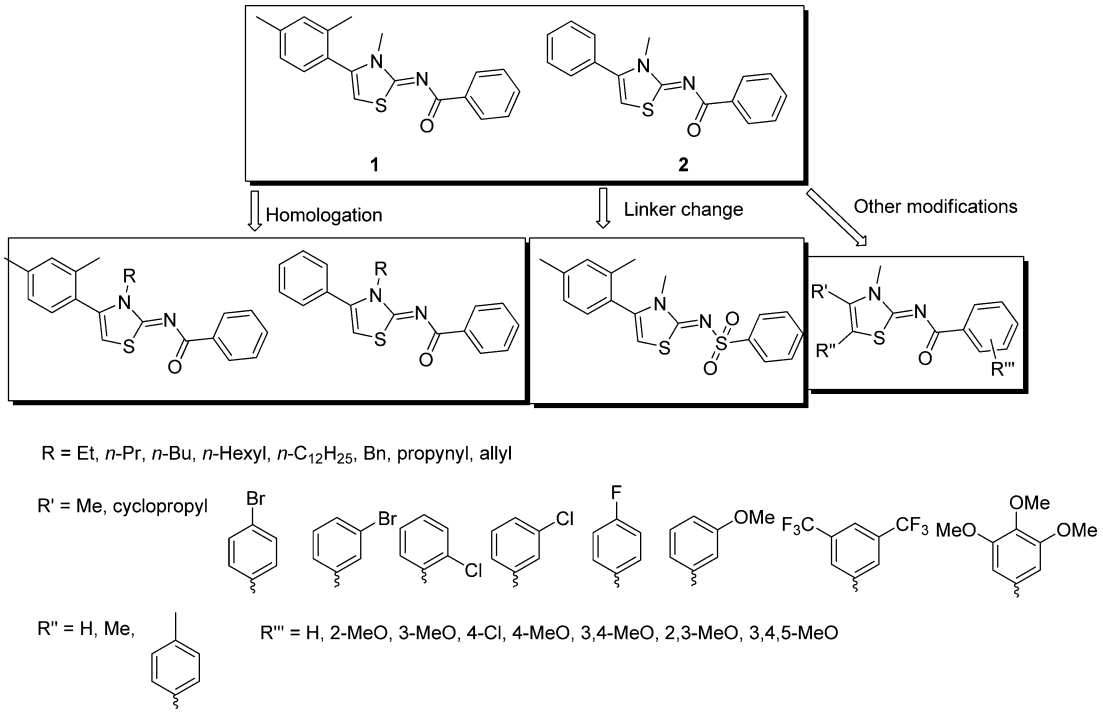
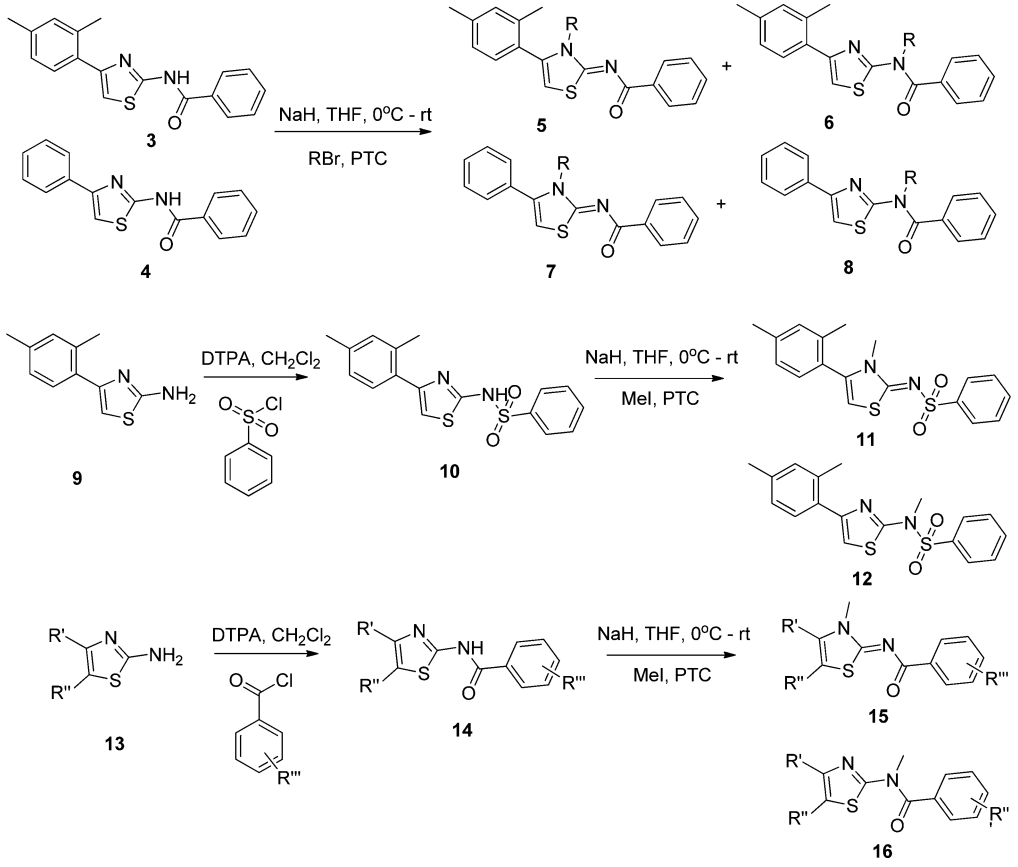


Figure 1. Structures of lead compounds **1** and **2** and their analogues from lead modifications.

Scheme 1. Synthesis of Analogues of Lead Compounds



RESULTS AND DISCUSSION

Chemistry. As shown in Scheme 1, compounds **5** and **7** and their isomers **6** and **8** were respectively obtained from the N-alkylation of the amides **3** and **4** which were prepared following the literature procedure.[10] 4-(2,4-Dimethylphenyl)thiazol-2-amine (**9**) was treated with benzenesulfonic chloride to give **10** which was transformed to the analogue **11** and its isomer **12** by the N-methylation reaction in THF. The acylation of the 2-

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Table 1. Antimigration Efficacy and Cytotoxicity of the Synthetic Thiazole Derivatives

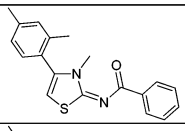
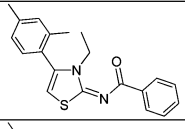
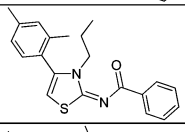
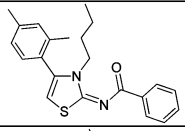
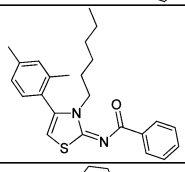
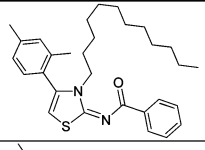
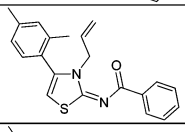
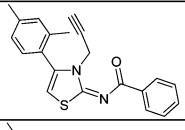
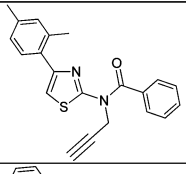
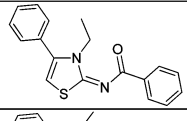
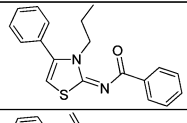
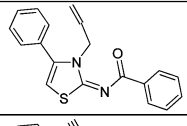
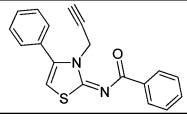
Thiazole compounds	Structures	IC_{50} (anti-migration, μM)	Cytotoxicity (Survival at 1 μM)
1		0.218	101%
5l		1.95	142%
5m		0.292	85.0%
5n		0.196	70.4%
5o		0.045	90.0%
5p		0.024	110%
5q		0.456	115%
5r		3.89	70.2%
6		0.321	106%
7a		0.0515	68.5%
7b		0.657	100%
7c		1.20	64.8%
7d		2.78	63.0%

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Table 1. continued

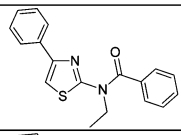
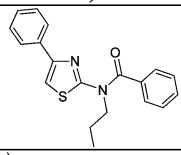
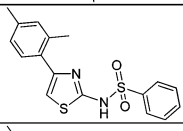
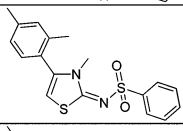
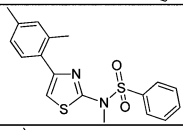
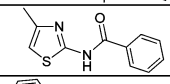
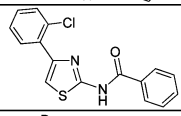
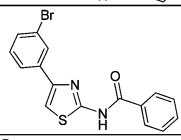
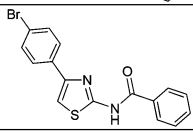
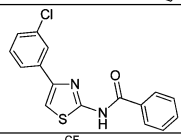
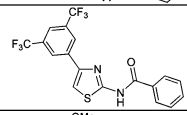
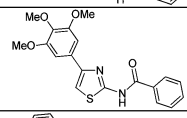
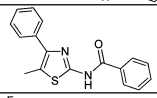
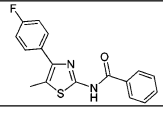
Thiazole compounds	Structures	IC_{50} (anti-migration, μM)	Cytotoxicity (Survival at 1 μM)
8a		0.533	129%
8b		0.077	116%
10		11.0	137%
11		1.46	92.4%
12		>25.0	117%
14a		12.0	103%
14b		15.0	102%
14c		10.0	78.0%
14d		9.50	82.2%
14e		0.123	71.4%
14f		0.336	85.8%
14g		>50.0	95.0%
14h		>25.0	99.0%
14i		9.10	53.7%

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Table 1. continued

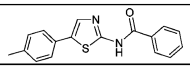
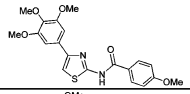
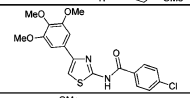
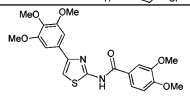
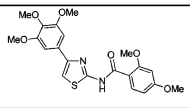
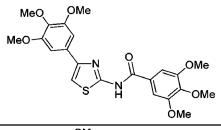
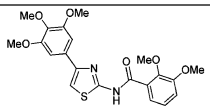
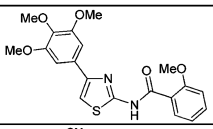
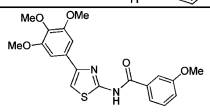
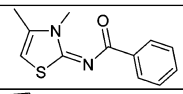
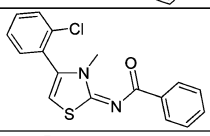
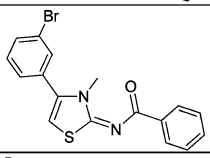
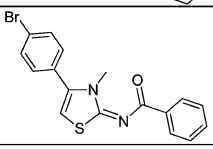
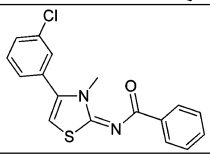
Thiazole compounds	Structures	IC_{50} (anti-migration, μM)	Cytotoxicity (Survival at 1 μM)
14j		0.308	55.6%
14k		>25.0	93.8%
14l		>25.0	85.6%
14m		>25.0	87.3%
14n		>50.0	93.8%
14o		1.03	77.6%
14p		15.0	90.1%
14q		8.50	104%
14r		14.0	92.6%
15a		15.0	103%
15b		0.268	72.2%
15c		0.302	94.4%
15d		0.125	59.3%
15e		0.367	35.2%

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Table 1. continued

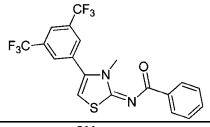
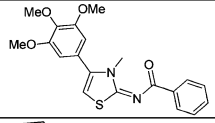
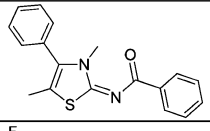
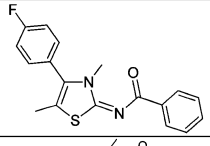
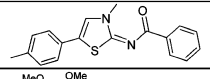
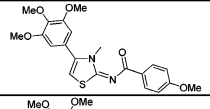
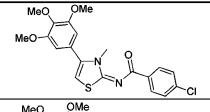
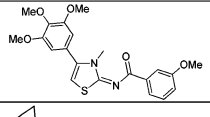
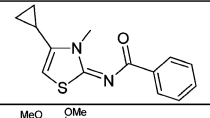
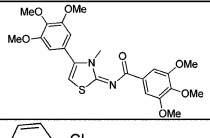
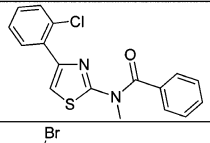
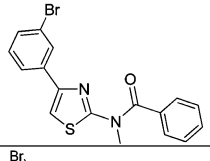
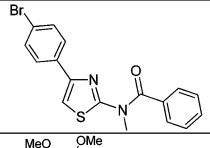
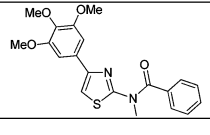
Thiazole compounds	Structures	IC_{50} (anti-migration, μM)	Cytotoxicity (Survival at 1 μM)
15f		0.0961	79.8%
15g		1.07	88.4%
15h		0.197	68.4%
15i		0.847	83.8%
15j		>25.0	113%
15k		8.04	106%
15l		0.758	77.6%
15m		>50.0	104%
15n		0.104	90.7%
15o		1.96	81.5%
16a		0.0416	88.9%
16b		0.437	104%
16c		0.317	65.4%
16d		10.0	96.4%

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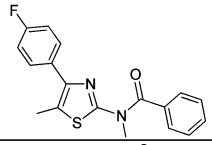
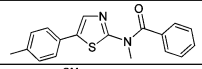
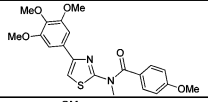
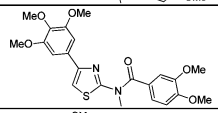
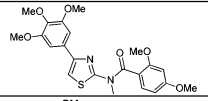
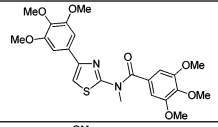
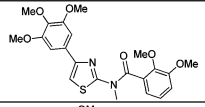
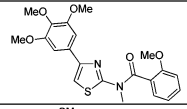
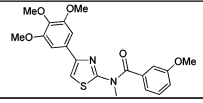
Table 1. continued

Thiazole compounds	Structures	IC_{50} (anti-migration, μM)	Cytotoxicity (Survival at 1 μM)
16e		1.62	108%
16f		1.09	59.3%
16g		0.312	100%
16h		>25.0	102%
16i		5.04	119%
16j		0.470	115%
16k		0.108	118%
16l		>25.0	109%
16m		0.646	88.9%

aminothiazoles **13** by acyl chloride provided the amides **14** at room temperature in dichloromethane, and further methylation of **14** led to the desired analogues **15** and their corresponding isomers **16**.

Antimigration Activity and Cytotoxicity. A Transwell migration assay was used to determine the effects of the synthesized thiazole derivatives on the migratory capacity of MDA-MB-231, an invasive and metastatic breast cancer cell line. The cancer cells were seeded at a density of 2.5×10^4 per well in the upper chamber in serum-free media. In the presence of varying concentrations of the thiazole derivatives, the cells' ability to migrate to the lower chamber with media containing 5% FBS was measured by counting the total number of cells in the lower chamber after 24 h. The concentration of individual thiazole compounds at which inhibition of migration is observed at 50% is defined as the IC_{50} value listed in Table 1. To evaluate possible contributions of cell viability loss to reduced migration, the thiazole derivatives were also subjected to cell survival assays to determine their cytotoxicities. These were conducted by treating the MDA-MB-231 cells with individual thiazole compounds for 5 days and counting the surviving cells.

The 63 newly synthesized thiazole derivatives displayed a wide range of antimigration activities as reflected in the IC_{50} values going from a low of 24 nM to greater than 50 μM or no apparent activity. Such variations in activity appears to be dependent on minor structural modifications, revealing some interesting trends that may help our understanding of the structure−activity-relationships for further optimization of pharmacological index of thiazole derivatives.

Compounds **5l−r** are homologues from the lead structure **1** that were designed and prepared to investigate the effect of thiazole-N substitution on gain or loss of the antimigration activity. For comparison, **1**, the most potent thiazole derivative from our previous study,[10] was also included in the assay. When the thiazole *N*-methyl is changed to an ethyl group (**5l**), there was a marked decrease of activity from 0.218 to 1.945 μM. Interestingly, an *n*-propyl substitution at the same position (**5m**) confers similar antimigration activity with an IC_{50} of 0.292 μM. This trend continues as the alkyl chain length increased to *n*-butyl (**5n**, IC_{50} = 0.196 μM), *n*-hexyl (**5o**, IC_{50} = 0.045 μM), and *n*-dodecyl (**5p**, IC_{50} = 0.024 μM). In addition, allyl substitution at the thiazole nitrogen (**5q**) yielded activity comparable to *n*-propyl (**5m**), but changing the substitution (**5r**) to propynyl leads to a significant loss of the activity, resulting in an IC_{50} value of 3.89 μM. On another note, putting the propynyl group on the amide nitrogen (**6**) appears to restore the activity.

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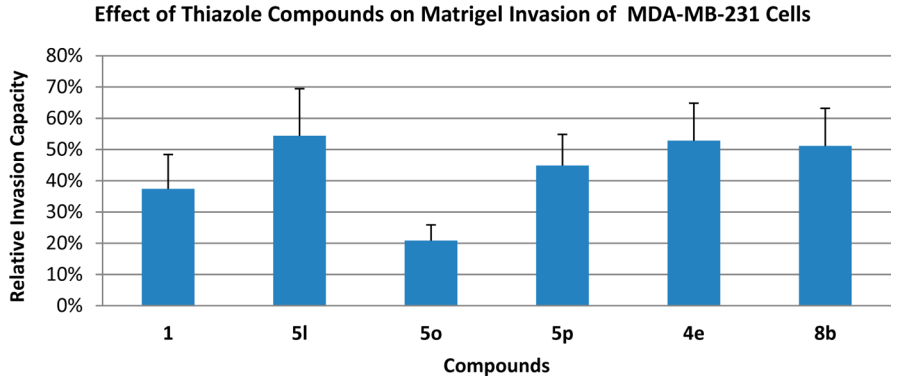

Figure 2. Antiinvasion activities of selected thiazole compounds.

Attempt to modify the amide linker leads to mixed results. If the amide linker is replaced by a sulfamide structure, antimigration activity was largely lost in **12** but not in **11**. The single structural difference between **11** and **12** is the methyl substitution. Thiazole *N*-methyl substitution confers, whereas the amide *N*-methyl substitution deprives, antimigration activity.

The lead compound **2** was a potent thiazole derivative discovered in a previous study, which also displayed significant cytotoxicity in several cancer cell lines at 10 μM.[10] In the current study we further investigated if modifications on *N*-alkyl substitutions could improve the activity and toxicity profile. Thus, when the alkyl substitution at the thiazole nitrogen varied from methyl (**5j**), ethyl (**7a**), propyl (**7b**) to allyl (**7c**) and propynyl (**7d**), the cytotoxicity of the compounds remained significant, with relative survival ratio after 5-day treatment at 10 μM ranging from 20.4% to 70.9%. However, the cytotoxicity is largely abrogated when the methyl and ethyl substitution is on the amide nitrogen (**4e**, **8a**, **8b**) while strong antimigration activity was retained. It was also noted that these compounds (**7a**–**d**) exhibited moderate to strong inhibition of cell growth, suggesting that, in addition to blocking fascin-mediated migration, these compounds may have other molecular targets that regulate cell survival and growth.

Next we examine how modifications on the thiazole ring affect antimigration activity. A methyl substitution on the 5-position of the thiazole ring (**14h**) results in a near complete loss of activity. Adding a fluorine atom at the para-position of the 1-thiazole phenyl brings back cytotoxicity without a significant gain of antimigration activity (**14i**). An attempt to move the thiazole phenyl ring to the 5-position results in a more linear analogue that has stronger growth inhibitory effect but also more antimigratory (**14j**). Interestingly, adding a thiazole *N*-methyl group to the above three analogues gives a more potent **15h** (compared to **14h**) and **15i** (compared to **15i**) but an inactive **15j** (compared to **14j**).

In comparison of **15j** (thiazole *N*-methyl) to **16f** (amide *N*-methyl), it was noted that the analogue with amide *N*-methyl substitution displayed significantly greater toxicity and antimigration activity than that with a thiazole *N*-methyl group.

Modifications on the thiazole phenyl ring by halogen and trifluoromethyl substitutions afforded six analogues **14b**, **14c**, **14d**, **14e**, **14f**, **14g**, of which only **14e** and **14f** showed strong antimigration activities with moderate inhibition of cell growth at the same time. It was noted that chlorine substitution at meta position (**14e**) is far superior to ortho position (**14b**). Adding a methyl group on the thiazole nitrogen to some of these analogues appears to endow more potent cytotoxicity while increasing the overall inhibitory effect on cell motility. Thus, **15b**. **15c**, **15d**, **15e**, **15f**, and **15g** all displayed potent antimigration activities with IC_{50} values ranging from 0.096 μM (**15f**) to 1.07 μM (**15g**).

Attempts to modify both thiazole phenyl and amide phenyl ring yielded eight analogues (**14k** through **14r**). These compounds showed almost no antimigration activities except **14o** with an IC_{50} value of 1.03 μM. However, if these analogues are modified further by adding a thiazole *N*-methyl substitution (**15k** through **15m**), some modest increases in the activity are obtained. For example, **15l** exhibited an IC_{50} value of 0.758 μM. On the other hand, if the methyl substitution is on the amide nitrogen (**16g** through **16m**), the results are mixed. For instance, compared to **14k**, IC_{50} value of **16g** improved from >25 to 0.312 μM, an increase of potency by nearly 100-fold. Also, from **15o** to **16j**, the position change of methyl substitution from thiazole nitrogen to amide nitrogen resulted in an increase of antimigration activity by 4 times.

Antiinvasion. To determine the antiinvasion activities of the thiazole compounds, the most potent antimigration analogues were selected for a Matrigel invasion assay. As shown in Figure 2, at 10 μM, all tested compounds demonstrate 50% or greater inhibition of cell invasion through the Matrigel. Notably, compound **5o** exhibited the greatest potency in blocking the cancer cell invasion. It is also noted that the antiinvasion activities of the selected thiazole derivatives do not always correlate with their antimigration activities.

Antiangiogenesis. Given the association of cell migration with angiogenesis[11−14] and the possible involvement of fascin in angiogenesis,[15] we next investigated if the thiazole compounds might have any antiangiogenic properties using the chick chorioallantoic membrane (CAM) assay. In this test the vascular system of a fertilized chicken embryo is used as a model. Figure 3 demonstrates the effects of **5o** and **5p** on the development of embryonal blood vessels compared to a negative control (PBS) and a positive control (10 ng/plug basic fibroblast growth factor (bFGF) and 30 ng/plug vascular endothelial growth factor (VEGF)) without the thiazole compounds. The antiangiogenesis activity of the three thiazole compounds was determined by the suppression of angiogenic action of BV (bFGF + VEGF) when each compound was added to a collagen containing BV and placed on the chorioallantoic membrane of 10-day old chick embryos for 3 days. As shown in Figure 3, the analogues **5o** and **5p** were seen to potently block the angiogenic action of BV.

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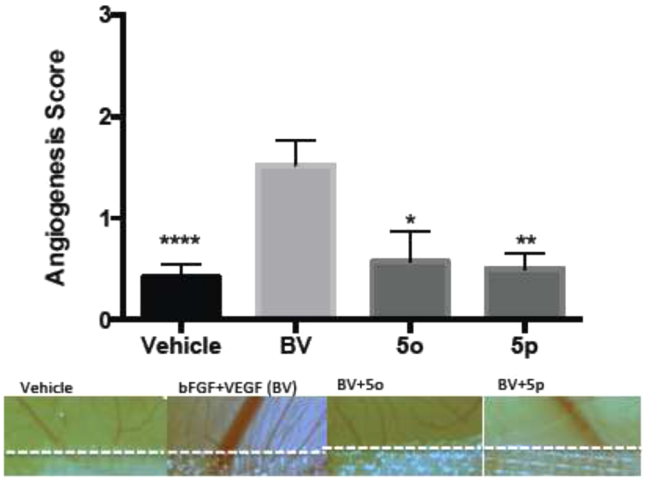

Figure 3. Effect of **5o** and **5p** on angiogenesis in chick embryo ex ovo chorioallantoic membrane (CAM) assay. Angiogenesis was scored by two scorers in a blinded fashion on a scale from 0 to 3 where 0 contained no new vessels and 3 exhibited extreme angiogenesis determined by the presence of new vessels radiating out from the plug in addition to the regular repeating pattern of the normal CAM vasculature. Dashed lines in representative images (below) indicate the top edges of the collagen and drug-containing plugs from which angiogenesis was determined. In these experiments, the thiazole analogues **5o** and **5p** significantly inhibited the induction of angiogenesis by bFGF and VEGF (BV) after 3 days.

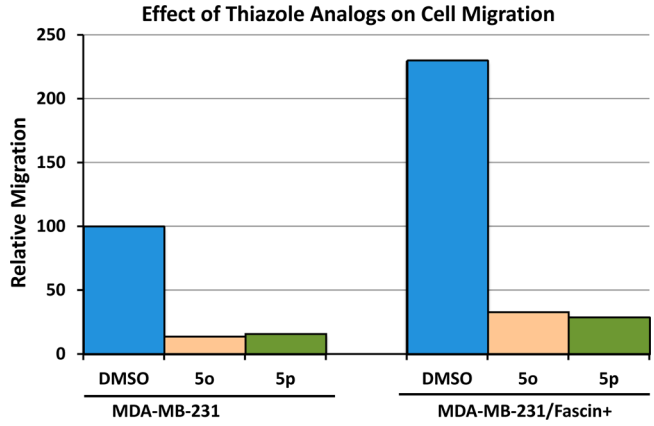

Figure 5. Enhanced cell migration induced by fascin-overexpression is abrogated by thiazole analogues **5l**, **5o**, and **5p**.

Mechanism of Action. Our previous study[10] found that the thiazole derivatives blocked migration and invasion of cancer cells by impairing the cytoskeleton dynamics accompanied by reduced colocalization of the actin-bundling protein fascin. To provide further evidence that fascin might be involved in the antimigratory action of the newly synthesized thiazole analogues, we overexpressed fascin in MDA-MB-231 cells to investigate the resulting cell motility and the effect of the thiazole compounds on the cells overexpressing fascin. Figure 4 shows the higher level of fascin expression in the transfected cells than the native level of fascin in the control cells (Figure 4A). Consistent with previous findings,[3,16−23] the fascin+ cells exhibited significantly higher (2.4-fold increase) migration than the control cells (vector). Treatment of the fascin+ cells with our most potent thiazole analogues, **5o** and **5p**, nearly completely abrogated the enhanced migration as illustrated in Figure 5. At 10 μM **5o** and **5p**, the control MDA-MB-231 cells lost about 80% migratory capacity. The thiazole compounds also inhibited the fascin+ MDA-MB-231 cells by about the same percentage (Figure 5). These results provided additional evidence that the thiazole derivatives likely acted through interaction with fascin.

Effect on F-Actin Structure. To investigate if the thiazole analogues inhibit cell migration and invasion by impairing the F-actin network, actin filaments of HeLa cells treated with selected thiazole analogues were stained with phalloidin tagged with fluorescent agent (Acti-stain 488 fluorescent phalloidin). As shown in Figure 6, the control cells showed a distinct presence of F-actin filaments along the cells and had more evenly distributed F-actin structures in the cytoplasm. In contrast, after treatment with the three thiazole analogues **5o**, **5p**, and **8b** each at 10^{-6} M, the cells exhibited various degrees of disorganization of actin filaments and a decrease of F-actin staining intensity near the cell membranes. These results suggest that the antimigration and antiinvasion activities of the thiazole analogues may be mediated by interfering with the actin filament network.

■ CONCLUSION

In summary, the 63 new thiazole derivatives designed and prepared by further structural modifications of the previously discovered lead compounds **1** and **2** produced more potent fascin inhibitors. Among the active compounds, the **5** series of analogues with longer alkyl chain substitutions on the thiazole nitrogen exhibited greater antimigration activities than those with other structural motifs. The most potent analogue, **5p**, inhibited 50% of cell migration at a concentration of 24 nM. As expected, all thiazole derivatives that were selected for Matrigel

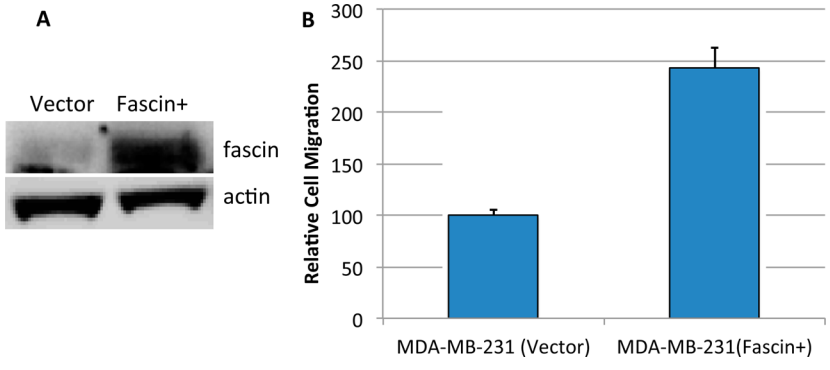

Figure 4. Overexpressing fascin in MDA-MB-231 leads to enhanced migration.

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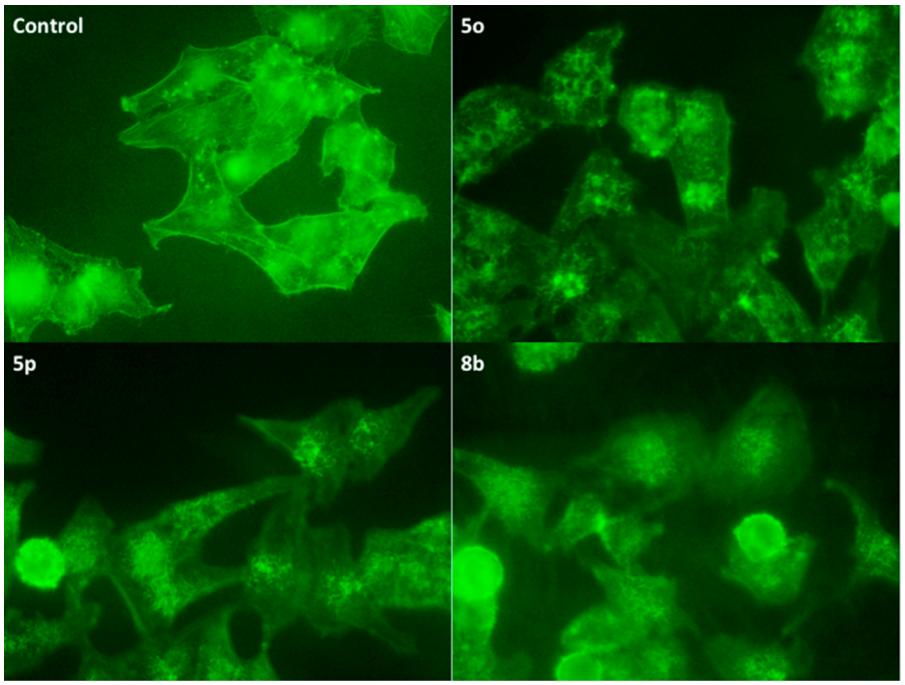

Figure 6. Effects of thiazole analogues **5o**, **5p**, and **8b** on the F-actin structure of HeLa cells. Cells were plated on glass coverslips and incubated with vehicle (DMSO), **5o**, **5p**, or **8b** for 24 h and were fixed and stained with Acti-stain 488 phalloidin. Cells were observed under a fluorescent microscope, a digital CCD camera, and 40× objective. Data are representative of three independent experiments.

invasion assays were found to have potent antiinvasion activities. In addition to antimigration and antiinvasion properties, two representative thiazole derivatives, **5o** and **5p**, also showed strong antiangiogenesis activity, blocking new blood vessel formation in a chicken embryo membrane assay. A functional study in which enhanced cell motility due to fascin overexpression was nearly completely abrogated by the most potent analogues **5o** and **5p** provided additional evidence that the action of the thiazole derivatives involved targeting the F-actin bundling protein fascin. This proposed mechanism of action is further supported by the observation that cellular F-actin structures were significantly impaired by the treatment of the thiazole analogues.

EXPERIMENTAL SECTION

Chemistry. All reagents and solvents were purchased from AK Scientific, Sigma-Aldrich Chemical Co., Fisher Scientific, ACROS, and Pharmco-AAPER and were used as received. All organic solvents (Pharmco-AAPER) used were of reagent grade quality and were used without further purification. NMR spectra were recorded on a Bruker Fourier-300 spectrometer (Bruker Inc., Billerica, MA) in ppm. Crude synthetic products were purified by the following methods: chromatography on silica gel (60−100 mesh, Fisher Scientific) column. Analytical thin layer chromatography (TLC) was performed on 250 μm fluorescent plates (Agela Technologies, DE, USA) and visualized by using UV light. For all products, the purity was ascertained to be greater than 95% by the HPLC method using a Shimadzu (Columbia, MD) 2010 HPLC−UV/MS system with a C-18 reverse phase column and by GC−MS analyses using an Agilent Technologies 5975C inert MSD mass spectrometer.

General Procedure for N-Alkylation of 3 and 4. To a cooled mixture of NaH (0.26 g, 60% in oil, 6.5 mmol) in THF (20 mL) was added a solution of compound **3** or **4** (5 mmol) in THF (10 mL) dropwise. The mixture was warmed to room temperature and stirred for 20 min. After that, the mixture was cooled to 0 °C again and MeI or RBr (6.5 mmol) was added dropwise. The mixture was then warmed to room temperature and stirred for 2 h. Water (5 mL) was added to quench the reaction, and the mixture was further diluted with water (50 mL). The mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phase was dried with anhydrous $MgSO_4$. After removal of all the solvent, the residue was purified by silica gel chromatography (hexane/EtOAc = 4:1) to afford products **5** (more polar) and **6** or **7** (more polar) and **8** as solids.

***N*-(4-(2,4-Dimethylphenyl)-3-ethylthiazol-2(3*H*)-ylidene)-benzamide (5l).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.37 (dd, J = 1.8 and 8.1 Hz, 2H), 7.49−7.42 (m, 3H), 7.18−7.10 (m, 3H), 6.45 (s, 1H), 4.35 (m, 1H), 3.82 (m, 1H), 1.24 (t, J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.1, 167.8, 140.1, 137.82, 137.75, 137.2, 131.3, 131.2, 130.6, 129.2, 128.0, 127.2, 126.8, 106.8, 42.1, 21.3, 19.6, 13.8. GC−MS: 336 (M^+). HRMS (ESI(+)) calcd for $C_{20}H_{21}N_2OS$ (M + H): 337.1375. Found: 337.1365.

***N*-(4-(2,4-Dimethylphenyl)-3-propylthiazol-2(3*H*)-ylidene)-benzamide (5m).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.36 (m, 2H), 7.52−7.42 (m, 3H), 7.17−7.09 (m, 3H), 6.45 (s, 1H), 4.28 (m, 1H), 3.71 (m, 1H), 2.41 (s, 3H), 2.13 (s, 3H), 1.70 (m, 2H), 0.81 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.0, 168.1, 140.0, 138.2, 137.7, 137.2, 131.3, 131.2, 130.7, 129.2, 128.0, 127.3, 126.8, 106.8, 48.5, 21.8, 21.3, 19.6, 11.2. GC−MS: 350 (M^+). HRMS (ESI(+)) calcd for $C_{21}H_{23}N_2OS$ (M + H): 351.1531. Found: 351.1525.

***N*-(3-Butyl-4-(2,4-dimethylphenyl)thiazol-2(3*H*)-ylidene)-benzamide (5n).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.36 (m, 2H), 7.52−7.42 (m, 3H), 7.17−7.09 (m, 3H), 6.45 (s, 1H), 4.33 (m, 1H), 3.73 (m, 1H), 2.40 (s, 3H), 2.14 (s, 3H), 1.64 (m, 2H), 1.22 (m, 2H), 0.81 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.1, 168.1, 140.1, 138.2, 137.7, 137.2, 131.3, 131.2, 130.7, 129.2, 128.0, 127.3, 126.8, 106.9, 46.7, 30.4, 21.3, 19.9, 19.6, 13.6. GC−MS: 364 (M^+). HRMS (ESI(+)) calcd for $C_{22}H_{25}N_2OS$ (M + H): 365.1688. Found: 365.1682.

***N*-(4-(2,4-Dimethylphenyl)-3-hexylthiazol-2(3*H*)-ylidene)-benzamide (5o).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.36 (dd, J = 1.5 and 7.8 Hz, 2H), 7.52−7.42 (m, 3H), 7.17−7.09 (m, 3H), 6.45 (s, 1H), 4.33 (m, 1H), 3.72 (m, 1H), 2.41 (s, 3H), 2.14 (s, 3H), 1.61 (m, 2H), 1.23−1.17 (m, 6H), 0.81 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.1, 168.0, 140.1, 138.1, 137.7, 137.2, 131.3, 131.2, 130.7, 129.2, 128.0, 127.3, 126.8, 106.9, 46.9, 31.1, 28.2, 26.2, 22.4, 21.3, 19.6,

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13.9. GC−MS: 392 (M^+). HRMS (ESI(+)) calcd for $C_{24}H_{29}N_2OS$ (M + H): 393.2001. Found: 393.1986.

***N*-(4-(2,4-Dimethylphenyl)-3-dodecylthiazol-2(3*H*)-ylidene)-benzamide (5p).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.35 (m, 2H), 7.49−7.26 (m, 3H), 7.17−7.09 (m, 3H), 6.44 (s, 1H), 4.32 (m, 1H), 3.73 (m, 1H), 2.40 (s, 3H), 2.13 (s, 3H), 1.59−1.50 (m, 4H), 1.28−1.17 (m, 16H), 0.88 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.1, 168.1, 140.0, 138.1, 137.7, 137.3, 131.3, 131.2, 130.7, 129.2, 128.0, 127.3, 126.8, 106.9, 46.9, 32.8, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 28.9, 28.2, 26.5, 25.8, 22.7, 21.3, 19.6, 14.1. MS (ESI): 477 (M + H). HRMS (ESI(+)) calcd for $C_{30}H_{41}N_2OS$ (M + H): 477.2940. Found: 477.2945.

***N*-(3-Allyl-4-(2,4-dimethylphenyl)thiazol-2(3*H*)-ylidene)-benzamide (5q).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.35 (m, 2H), 7.51−7.41 (m, 3H), 7.14−7.07 (m, 3H), 6.46 (s, 1H), 5.87 (m, 1H), 5.10 (dd, J = 1.2 and 10.2 Hz, 1H), 4.98 (dd, J = 1.2 and 17.1 Hz, 2H), 4.42 (m, 1H), 2.40 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.2, 168.1, 140.1, 137.8, 137.1, 131.3, 131.1, 130.8, 129.2, 128.0, 127.0, 126.6, 118.4, 106.8, 49.1, 21.3, 19.7. GC−MS: 348 (M^+). HRMS (ESI(+)) calcd for $C_{21}H_{21}N_2OS$ (M + H): 349.1375. Found: 349.1370.

***N*-(4-(2,4-Dimethylphenyl)-3-(prop-2-yn-1-yl)thiazol-2(3*H*)-ylidene)benzamide (5r).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.40 (m, 2H), 7.52−7.42 (m, 3H), 7.23−7.11 (m, 3H), 6.47 (s, 1H), 4.80 (m, 2H), 2.40 (s, 3H), 2.21 (m, 1H), 2.19 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.4, 168.1, 140.4, 138.1, 137.1, 136.9, 131.6, 131.4, 130.8, 129.4, 128.1, 126.9, 126.4, 106.9, 77.3, 72.4, 36.0, 21.4, 19.9. MS (ESI): 347 (M + H). HRMS (ESI(+)) calcd for $C_{21}H_{19}N_2OS$ (M + H): 347.1218. Found: 347.1216.

***N*-(4-(2,4-Dimethylphenyl)thiazol-2-yl)-*N*-(prop-2-yn-1-yl)-benzamide (6).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.36 (dd, J = 1.5 and 8.1 Hz, 2H), 7.52−7.43 (m, 4H), 7.20 (s, 1H), 7.18 (s, 2H), 4.75 (m, 2H), 2.42 (s, 3H), 2.22(m, 1H), 2.21 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.7, 167.3, 141.0, 138.5, 136.2, 135.9, 131.9, 131.6, 130.5, 129.5, 128.1, 127.3, 124.8, 98.0, 76.97, 72.7, 37.1, 21.5, 19.6. MS (ESI): 347 (M + H). HRMS (ESI(+)) calcd for $C_{21}H_{19}N_2OS$ (M + H): 347.1218. Found: 347.1210.

***N*-(4-Phenyl-3-ethylthiazol-2(3*H*)-ylidene)benzamide (7a).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.37 (dd, J = 1.5 and 7.5 Hz, 2H), 7.51−7.40 (m, 8H), 6.52 (s, 1H), 4.26 (q, J = 6.9 Hz, 2H), 1.34 (t, J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.2, 168.1, 139.1, 137.1, 130.9, 129.6, 129.4, 129.2, 128.9, 128.0, 107.2, 42.5, 14.0. GC−MS: 308 (M^+). HRMS (ESI(+)) calcd for $C_{18}H_{17}N_2OS$ (M + H): 309.1062. Found: 309.1052.

***N*-(4-Phenyl-3-propylthiazol-2(3*H*)-ylidene)benzamide (7b).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.36 (dd, J = 1.2 and 7.5 Hz, 2H), 7.50−7.39 (m, 8H), 6.52 (s, 1H), 4.19 (q, J = 7.5 Hz, 2H), 1.76 (m, 2H), 0.83 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.1, 168.5, 139.3, 137.2, 131.3, 130.9, 129.6, 129.5, 129.2, 128.8, 128.7, 128.0, 126.0, 107.2, 48.8, 21.9, 11.1. GC−MS: 322 (M^+). HRMS (ESI(+)) calcd for $C_{19}H_{19}N_2OS$ (M + H): 323.1218. Found: 323.1210.

***N*-(3-Allyl-4-phenylthiazol-2(3*H*)-ylidene)benzamide (7c).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.35 (dd, J = 1.8 and 6.3 Hz, 2H), 7.49−7.43 (m, 8H), 6.55 (s, 1H), 5.98 (m, 1H), 5.19 (d, J = 10.2 Hz, 1H), 4.98 (d, J = 17.4 Hz, 1H), 4.82 (d, J = 5.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.3, 168.5, 139.3, 137.0, 131.9, 131.4, 130.6, 129.7, 129.6, 129.2, 128.7, 128.0, 118.0, 107.1, 49.6. GC−MS: 320 (M^+). HRMS (ESI(+)) calcd for $C_{19}H_{17}N_2OS$ (M + H): 321.1062. Found: 321.1054.

***N*-(4-Phenyl-3-(prop-2-yn-1-yl)thiazol-2(3*H*)-ylidene)-benzamide (7d).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.40 (d, J = 6.6 Hz, 2H), 7.57−7.44 (m, 8H), 6.56 (s, 1H), 4.92 (d, J = 2.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.4, 168.5, 138.6, 136.8, 131.6, 130.1, 129.9, 129.44, 129.37, 129.0, 128.1, 77.8, 72.8, 36.9. LC−MS (ESI): 319 (M + H). HRMS (ESI(+)) calcd for $C_{19}H_{15}N_2OS$ (M + H): 319.0905. Found: 319.0897.

***N*-Ethyl-*N*-(4-phenylthiazol-2-yl)benzamide (8a).** ^{1}H NMR (300 MHz, $CDCl_3$): 7.92 (m, 2H), 7.55−7.48 (m, 5H), 7.42 (m, 2H), 7.34 (m, 1H), 7.25 (s, 1H), 4.27 (q, J = 7.5 Hz, 2H), 1.38 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 170.6, 159.0, 149.5, 135.2, 134.8, 130.4, 128.68, 128.65, 127.8, 126.7, 126.0, 109.2, 45.2, 14.1. GC−MS: 308 (M^+). HRMS (ESI(+)) calcd for $C_{18}H_{17}N_2OS$ (M + H): 309.1062. Found: 309.1053.

***N*-(4-Phenylthiazol-2-yl)-*N*-propylbenzamide (8b).** ^{1}H NMR (300 MHz, $CDCl_3$): 7.91 (dd, J = 1.5 and 6.9 Hz, 2H), 7.53−7.32 (m, 8H), 7.24 (s, 1H), 4.18 (q, J = 7.5 Hz, 2H), 1.83 (m, 2H), 0.83 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 170.8, 159.3, 149.5, 135.2, 134.8, 130.4, 128.68, 128.66, 128.5, 127.9, 127.0, 126.0, 109.2, 51.6, 21.9, 11.2. GC−MS: 322 (M^+). HRMS (ESI(+)) calcd for $C_{19}H_{19}N_2OS$ (M + H): 323.1218. Found: 323.1211.

Synthesis of *N*-(4-(2,4-Dimethylphenyl)thiazol-2-yl)-benzenesulfonamide (10). To a solution of **9** (0.61g, 3.0 mmol) and pyridine (0.96 mL, 12 mmol) in anhydrous dichloromethane was added benzenesulfonic chloride (0.76 mL, 6 mmol) in dichloromethane dropwise at 0 °C. After being stirred at room temperature for 2 h under nitrogen atmosphere, the reaction mixture was concentrated in vacuo. The saturated Na_2CO_3 solution was added to quench the reaction, and the solution was extracted with ethyl acetate, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash chromatography to give product **10** (0.57 g, 55% yield) as a solid. ^{1}H NMR (300 MHz, $CDCl_3$): 9.85 (bs, 1H), 7.93 (d, J = 7.8 Hz, 2H), 7.53−7.43 (m, 3H), 7.16 (d, J = 7.8 Hz, 1H), 7.08−7.04 (m, 3H), 6.26 (s, 1H), 2.34 and 2.32 (ds, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): 168.7, 141.9, 140.1, 136.3, 136.1, 132.2, 132.0, 128.9, 128.7, 127.2, 126.5, 125.7, 103.6, 21.2, 20.4. GC−MS: 344 (M^+). HRMS (ESI(+)) calcd for $C_{17}H_{17}N_2O_2S_2$ (M + H): 345.0731. Found: 345.0727.

Methylation of 10 To Afford *N*-(4-(2,4-Dimethylphenyl)-3-methylthiazol-2(3*H*)-ylidene)benzenesulfonamide (11) and *N*-(4-(2,4-Dimethylphenyl)thiazol-2-yl)-*N*-methylbenzene-sulfonamide (12). To a cooled mixture of NaH (0.07 g, 60% in oil, 1.6 mmol) in THF (5 mL) was added a solution of compound **10** (0.34g, 1 mmol) in THF (5 mL) dropwise. The mixture was warmed to room temperature and stirred for 20 min. After that, the mixture was cooled to 0 °C again and MeI (0.25 mL, 4.0 mmol) was added dropwise. The mixture was then warmed to room temperature and stirred for 2 h. Water (3 mL) was added to quench the reaction, and the mixture was further diluted with water (10 mL). The mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phase was dried with anhydrous $MgSO_4$. After removal of all the solvent, the residue was purified by silica gel chromatography (hexane/EtOAc = 4:1) to afford products **11** (more polar) (0.27 g, 75% yield) and **12** (0.034 g, 9% yield) as solids.

11: ^{1}H NMR (300 MHz, $CDCl_3$): 8.05−8.02 (m, 2H), 7.58−7.43 (m, 3H), 7.12−7.01 (m, 3H), 6.27 (s, 1H), 3.19 (s, 3H), 2.36 (s, 3H), 2.11 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 166.8, 142.3, 140.6, 139.1, 137.6, 131.9, 131.4, 130.5, 128.6, 127.1, 126.6, 126.4, 103.5, 33.6, 21.3, 19.5. GC−MS: 358 (M^+). HRMS (ESI(+)) calcd for $C_{18}H_{19}N_2O_2S_2$ (M + H): 359.0888. Found: 359.0879.

12: ^{1}H NMR (300 MHz, $CDCl_3$): 7.83 (d, J = 7.2 Hz, 2H), 7.58−7.38 (m, 4H), 7.02−6.98 (m, 2H), 6.88 (s, 1H), 3.45(s, 3H), 2.32 and 2.30 (ds, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): 160.0, 151.2, 137.9, 136.8, 135.9, 133.8, 131.8, 131.4, 129.4, 129.3, 127.4, 126.6, 112.0, 36.7, 21.2, 21.1. GC−MS: 358 (M^+). HRMS (ESI(+)) calcd for $C_{18}H_{19}N_2O_2S_2$ (M + H): 359.0888. Found: 359.0880.

General Procedure for the Acylation of 13 To Afford 14. To a solution of **13** (0.01 mol) and DMAP (1.24 g, 0.01 mol) in anhydrous dichloromethane was added the acyl chloride in dichloromethane dropwise at 0 °C. After being stirred at room temperature for 2 h under nitrogen atmosphere, the reaction mixture was concentrated in vacuo. The saturated Na_2CO_3 solution was added to quench the reaction, and the solution was extracted with ethyl acetate, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash chromatography to give product **14** as a solid.

***N*-(4-Methylthiazol-2-yl)benzamide (14a).** ^{1}H NMR (300 MHz, $CDCl_3$): 7.99 (dd, J = 1.5 and 8.4 Hz, 2H), 7.52−7.47 (m, 3H), 6.55 (d, J = 0.9 Hz, 1H), 2.11 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 172.0, 165.3, 159.3, 146.8, 133.1, 132.8, 132.3, 130.0, 128.9, 128.3, 127.8, 108.4, 16.4. GC−MS: 218 (M^+). HRMS (ESI(+)) calcd for $C_{11}H_{11}N_2OS$ (M + H): 219.0592. Found: 219.0589.

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***N*-(4-(2-Chlorophenyl)thiazol-2-yl)benzamide (14b).** ^{1}H NMR (300 MHz, $CDCl_3$): 11.5 (bs, 1H), 7.75 (dd, J = 1.2 and 8.4 Hz, 2H), 7.63 (dd, J = 2.1 and 7.2 Hz, 1H), 7.51−7.45 (m, 2H), 7.38−7.28 (m, 3H), 7.19−7.08 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 165.3, 158.5, 146.3, 132.8, 132.7, 132.0, 131.7, 130.8, 130.4, 128.9, 128.7, 127.4, 126.8, 113.2. GC−MS: 314 (M^+). HRMS (ESI(+)) calcd for $C_{16}H_{12}ClN2OS$ (M + H): 315.0359. Found: 315.0351.

***N*-(4-(3-Bromophenyl)thiazol-2-yl)benzamide (14c).** ^{1}H NMR (300 MHz, $CDCl_3$): 11.68 (bs, 1H), 8.17−7.15 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$): 171.3, 165.3, 160.1, 148.3, 135.9, 133.5, 133.1, 133.0, 131.7, 131.1, 130.3, 130.2, 130.1, 129.5, 129.3, 128.9, 128.4, 127.8, 124.9, 122.9, 109.3. GC−MS: 360, 358 (M^+). HRMS (ESI(+)) calcd for $C_{16}H_{12}BrN_2OS$ (M + H): 358.9854. Found: 358.9847.

***N*-(4-(4-Bromophenyl)thiazol-2-yl)benzamide (14d).** ^{1}H NMR (300 MHz, $CDCl_3$): 10.34 (bs, 1H), 7.90 (m, 2H), 7.67−7.41 (m, 7H), 7.20 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 164.9, 158.7, 149.1, 133.1, 133.0, 131.83, 131.79, 129.0, 127.6, 127.4, 122.1, 108.5. GC−MS: 358, 360 (M^+). HRMS (ESI(+)) calcd for $C_{16}H_{12}BrN_2OS$ (M + H): 358.9854. Found: 358.9848.

***N*-(4-(3-Chlorophenyl)thiazol-2-yl)benzamide (14e).** ^{1}H NMR (300 MHz, $CDCl_3$): 10.19 (bs, 1H), 7.90 (m, 2H), 7.77 (t, J = 1.8 Hz, 1H), 7.66−7.56 (m, 2H), 7.49−7.44 (m, 1H), 7.32−7.26 (m, 2H), 7.20 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 164.8, 158.5, 148.7, 135.9, 134.7, 133.0, 131.8, 130.0, 129.0, 128.0, 127.4, 126.2, 124.1, 109.1. GC−MS: 314 (M^+). HRMS (ESI(+)) calcd for $C_{16}H_{12}ClN_2OS$ (M + H): 315.0359. Found: 315.0353.

***N*-(4-(3,5-Bis(trifluoromethyl)phenyl)thiazol-2-yl)benzamide (14f).** ^{1}H NMR (300 MHz, $CDCl_3$): 10.08 (bs, 1H), 8.24 (s, 2H), 7.93(m, 2H), 7.77 (s, 1H), 7.60 (m, 1H), 7.51−7.45 (m, 2H), 7.39 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 165.0, 159.1, 147.3, 136.3, 133.4, 132.5, 132.0, 131.7, 129.3, 127.6, 126.2, 125.3, 121.7, 110.9. GC−MS: 416 (M^+). HRMS (ESI(+)) calcd for $C_{18}H_{11}F_6N_2OS$ (M + H): 417.0496. Found: 417.0486.

***N*-(4-(3,4,5-Trimethoxyphenyl)thiazol-2-yl)benzamide (14g).** Yield: 1.9 g, 51%. ^{1}H NMR (300 Hz, $CDCl_3$): 10.10 (1H, bs), 7.93 (2H, J = 7.2 Hz, d), 7.59 (1H, m), 7.52−7.47 (2H, m), 7.13 (1H, s), 7.04 (2H, s), 3.92 (6H, s), 3.87 (3H,s). ^{13}C NMR (75 Hz, $CDCl_3$): 164.6, 158.1, 153.4, 150.0, 138.1, 133.0, 131.8, 130.0, 129.0, 127.3, 107.6, 103.3, 61.0, 56.2. MS-EI: 370 (M^+). HRMS (ESI(+)) calcd for $C_{19}H_{19}N_2O_4S$ (M + H): 371.1066. Found: 371.1052. HRMS (ESI(+)) calcd for $C_{19}H_{19}N_2O_4S$ (M + H): 371.1066. Found: 371.1060.

***N*-(5-Methyl-4-phenylthiazol-2-yl)benzamide (14h).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.20 (d, J = 6.9 Hz, 2H), 8.09 (d, J = 7.2 Hz, 2H), 7.62−7.43 (m, 6H), 2.51 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 171.3, 165.2, 157.1, 144.4, 133.7, 133.3, 132.8, 131.9, 130.3, 130.1, 128.8, 128.4, 128.3, 128.1, 127.9, 122.0, 12.0. GC−MS: 294 (M^+). HRMS (ESI(+)) calcd for $C_{17}H_{15}N_2OS$ (M + H): 295.0905. Found: 295.0897.

***N*-(4-(4-Fluorophenyl)-5-methylthiazol-2-yl)benzamide (14i).** ^{1}H NMR (300 MHz, $CDCl_3$): 10.6 (bs, 1H), 7.82 (dd, J = 1.2 and 8.1 Hz, 2H), 7.53−7.41 (m, 5H), 7.01 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 164.7, 155.1, 144.2, 132.7, 132.0, 130.7, 130.0, 129.9, 128.8, 127.4, 122.2, 115.4, 115.2, 12.1. GC−MS: 312 (M^+). HRMS (ESI(+)) calcd for $C_{17}H_{14}FN_2OS$ (M + H): 313.0811. Found: 313.0802.

***N*-(5-(*p*-Tolyl)thiazol-2-yl)benzamide (14j).** ^{1}H NMR (300 MHz, $CDCl_3$): 10.01 (bs, 1H), 7.92 (d, J = 7.5 Hz, 2H), 7.69 (d, J = 8.1 Hz, 2H), 7.57−7.45 (m, 3H), 7.19 (d, J = 8.1 Hz, 2H), 7.14 (s, 1H), 2.37 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 165.2, 158.9, 150.3, 137.9, 132.7, 131.9, 131.4, 129.4, 128.7, 127.4, 126.0, 107.3, 21.2. GC−MS: 294 (M^+). HRMS (ESI(+)) calcd for $C_{17}H_{15}N_2OS$ (M + H): 295.0905. Found: 295.0899.

4-Methoxy-*N*-(4-(3,4,5-trimethoxyphenyl)thiazol-2-yl)-benzamide (14k). ^{1}H NMR (300 MHz, $CDCl_3$): 10.92 (bs, 1H), 7.80 (d, J = 8.7 Hz, 2H), 7.10 (s, 1H), 6.94 (s, 2H), 6.84 (d, J = 8.7 Hz, 2H), 3.86 (s, 6H), 3.84(s, 3H), 3.83 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 164.6, 163.1, 159.1, 153.2, 149.9, 137.9, 130.0, 129.4, 124.0, 114.0, 107.5, 103.3, 60.8, 56.0, 55.4. HRMS (ESI(+)) calcd for $C_{20}H_{21}N_2O_5S$ (M + H): 401.1171. Found: 401.1165.

4-Chloro-*N*-(4-(3,4,5-trimethoxyphenyl)thiazol-2-yl)-benzamide (14l). ^{1}H NMR (300 MHz, $CDCl_3$): 11.43 (bs, 1H), 7.73 (m, 2H), 7.30 (m, 2H), 7.12 (s, 1H), 6.88 (s, 2H), 3.85 (s, 6H), 3.83(s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 164.4, 159.0, 153.3, 150.1, 139.1, 138.0, 130.3, 129.7, 129.0, 128.8, 107.9, 103.3, 60.8, 56.0. GC−MS: 404 (M^+). HRMS (ESI(+)) calcd for $C_{19}H_{18}ClN_2O_4S$ (M + H): 405.0676. Found: 405.0664.

3,4-Dimethoxy-*N*-(4-(3,4,5-trimethoxyphenyl)thiazol-2-yl)-benzamide (14m). ^{1}H NMR (300 MHz, $CDCl_3$): 11.07 (bs, 1H), 7.44 (dd, J = 1.8 and 8.1 Hz, 1H), 7.39 (s, 1H), 7.11 (s, 1H), 6.92 (s, 2H), 6.78 (d, J = 8.4 Hz, 1H), 3.92 (s, 3H), 3.86 (s, 6H), 3.83(s, 3H), 3.82 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 164.8, 159.2, 153.3, 152.8, 150.1, 148.9, 138.0, 129.9, 124.2, 120.8, 110.4, 110.3, 107.6, 103.3, 60.9, 56.0, 55.8. GC−MS: 430 (M^+). HRMS (ESI(+)) calcd for $C_{21}H_{23}N_2O_6S$ (M + H): 431.1277. Found: 431.1265.

2,4-Dimethoxy-*N*-(4-(3,4,5-trimethoxyphenyl)thiazol-2-yl)-benzamide (14n). ^{1}H NMR (300 MHz, $CDCl_3$): 11.03 (bs, 1H), 8.28 (d, J = 9.0 Hz, 1H), 7.10 (s, 2H), 7.09 (s, 1H), 6.68 (dd, J = 2.1 and 9.0 Hz, 1H), 6.56 (d, J = 2.1 Hz, 1H), 4.11 (s, 3H), 3.96(s, 6H), 3.90 (s, 3H), 3.89 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 164.8, 162.6, 159.2, 158.1, 153.4, 149.9, 138.0, 134.5, 130.4, 112.0, 107.5, 106.1, 103.4, 98.7, 60.9, 56.4, 56.2, 55.7. HRMS (ESI(+)) calcd for $C_{21}H_{23}N_2O_6S$ (M + H): 431.1277. Found: 431.1270.

3,4,5-Trimethoxy-*N*-(4-(3,4,5-trimethoxyphenyl)thiazol-2-yl)benzamide (14o). ^{1}H NMR (300 MHz, $CDCl_3$): 11.50 (bs, 1H), 7.13 (d, J = 0.3 Hz, 1H), 7.06 (s, 2H), 6.90 (s, 2H), 3.87 (s, 3H), 3.84(s, 6H), 3.82 (s, 3H), 3.78 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): 165.1, 159.3, 153.3, 153.1, 150.2, 141.9, 138.1, 129.6, 126.8, 107.8, 104.8, 103.2, 60.9, 60.8, 56.1, 56.0. HRMS (ESI(+)) calcd for $C_{22}H_{25}N_2O_7S$ (M + H): 461.1382. Found: 461.1374.

2,3-Dimethoxy-*N*-(4-(3,4,5-trimethoxyphenyl)thiazol-2-yl)-benzamide (14p). ^{1}H NMR (300 MHz, $CDCl_3$): 11.38 (bs, 1H), 7.84 (dd, J = 1.5 and 7.8 Hz, 1H), 7.24−7.15 (m, 3H), 7.12 (s, 2H), 4.11 (s, 3H), 3.96(s, 6H), 3.92 (s, 3H), 3.89 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 162.6, 157.5, 153.4, 152.6, 150.1, 148.0, 138.1, 130.3, 124.8, 124.0, 123.0, 117.0, 107.6, 103.4, 61.9, 60.9, 56.2. HRMS (ESI(+)) calcd for $C_{21}H_{23}N_2O_6S$ (M + H): 431.1277. Found: 431.1268.

2-Methoxy-*N*-(4-(3,4,5-trimethoxyphenyl)thiazol-2-yl)-benzamide (14q). ^{1}H NMR (300 MHz, $CDCl_3$): 11.19 (bs, 1H), 8.33 (dd, J = 1.5 and 7.8 Hz, 1H), 7.57 (dt, J = 1.5 and Hz, 1H), 7.19−7.07 (m, 5H), 4.14 (s, 3H), 3.96 (s, 6H), 3.89 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 162.7, 157.8, 157.7, 153.4, 150.0, 138.0, 134.5, 132.6, 130.3, 121.7, 119.0, 111.6, 107.7, 103.4, 60.9, 56.4, 56.2. HRMS (ESI(+)) calcd for $C_{20}H_{21}N_2O_5S$ (M + H): 401.1171. Found: 401.1165.

3-Methoxy-*N*-(4-(3,4,5-trimethoxyphenyl)thiazol-2-yl)-benzamide (14r). ^{1}H NMR (300 MHz, $CDCl_3$): 10.75 (bs, 1H), 7.41−7.39 (m, 2H), 7.31 (m, 1H), 7.12 (s, 1H), 7.05 (m, 1H), 6.96 (s, 2H), 3.88 (s, 6H), 3.84 (s, 3H), 3.77 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 164.8, 159.8, 158.5, 153.3, 150.1, 138.0, 133.1, 129.9, 129.8, 119.4, 119.2, 112.2, 107.6, 103.3, 60.9, 56.0, 55.3. HRMS (ESI(+)) calcd for $C_{20}H_{21}N_2O_5S$ (M + H): 401.1171. Found: 401.1164.

General Procedure for Methylation of 14. To a cooled mixture of NaH (0.26 g, 60% in oil, 6.5 mmol) in THF (20 mL) was added a solution of compound **14** (5 mmol) in THF (10 mL) dropwise. The mixture was warmed to room temperature and stirred for 20 min. After that, the mixture was cooled to 0 °C again and MeI (6.5 mmol) was added dropwise. The mixture was then warmed to room temperature and stirred for 2 h. Water (5 mL) was added to quench the reaction, and the mixture was further diluted with water (50 mL). The mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phase was dried with anhydrous $MgSO_4$. After removal of all the solvent, the residue was purified by silica gel chromatography (hexane/EtOAc = 4:1) to afford products **15** (more polar) and **16** as solids.

***N*-(3,4-Dimethylthiazol-2(3*H*)-ylidene)benzamide (15a).** ^{1}H NMR (300 MHz, $CDCl_3$): 7.83 (dd, J = 1.8 and 7.8 Hz, 2H), 7.47−7.43 (m, 3H), 6.29 (d, J = 1.2 Hz, 1H), 3.79 (s, 3H), 2.31 (d, J = 1.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.0, 169.0, 137.1, 134.4, 131.3, 129.2, 128.0, 104.3, 32.9, 14.4. GC−MS: 232 (M^+). HRMS

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(ESI(+)) calcd for $C_{12}H_{13}N_2OS$ (M + H): 233.0749. Found: 233.0741.

***N*-(4-(2-Chlorophenyl)-3-methylthiazol-2(3*H*)-ylidene)-benzamide (15b).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.38 (dd, *J* = 1.8 and 7.8 Hz, 2H), 7.54−7.39 (m, 7H), 6.60 (s, 1H), 3.63 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.3, 168.4, 136.9, 136.3, 134.8, 132.3, 131.53, 131.49, 130.0, 129.9, 129.2, 128.1, 127.3, 108.1, 34.0. GC−MS: 328 (M^+). HRMS (ESI(+)) calcd for $C_{17}H_{14}ClN_2OS$ (M + H): 329.0515. Found: 329.0505.

***N*-(4-(3-Bromophenyl)-3-methylthiazol-2(3*H*)-ylidene)-benzamide (15c).** ^{1}H NMR (300 MHz, $CDCl_3$): 7.83 (dd, *J* = 1.8 and 8.1 Hz, 2H), 7.64 (m, 1H), 7.59 (m, 1H), 7.51−7.36 (m, 6H), 6.60 (s, 1H), 3.75 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.4, 168.9, 137.8, 136.8, 132.8, 132.5, 132.2, 131.6, 130.5, 129.3, 128.1, 127.8, 123.0, 107.8, 34.9. GC−MS: 374, 372 (M^+). HRMS (ESI(+)) calcd for $C_{17}H_{14}BrN_2OS$ (M + H): 373.0010. Found: 373.0006.

***N*-(4-(4-Bromophenyl)-3-methylthiazol-2(3*H*)-ylidene)-benzamide (15d).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.37 (dd, *J* = 1.5 and 8.1 Hz, 2H), 7.65 (d, *J* = 6.6 Hz, 2H), 7.51−7.43 (m, 4H), 7.28 (d, *J* = 7.2 Hz, 1H), 6.58 (d, *J* = 0.9 Hz, 1H), 3.74 (d, *J* = 2.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.3, 168.9, 138.2, 136.9, 132.3, 131.9, 131.6, 130.8, 129.5, 128.1, 124.2, 107.4, 34.9. GC−MS: 372, 374 (M^+). HRMS (ESI(+)) calcd for $C_{17}H_{14}BrN_2OS$ (M + H): 373.0010. Found: 373.0000.

***N*-(4-(3-Chlorophenyl)-3-methylthiazol-2(3*H*)-ylidene)-benzamide (15e).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.37 (d, *J* = 7.8 Hz, 2H), 7.51−7.43 (m, 6H), 7.32 (m, 1H), 6.59(s, 1H), 3.75 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.4, 168.9, 137.9, 136.8, 135.0, 132.3, 131.6, 130.3, 129.9, 129.4, 129.3, 128.1, 127.4, 107.8, 34.9. GC−MS: 328 (M^+). HRMS (ESI(+)) calcd for $C_{17}H_{14}ClN_2OS$ (M + H): 329.0515. Found: 329.0508.

***N*-(4-(3,5-Bis(trifluoromethyl)phenyl)-3-methylthiazol-2(3*H*)-ylidene)benzamide (15f).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.37 (dd, *J* = 1.5 and 8.1 Hz, 2H), 8.03 (s, 1H), 7.91 (s, 2H), 7.50−7.46 (m, 3H), 6.73 (s, 1H), 3.77 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.4, 168.9, 136.4, 136.0, 132.8, 132.7, 132.4, 131.7, 129.2, 128.0, 123.3, 120.9, 109.4, 34.8. GC−MS: 430 (M^+). HRMS (ESI(+)) calcd for $C_{19}H_{13}F_6N_2OS$ (M + H): 431.0653. Found: 431.0646.

***N*-(4-(3,4,5-Trimethoxyphenyl)-3-methylthiazol-2(3*H*)-yliene)benzamide (15g).** Yield: 0.69 g, 36%. ^{1}H NMR (300 Hz, $CDCl_3$): 8.37 (2H, *J* = 7.8 Hz, d), 7.50−7.43 (3H, m), 6.60 (2H, s), 6.56 (1H, s), 3.92 (3H, s), 3.90 (6H, s), 3.76 (3H, s). ^{13}C NMR (75 Hz, $CDCl_3$): 174.3, 168.7, 153.5, 139.4, 139.1, 137.0, 131.5, 129.2, 128.1, 125.9, 106.7, 106.6, 61.0, 56.4, 35.0. MS-EI: 384 (M^+). HRMS (ESI(+)) calcd for $C_{20}H_{21}N_2O_4S$ (M + H): 385.1222. Found: 385.1215.

***N*-(3,5-Dimethyl-4-phenylthiazol-2(3*H*)-ylidene)benzamide (15h).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.37 (dd, *J* = 1.8 and 8.1 Hz, 2H), 7.53−7.42 (m, 6H), 7.33−7.30 (m, 2H), 3.61 (s, 3H), 2.17 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.0, 167.0, 137.2, 134.1, 131.3, 130.4, 129.8, 129.5, 129.2, 129.1, 128.0, 117.8, 35.0. GC−MS: 308 (M^+). HRMS (ESI(+)) calcd for $C_{18}H_{17}N_2OS$ (M + H): 309.1062. Found: 309.1055.

***N*-(4-(4-Fluorophenyl)-3,5-dimethylthiazol-2(3*H*)-ylidene)-benzamide (15i).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.36 (dd, *J* = 0.9 and 7.8 Hz, 2H), 7.47−7.44 (m, 3H), 7.32−7.22 (m, 4H), 3.60 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174. 0, 167.0, 164.9, 161.6, 137.1, 133.0, 132.4, 132.2, 131.4, 129.2, 128.0, 125.74, 125.70, 118.2, 116.5, 116.2, 34.9, 12.2. GC−MS: 326 (M^+). HRMS (ESI(+)) calcd for $C_{18}H_{16}FN_2OS$ (M + H): 327.0967. Found: 327.0957.

***N*-(3-Methyl-5-(*p*-toyl)thiazol-2(3*H*)-ylidene)benzamide (15j).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.38 (dd, *J* = 1.5 and 7.8 Hz, 2H), 7.48−7.44 (m, 3H), 7.30 (s, 4H), 6.53 (s, 1H), 3.74 (s, 3H), 2.44 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.2, 168.9, 139.8, 139.6, 137.1, 131.4, 129.6, 129.23, 129.17, 128.0, 127.7, 106.6, 34.9, 21.4. GC−MS: 308 (M^+). HRMS (ESI(+)) calcd for $C_{18}H_{17}N2OS$ (M + H): 309.1062. Found: 309.1054.

4-Methoxy-*N*-(3-methyl-4-(3,4,5-trimethoxyphenyl)thiazol-2(3*H*)-ylidene)benzamide (15k). ^{1}H NMR (300 MHz, $CDCl_3$): 8.33 (d, *J* = 9.0 Hz, 2H), 6.95 (d, *J* = 9.0 Hz, 2H), 6.60 (s, 2H), 6.53 (s, 1H), 3.92 (s, 3H), 3.90(s, 6H), 3.88 (s, 3H), 3.74 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 173.9, 168.4, 162.4, 153.5, 139.2, 139.1, 131.1, 129.7, 126.0, 113.2, 106.6, 106.4, 61.0, 56.3, 55.3, 34.9, 29.7. HRMS (ESI(+)) calcd for $C_{21}H_{23}N_2O_5S$ (M + H): 415.1328. Found: 415.1321.

4-Chloro-*N*-(3-methyl-4-(3,4,5-trimethoxyphenyl)thiazol-2(3*H*)-ylidene)benzamide (15l). ^{1}H NMR (300 MHz, $CDCl_3$): 8.30 (d, *J* = 8.7 Hz, 2H), 7.42 (d, *J* = 8.7 Hz, 2H), 6.60 (s, 2H), 6.58 (s, 1H), 3.93 (s, 3H), 3.90 (s, 6H), 3.75 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 173.2, 168.8, 153.6, 139.5, 139.3, 137.6, 135.5, 130.7, 128.3, 125.7, 106.8, 106.7, 61.0, 56.4, 35.0. HRMS (ESI(+)) calcd for $C_{20}H_{20}ClN_2O_4S$ (M + H): 419.0832. Found: 419.0824.

3-Methoxy-*N*-(3-methyl-4-(3,4,5-trimethoxyphenyl)thiazol-2(3*H*)-ylidene)benzamide (15m). ^{1}H NMR (300 MHz, $CDCl_3$): 8.00 (m, 1H), 7.91 (m, 1H), 7.37 (t, *J* = 7.8 Hz, 1H), 7.05 (m, 1H), 6.60 (s, 2H), 6.57 (s, 1H), 3.93 (s, 3H), 3.90 (s, 6H), 3.89 (s, 3H), 3.76 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.1, 168.8, 159.5, 153.5, 139.4, 139.2, 138.5, 129.0, 125.8, 121.8, 118.0, 113.6, 106.7, 106.6, 61.0, 56.4, 55.4, 35.0. HRMS (ESI(+)) calcd for $C_{21}H_{23}N2O_5S$ (M + H): 415.1328. Found: 415.1320.

***N*-(4-Cyclopropyl-3-methylthiazol-2(3*H*)-ylidene)benzamide (15n).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.35 (dd, *J* = 1.8 and 7.8 Hz, 2H), 7.49−7.41 (m, 3H), 6.23 (d, *J* = 1.2 Hz, 1H), 3.95 (s, 3H), 1.75 (m, 1H), 1.02 (m, 2H), 0.73 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 174.0, 169.1, 140.6, 137.1, 131.3, 129.2, 128.0, 103.9, 33.2, 8.7, 5.9. GC−MS: 258 (M^+). HRMS (ESI(+)) calcd for $C_{14}H_{15}N2OS$ (M + H): 259.0509. Found: 259.0519.

3,4,5-Trimethoxy-*N*-(3-methyl-4-(3,4,5-trimethoxyphenyl)-thiazol-2(3*H*)-ylidene)benzamide (15o). ^{1}H NMR (300 MHz, $CDCl_3$): 7.68 (s, 2H), 6.61 (s, 2H), 6.57 (s, 1H), 3.96 (s, 6H), 3.93 (s, 3H), 3.92(s, 3H), 3.91 (s, 6H), 3.77 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 173.7, 168.8, 153.5, 152.7, 141.1, 139.4, 139.2, 132.3, 125.8, 106.7, 106.6, 106.4, 61.0, 60.9, 56.3, 56.1, 34.9. HRMS (ESI(+)) calcd for $C_{23}H_{27}N_2O_7S$ (M + H): 475.1539. Found: 475.1526.

***N*-(4-(2-Chlorophenyl)thiazol-2-yl)-*N*-methylbenzamide (16a).** ^{1}H NMR (300 MHz, $CDCl_3$): 7.97 (dd, *J* = 1.8 and 7.8 Hz, 1H), 7.60−7.46 (m, 7H), 7.37−7.23 (m, 2H), 3.74 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 170.4, 159.0, 146.0, 134.5, 133.5, 132.1, 131.3, 130.9, 130.6, 128.73, 128.66, 127.6, 126.9, 114.6, 38.5. GC−MS: 328 (M^+). HRMS (ESI(+)) calcd for $C_{17}H_{14}ClN_2OS$ (M + H): 329.0515. Found: 329.0515.

***N*-(4-(3-Bromophenyl)thiazol-2-yl)-*N*-methylbenzamide (16b).** ^{1}H NMR (300 MHz, $CDCl_3$): 8.1 (s, 1H), 7.83 (d, *J* = 7.8 Hz, 1H), 7.59−7.44 (m, 6H), 7.31−7.28 (m, 2H), 3.76 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 170.5, 160.2, 147.9, 136.7, 134.4, 131.0, 130.7, 130.2, 129.1, 128.7, 127.6, 124.5, 122.9, 110.2, 38.5. GC−MS: 374, 372 (M^+). HRMS (ESI(+)) calcd for $C_{17}H_{14}BrN_2OS$ (M + H): 373.0010. Found: 373.0001.

***N*-(4-(4-Bromophenyl)thiazol-2-yl)-*N*-methylbenzamide (16c).** ^{1}H NMR (300 MHz, $CDCl_3$): 7.80 (d, *J* = 8.7 Hz, 2H), 7.59−7.50 (m, 7H), 3.75 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 170.4, 160.2, 148.3, 134.5, 133.7, 131.8, 131.0, 128.7, 127.6, 121.8, 109.6, 38.5. GC−MS: 372, 374 (M^+). HRMS (ESI(+)) calcd for $C_{17}H_{14}BrN_2OS$ (M + H): 373.0010. Found: 373.0005.

***N*-Methyl-*N*-(4-(3,4,5-trimethoxyphenyl)thiazol-2-yl)-benzamide (16d).** Yield: 0.35 g, 18%. ^{1}H NMR ($CDCl_3$): 7.61−7.57 (2H, m), 7.55−7.50 (3H, m), 7.19 (1H, s), 7.15 (2H, s), 3.95 (6H, s), 3.89 (3H, s), 3.77 (3H, s). ^{13}C NMR ($CDCl_3$): 170.4, 160.0, 153.5, 149.5, 138.3, 134.5, 131.0, 130.5, 128.7, 127.6, 108.9, 103.4, 61.0, 56.2, 38.5. MS-EI: 384 (M^+). HRMS (ESI(+)) calcd for $C_{20}H_{21}N_2O_4S$ (M + H): 385.1222. Found: 385.1216.

***N*-(4-(4-Fluorophenyl)-5-methylthiazol-2-yl)-*N*-methylbenzamide (16e).** ^{1}H NMR (300 MHz, $CDCl_3$): 7.69−7.64 (m, 2H), 7.57−7.48 (m, 5H), 7.16−7.10 (m, 2H), 3.67 (s, 3H), 2.52 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 170.2, 163.7, 160.5, 156.2, 143.8, 134.6, 131.6, 130.8, 130.1, 130.0, 128.6, 127.6, 123.3, 115.4, 115.1, 37.9, 12.1. GC−MS: 326 (M^+). HRMS (ESI(+)) calcd for $C_{18}H_{16}FN_2OS$ (M + H): 327.0967. Found: 327.0959.

***N*-Methyl-*N*-(5-(*p*-tolyl)thiazol-2-yl)benzamide (16f).** ^{1}H NMR (300 MHz, $CDCl_3$): 7.82 (d, *J* = 8.1 Hz, 2H), 7.59−7.49 (m, 5H), 7.24−7.19 (m, 3H), 3.76 (s, 3H), 2.39 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 170.4, 159.9, 149.5, 137.7, 134.7, 132.0, 130.8, 129.4,

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128.6, 127.6, 126.0, 108.4, 38.4, 21.3. GC−MS: 308 (M^+). HRMS (ESI(+)) calcd for $C_{18}H_{17}N_2OS$ (M + H): 309.1062. Found: 309.1055.

4-Methoxy-*N*-methyl-*N*-(4-(3,4,5-trimethoxyphenyl)thiazol-2-yl)benzamide (16g). ^{1}H NMR (300 MHz, $CDCl_3$): 7.58 (d, J = 8.4 Hz, 2H), 7.16 (s, 1H), 7.16 (s, 2H), 7.00 (d, J = 8.7 Hz, 2H), 3.95 (s, 6H), 3.89(s, 6H), 3.81 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 170.2, 161.8, 160.4, 153.4, 149.2, 138.0, 130.6, 130.0, 126.4, 113.9, 108.7, 103.4, 60.9, 56.2, 55.4, 38.8, 29.7. HRMS (ESI(+)) calcd for $C_{21}H_{23}N_2O_5S$ (M + H): 415.1328. Found: 415.1317.

3,4-Dimethoxy-*N*-methyl-*N*-(4-(3,4,5-trimethoxyphenyl)-thiazol-2-yl)benzamide (16h). ^{1}H NMR (300 MHz, $CDCl_3$): 7.23−7.17 (m, 3H), 7.16 (s, 2H), 6.95 (d, J = 8.4 Hz, 1H), 3.96 (s, 3H), 3.95(s, 6H), 3.93 (s, 3H), 3.88 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 170.1, 160.4, 153.4, 151.4, 149.3, 149.0, 138.1, 130.5, 126.5, 121.3, 111.3, 110.4, 108.7, 103.4, 61.0, 56.2, 56.0, 38.8, 29.7. GC−MS: 444 (M^+). HRMS (ESI(+)) calcd for $C_{22}H_{25}N_2O_6S$ (M + H): 445.1433. Found: 445.1426.

2,4-Dimethoxy-*N*-methyl-*N*-(4-(3,4,5-trimethoxyphenyl)-thiazol-2-yl)benzamide (16i). ^{1}H NMR (300 MHz, $CDCl_3$): 7.35 (d, J = 8.4 Hz, 1H), 7.16 (s, 3H), 6.60 (dd, J = 2.4 and 8.4 Hz, 1H), 6.52 (d, J = 2.1 Hz, 1H), 3.94(s, 6H), 3.88 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H), 3.64 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 168.9, 162.8, 159.9, 157.1, 153.4, 149.0, 138.0, 130.7, 129.9, 117.2, 108.5, 105.0, 103.4, 98.5, 60.9, 56.2, 55.6, 55.5, 36.7. HRMS (ESI(+)) calcd for $C_{22}H_{25}N_2O_6S$ (M + H): 445.1433. Found: 445.1424.

3,4,5-Trimethoxy-*N*-methyl-*N*-(4-(3,4,5-trimethoxyphenyl)-thiazol-2-yl)benzamide (16j). ^{1}H NMR (300 MHz, $CDCl_3$): 7.19 (s, 1H), 7.16 (s, 2H), 6.82 (s, 2H), 3.95 (s, 6H), 3.92 (s, 3H), 3.90 (s, 6H), 3.89(s, 3H), 3.80 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 170.1, 160.2, 153.5, 153.3, 149.4, 140.3, 138.2, 130.5, 129.5, 108.9, 105.1, 103.4, 61.01, 60.98, 56.3, 56.2, 38.7. HRMS (ESI(+)) calcd for $C_{23}H_{27}N_2O_7S$ (M + H): 475.1539. Found: 475.1530.

2,3-Dimethoxy-*N*-methyl-*N*-(4-(3,4,5-trimethoxyphenyl)-thiazol-2-yl)benzamide (16k). ^{1}H NMR (300 MHz, $CDCl_3$): 7.20−7.18 (m, 2H), 7.15 (s, 2H), 7.05 (dd, J = 1.5 and 8.1 Hz, 1H), 6.93 (dd, J = 1.5 and 7.8 Hz, 1H), 3.95(s, 6H), 3.93 (s, 3H), 3.88 (s, 3H), 3.86 (s, 3H), 3.63 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 168.4, 159.4, 153.4, 152.7, 149.1, 145.2, 138.0, 130.5, 130.0, 124.9, 119.0, 114.0, 108.7, 103.4, 61.7, 60.9, 56.2, 55.9, 36.7. HRMS (ESI(+)) calcd for $C_{22}H_{25}N_2O_6S$ (M + H): 445.1433. Found: 445.1428.

2-Methoxy-*N*-methyl-*N*-(4-(3,4,5-trimethoxyphenyl)thiazol-2-yl)benzamide (16l). ^{1}H NMR (300 MHz, $CDCl_3$): 7.47 (m, 1H), 7.38 (dd, J = 1.8 and 7.5 Hz, 1H), 7.18 (s, 1H), 7.16 (s, 2H), 7.08 (dt, J = 0.9 and 7.5 Hz, 1H), 7.00 (d, J = 8.4 Hz, 1H), 3.95 (s, 6H), 3.88 (s, 3H), 3.86 (s, 3H), 3.63 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 168.9, 155.5, 153.4, 149.1, 138.0, 131.7, 130.6, 128.3, 124.5, 121.1, 111.0, 108.7, 103.4, 60.9, 56.2, 55.6, 36.5. HRMS (ESI(+)) calcd for $C_{21}H_{23}N_2O_5S$ (M + H): 415.1328. Found: 415.1319.

3-Methoxy-*N*-methyl-*N*-(4-(3,4,5-trimethoxyphenyl)thiazol-2-yl)benzamide (16m). ^{1}H NMR (300 MHz, $CDCl_3$): 7.41 (m, 1H), 7.19 (s, 1H), 7.15 (s, 2H), 7.15−7.05 (m, 3H), 3.95 (s, 6H), 3.89 (s, 3H), 3.86 (s, 3H), 3.76 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 170.2, 159.7, 153.4, 149.3, 138.1, 135.7, 130.5, 129.8, 119.5, 116.8, 112.8, 108.9, 103.4, 60.9, 56.2, 55.4, 38.4. HRMS (ESI(+)) calcd for $C_{21}H_{23}N_2O_5S$ (M + H): 415.1328. Found: 415.1322.

Cell Culture. MDA-MB-231 cell line was a gift from Dr. KiTani Johnson, and HeLa cell line was a gift from Dr. Thomas Wiese, both of Xavier University of Louisiana College of Pharmacy. A549 cells were generously provided by Dr. Mandip Sachdeva of Florida A&M University College of Pharmacy. These cancer cell lines were routinely cultured in DMEM medium supplemented with 10% FBS, 4 mM glutamine, 1 mM sodium pyruvate, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin. Cultures were maintained in 5% carbon dioxide at a temperature of 37 °C.

In Vitro Migration Assays. Migration assays were performed following the manufacturer's instructions (BD Falcon). Briefly, MDA-MB-231, A549, or HeLa cells were seeded at a density of 2.5 × 10^4 in 500 μL of serum-free and phenol red-free media in the upper chamber of a 24-well Transwell system. DMEM supplemented with FBS (5%) was used as a chemoattractant in the lower wells. After 24 h, membranes were scrubbed, fixed with 10% phospho-buffered formalin, permeabilized with 100% ice-cold methanol, and stained with 0.1% crystal violet in 20% methanol. Membranes were removed and mounted on glass slides for visualization by light microscopy. For dose dependent migration assays, MDA-MB-231 cells were seeded in the Transwell migration chambers as described above and treated with 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M concentrations of each thiazole compound for measurement of resulting cell migration; 0.1% DMSO was used as vehicle control. Data are represented as % of migrated cells treated with vehicle per 100× field of view (100×).

Cell Survival/Growth Assay. For growth assay in the presence of 10^{-5} M individual thiazole compound, MDA-MB-231 cells were plated in six-well plates at a density of 50 000 per well in DMEM medium supplemented with 10% FBS. The cells were then cultured for 5 days, while equal treatment volumes of DMSO were used as vehicle control. Cell numbers were counted with a Coulter instrument (Beckman-Coulter). The ratio of thiazole compound treated cell numbers to vehicle treated cell numbers was defined as survival ratio. Experiments were conducted in triplicate and data represented as the mean ± SD.

Invasion Assays. Matrigel-coated invasion chambers (BD Biosciences, NJ) were used to determine the inhibitory effect of the thiazole derivatives on MDA-MB-231 cells. Briefly, cells in exponential phase of growth were serum-starved for 24 h prior to seeding, detached by brief trypsinization, and resuspended in medium containing DMSO or thiazole derivatives. The Matrigel invasion inserts were prepared following the manufacturer's instructions. MDA-MB-231 cells (2.5 × 10^4 cells/well) were seeded in the upper chamber, and medium supplemented with 10% FBS as chemoattractant containing DMSO or 10 μM thiazole derivatives was added to the bottom well. After incubation for 24 h, the noninvasive cells were removed from the upper surface of the membrane by "scrubbing", and the invasive cells on the under surface of the membrane were fixed with 4% formaldehyde for 10 min at room temperature and stained with 0.04% crystal violet, counted microscopically at 100× magnification. Five fields per membrane were randomly selected and counted in each group. The relative invasion of cells was calculated as the percentage invasion through the Matrigel membrane relative to that of DMSO treated cells.

Overexpression of Human Fascin 1. Construction of Fascin Vector. The fascin total RNA samples were extracted from HeLa cells using a PureLink total RNA purification system (Invitrogen) and quantitatively analyzed using a Nanodrop spectrophotometer (Thermo Scientific). The fascin cDNA was generated using a Superscript III one-step RT-PCR system (Invitrogen) with the following primers: fascin1-F (sense) 5′-GAA TTC ATG ACC GCC AAC GGC ACA GC-3′ and fascin1-R (antisense) 5′-AAG CTT CTA GTA CTC CCA GAG CGA GGC-3′. The RT-PCR reaction was carried out as follows: step 1, 45 °C for 30 min and 94 °C for 2 min; step 2, for 35 cycles 94 °C for 15 s, 51 °C for 30 s, and 72 °C for 1 min and 30 s; step 3, 72 °C for 5 min and hold at 4 °C. Human fascin cDNA (1.5 kilobases [kb]) samples were then cloned into a pcDNA 3.1 vector (Invitrogen).

Overexpression of Fascin in MDA-MB-231 Cell. For fascin overepression, the MDA-MB-231 cells were seeded at a density of 5 × 10^5 cells par well in six-well plates. At 70−80% confluence, the cells were transiently tranfected with 4 μg of the fascin-expressing plasmid pcDNA-fascin. The transfection was achieved by incubating the cells overnight at 37 °C in a CO_2 incubator with Lipofectamine 2000 reagent (Invitrogen). The next day, cell medium was replaced with 1000 μg/m G418 followed by medium replacement every 2−3 days for a total 10−12 days. Stable clones were isolated after selection with 500 μg/mL G418 for 2−3 weeks, and fascin expression levels were determined by Western blot.

Chick Embryo Chorioallantoic Membrane (CAM) Assay. Fertilized embryos (Charles River Laboratories, Charleston, SC) were incubated at 37.5 °C for 3 days, removed from their shell using a Dremel tool, and placed into a covered weighing boat for 7 further days of incubation. Solidified 30 μL onplants containing 2.1 mg/mL rat tail collagen (BD Biosciences, Bedford, MA) and 10 ng of bFGF

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and 30 ng of VEGF in the presence or absence of thiazole analogues **5o** and **5p** were placed on the CAM over two pieces of nylon mesh approximately 0.5 cm^2. Four collagen onplants were added per egg on at least three separate eggs. After 3 additional days of incubation, images were taken of each plug on surviving embryos using a mini-Vid camera (LW Scientific; Lawrenceville, GA) and quantified in a masked fashion on a scale from 0 to 3 with 0 representing no angiogenesis and 3 representing extreme angiogenesis. Data from one scorer (confirmed by a second masked scorer) are presented as the mean ± standard error of the mean. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test (GraphPad Prism 6, La Jolla, CA).

Cell Phalloidin Staining. HeLa cells were grown on glass coverslips until about 50% confluent. Vehicle or thiazole analogues treated HeLa cells were fixed for 15 min with 3.7% paraformaldehyde in PBS. Cells were washed with PBS and permeabilized in pemeabilization buffer (0.5% Triton X-100 (w/v) in PBS) for 10 min and washed again with PBS. The cells were then treated for 30 min with 200 μL of 100 nM Acti-stain 488 phalloidin in the dark. The coverslips were rinsed with PBS and inverted on a drop of antifade mounting media on a glass slide. Cells were observed under a fluorescence microscope equipped with a 480Ex/535Em filter set, a digital CCD camera, and 40× objective.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

DMSO, dimethyl sulfoxide; CAM, chorioallantoic membrane; FBS, fetal bovine serum; DMEM, Dulbecco's modified Eagle medium; FITC, fluorescein isothiocyanate; PBS, phosphate buffered saline; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; BV, bFGF + VEGF

REFERENCES

(1) Pelosi, G.; Pasini, F.; Fraggetta, F.; Pastorino, U.; Iannucci, A.; Maisonneuve, P.; Arrigoni, G.; De Manzoni, G.; Bresaola, E.; Viale, G. Independent value of fascin immunoreactivity for predicting lymph node metastases in typical and atypical pulmonary carcinoids. *Lung Cancer* **2003**, *42*, 203−213.

(2) Vignjevic, D.; Schoumacher, M.; Gavert, N.; Janssen, K.-P.; Jih, G.; Laé, M.; Louvard, D.; Ben-Ze'ev, A.; Robine, S. Fascin, a novel target of β-catenin-TCF signaling, is expressed at the invasive front of human colon cancer. *Cancer Res.* **2007**, *67*, 6844−6853.

(3) Darnel, A. D.; Behmoaram, E.; Vollmer, R. T.; Corcos, J.; Bijian, K.; Sircar, K.; Su, J.; Jiao, J.; Alaoui-Jamali, M. A.; Bismar, T. A. Fascin regulates prostate cancer cell invasion and is associated with metastasis and biochemical failure in prostate cancer. *Clin. Cancer Res.* **2009**, *15*, 1376−1383.

(4) Yoder, B. J.; Tso, E.; Skacel, M.; Pettay, J.; Tarr, S.; Budd, T.; Tubbs, R. R.; Adams, J. C.; Hicks, D. G. The expression of fascin, an actin-bundling motility protein, correlates with hormone receptor−negative breast cancer and a more aggressive clinical course. *Clin. Cancer Res.* **2005**, *11*, 186−192.

(5) Al-Alwan, M.; Olabi, S.; Ghebeh, H.; Barhoush, E.; Tulbah, A.; Al-Tweigeri, T.; Ajarim, D.; Adra, C. Fascin is a key regulator of breast cancer invasion that acts via the modification of metastasis-associated molecules. *PLoS One* **2011**, *6*, e27339.

(6) Hashimoto, Y.; Kim, D. J.; Adams, J. C. The roles of fascins in health and disease. *J. Pathol.* **2011**, *224*, 289−300.

(7) Jayo, A.; Parsons, M. Fascin: a key regulator of cytoskeletal dynamics. *Int. J. Biochem. Cell Biol.* **2010**, *42*, 1614−1617.

(8) Chen, L.; Yang, S.; Jakoncic, J.; Zhang, J. J.; Huang, X.-Y. Migrastatin analogues target fascin to block tumour metastasis. *Nature* **2010**, *464*, 1062−1066.

(9) Kraft, R.; Kahn, A.; Medina-Franco, J. L.; Orlowski, M. L.; Baynes, C.; López-Vallejo, F.; Barnard, K.; Maggiora, G. M.; Restifo, L. L. A cell-based fascin bioassay identifies compounds with potential anti-metastasis or cognition-enhancing functions. *Dis. Models Mech.* **2013**, *6*, 217−235.

(10) Zheng, S.; Zhong, Q.; Jiang, Q.; Mottamal, M.; Zhang, Q.; Zhu, N.; Burow, M. E.; Worthylake, R. A.; Wang, G. Discovery of a series of thiazole derivatives as novel inhibitors of metastatic cancer cell migration and invasion. *ACS Med. Chem. Lett.* **2013**, *4*, 191−196.

(11) Arjonen, A.; Kaukonen, R.; Ivaska, J. Filopodia and adhesion in cancer cell motility. *Cell Adhes. Migr.* **2011**, *5*, 421−423.

(12) Merajver, S. D.; Usmani, S. Z. Multifaceted role of Rho proteins in angiogenesis. *J. Mammary Gland Biol. Neoplasia* **2005**, *10*, 291−298.

(13) Lipscomb, E. A.; Mercurio, A. M. Mobilization and activation of a signaling competent α6β4integrin underlies its contribution to carcinoma progression. *Cancer Metastasis Rev.* **2005**, *24*, 413−423.

(14) Zhou, W.; Wang, G.; Guo, S. Regulation of angiogenesis via Notch signaling in breast cancer and cancer stem cells. *Biochim. Biophys. Acta* **2013**, *1836*, 304−320.

(15) Ma, Y.; Reynolds, L. E.; Li, A.; Stevenson, R. P.; Hodivala-Dilke, K. M.; Yamashiro, S.; Machesky, L. M. Fascin 1 is dispensable for developmental and tumour angiogenesis. *Biol. Open* **2013**, *2*, 1187−1191.

(16) Shonukan, T.; Bagayogo, I.; McCrea, P.; Chao, M.; Hempstead, B. Neurotrophin-induced melanoma cell migration is mediated through the actin-bundling protein fascin. *Oncogene* **2003**, *22*, 3616−3623.

(17) Hwang, J. H.; Smith, C. A.; Salhia, B.; Rutka, J. T. The role of fascin in the migration and invasiveness of malignant glioma cells. *Neoplasia* **2008**, *10*, 149−159.

(18) Hayashi, Y.; Toda, K.; Saibara, T.; Okamoto, S.; Osanai, M.; Enzan, H.; Lee, G.-H. Expression of fascin-1, an actin-bundling protein, in migrating hepatoblasts during rat liver development. *Cell Tissue Res.* **2008**, *334*, 219−226.

(19) Alam, H.; Bhate, A. V.; Gangadaran, P.; Sawant, S. S.; Salot, S.; Sehgal, L.; Dange, P. P.; Chaukar, D. A.; D'cruz, A. K.; Kannanl, S. Fascin overexpression promotes neoplastic progression in oral squamous cell carcinoma. *BMC Cancer* **2012**, *12*, 32.

(20) Bi, J.; Zhu, Y.; Chen, X.; Yu, M.; Zhang, Y.; Li, B.; Sun, J.; Shen, H.; Kong, C. The role of fascin in migration and invasion of urothelial carcinoma of the bladder. *Urol. Int.* **2013**, *91*, 227−235.

(21) Jansen, S.; Collins, A.; Yang, C.; Rebowski, G.; Svitkina, T.; Dominguez, R. Mechanism of actin filament bundling by fascin. *J. Biol. Chem.* **2011**, *286*, 30087−30096.

(22) Flocco, M. M.; Mowbray, S. L. Planar stacking interactions of arginine and aromatic side-chains in proteins. *J. Mol. Biol.* **1994**, *235*, 709−717.

(23) Burley, S. K.; Petsko, G. A. Amino-aromatic interactions in proteins. *FEBS Lett.* **1986**, *203*, 139−143.