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Novel Flavonoids with Antiproliferative Activities against Breast Cancer Cells

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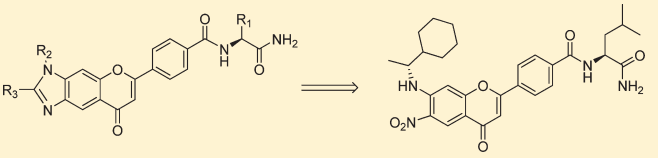
Supporting Information

ABSTRACT: A series of flavone analogues were synthesized and evaluated for their antiproliferation activity against breast cancer cells. The IC_{50} of compound **10** and **24** were determined to be at 5 μM. These compounds were used as baits to screen breast cancer cDNA expression phage display proteome library. DNA sequencing of the binding phages suggests that eEF1A1 is a target protein for **10** and **24**. Further optimization of these compounds led to the discovery of **39** with higher cytotoxic potency (IC_{50} = 1 μM) and binding to eEF1A2. Biological and biochemical data suggest that eEF1A2 might be a therapeutic target and that **39** is an excellent lead compound for further development.



INTRODUCTION

Breast cancer is the most common malignant tumor and the second most lethal cancer among women.[1] Women have a 1 in 8 lifetime risk of developing breast cancer and 1 in 35 risk of breast cancer causing death in the US. Many breast tumors express higher levels of estrogen receptors than normal breast tissues.[2] Estrogen receptor (ER)/progesterone receptor (PR) positive breast cancers often respond to hormonal therapy. Conventional chemotherapeutic drugs are used for the treatment of ER/PR negative breast cancer. Herception is useful for the treatment of Her-2/neu positive breast cancer. Despite of combination hormonal therapy, chemotherapy, and targeted therapy,[3,4] most metastatic breast cancer eventually becomes refractory to such treatments. There is an urgent need to explore new drug candidates with novel mechanisms of action.

Many current anticancer drugs are either natural products or their derivatives.[5,6] Natural products have also served as useful scaffolds for chemical diversification in the context of drug discovery.[7] Flavonoids are the most explored class of natural products because they are widely distributed among various plants and common components of the human diet.[8] They probably play an important role in cancer prevention by interfering with cell proliferation, survival, cell signaling, and regulating the immune system.[9−11] Additional studies have also indicated that some flavonoids exhibit aromatase inhibitory activity[12,13] and tyrosinase inhibitory activity.[14] We have previously developed several novel flavonoid scaffolds with three diversification points.[15] Here, based on the flavone template, a series of flavonoid derivatives were synthesized on solid phase and evaluated for their antiproliferative activities in breast cancer cell line MDA-MB-231 and MCF-7. Two compounds were found to exhibit potent cytotoxic effect in both ER negative and ER positive breast cancer cell lines. The molecular targets of these lead compounds were identified by using them as bait to screen cDNA expression phage display proteome library. Further optimization of the lead compounds resulted in the development of a relatively potent antiproliferative compound that selectively bind to eukaryotic elongation factor 2A (eEF1A2).

RESULTS AND DISCUSSION

A number of flavone scaffolds were developed with three functional groups (carboxy, fluoro, and nitro). Heterocycles or other pharmacophores can be readily introduced to the flavonone scaffolds. In our previous study, we reported the use of solid phase method to prepare several flavone derivatives (Figure 1, **1**−**6**).[15] As part of ongoing search for anticancer agents, we used MTT assay to evaluate their antiproliferactive activity in breast cancer cell line MDA-MB-231(ER−) and MCF-7(ER+). A compound was considered active if the IC_{50} was $\leq$10 μM during the initially screening. Only **5** showed cytotoxic activity in both breast cancer cell lines (Figure 2).

Design, Synthesis, and Biological Evaluation of Flavone Analogues 7−32. To optimize **5**, we designed and synthesized 26 flavone analogues (**7**−**32**) according to our published method (Scheme 1).[15] Various Fmoc amino acids, such as polar, acidic, and hydrophobic amino acids (R1 group in Table 1) were first introduced to Rink resin. After Fmoc deprotection, 4-(7-fluoro-6-nitro-4-oxo-4*H*-chromen-2-yl)-benzoic acid as a functionalized flavone scaffold was attached to the resin. The fluorine in the resin-bound flavone scaffold was subsequently replaced by a number of structurally diverse primary amines, such as aliphatic

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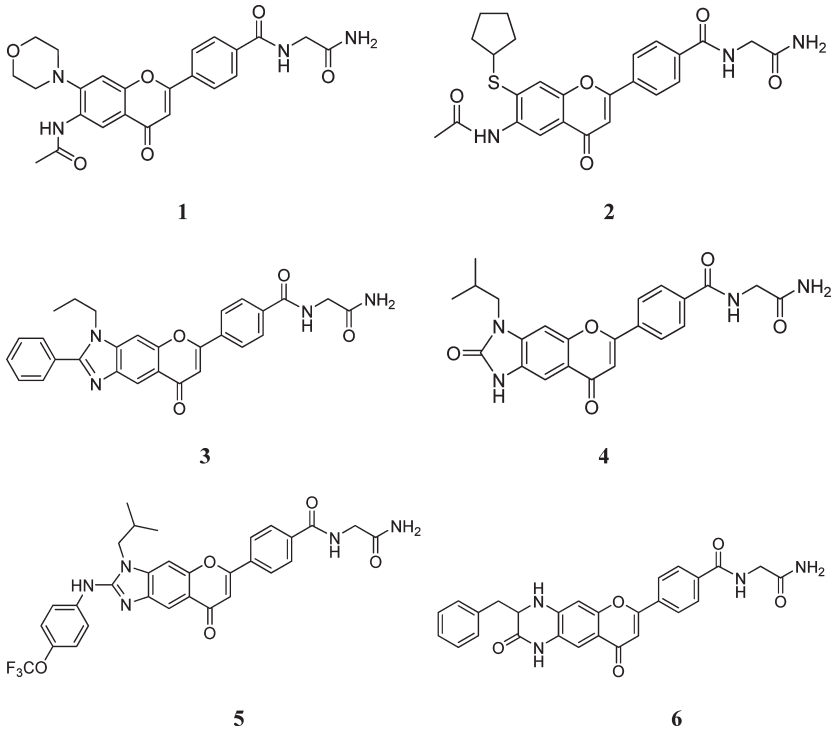


Figure 1. Structure of flavone model compounds **1**−**6**.

and aromatic amines (R2 group in Table 1). Reduction of the nitro group then afforded the *o*-phenylenediamine intermediates. The resin bound *o*-phenylenediamine flavone was treated with different isothiocyanates (R3 group in Table 1) in the presence of 1, 3-diisopropylcarbodiimide (DIC) to provide **7**−**17** and **18**−**20** (without introducing R1 group). To increase structural diversities, the resin bound *o*-phenylenediamine flavone was reacted with various aliphatic aldehydes and substituted aromatic aldehydes (R3 group in Table 1) to yield **21**−**28** and **29**−**32** (without introducing R1 group). Compounds **7**−**32** were tested for in vitro anticancer activity against two breast cancer cell lines MDA-MB-231(ER−) and MCF-7(ER+). Cells were treated with 10 μM concentration of each analogue for three days. **10** and **24** were among the most active compounds (Figure 3). Further MTT assays were carried out to test **10** and **24** in varying concentration, ranging from 0.08 μM to 50 μM (Supporting Information, Figure 1). Both **10** and **24** had high efficacy (IC_{50} = 5.0 μM) in MDA-MB-231cells. Additionally, **10** (IC_{50} = 5.0 μM) was higher than **24** (IC_{50} = 8.0 μM) in MCF-7 cells.

Identification of eEF1A1 as a Binding Protein for Compound 10 and 24. Phage display technology enables one to express and display recombinant peptides or proteins on the phage surface. Peptide and protein epitope for antibodies,[16] EGFR,[17,18] and binding proteins for bioactive small molecules[19−21] have been successfully identified by such technology. To identify the target proteins for **10** and **24**, these chemical compounds were synthesized on TentaGel beads (polystyrene with grafted polyoxyethylene) (Supporting Information Scheme 1) and used as bait to screen cDNA expression phage display proteome library constructed from human breast tumor cDNA. TentaGel resin was used because of its favorable swelling characteristics both in organic and aqueous media, its uniform size, and its nonstick property.[22] After four rounds of biopanning, 12 phage clones from each compound were randomly selected, amplified and sequenced. The DNA sequence of

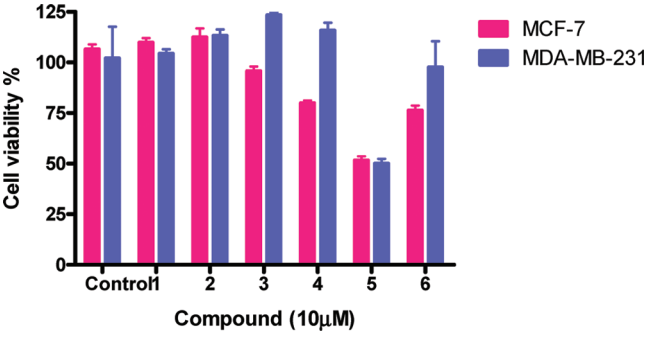
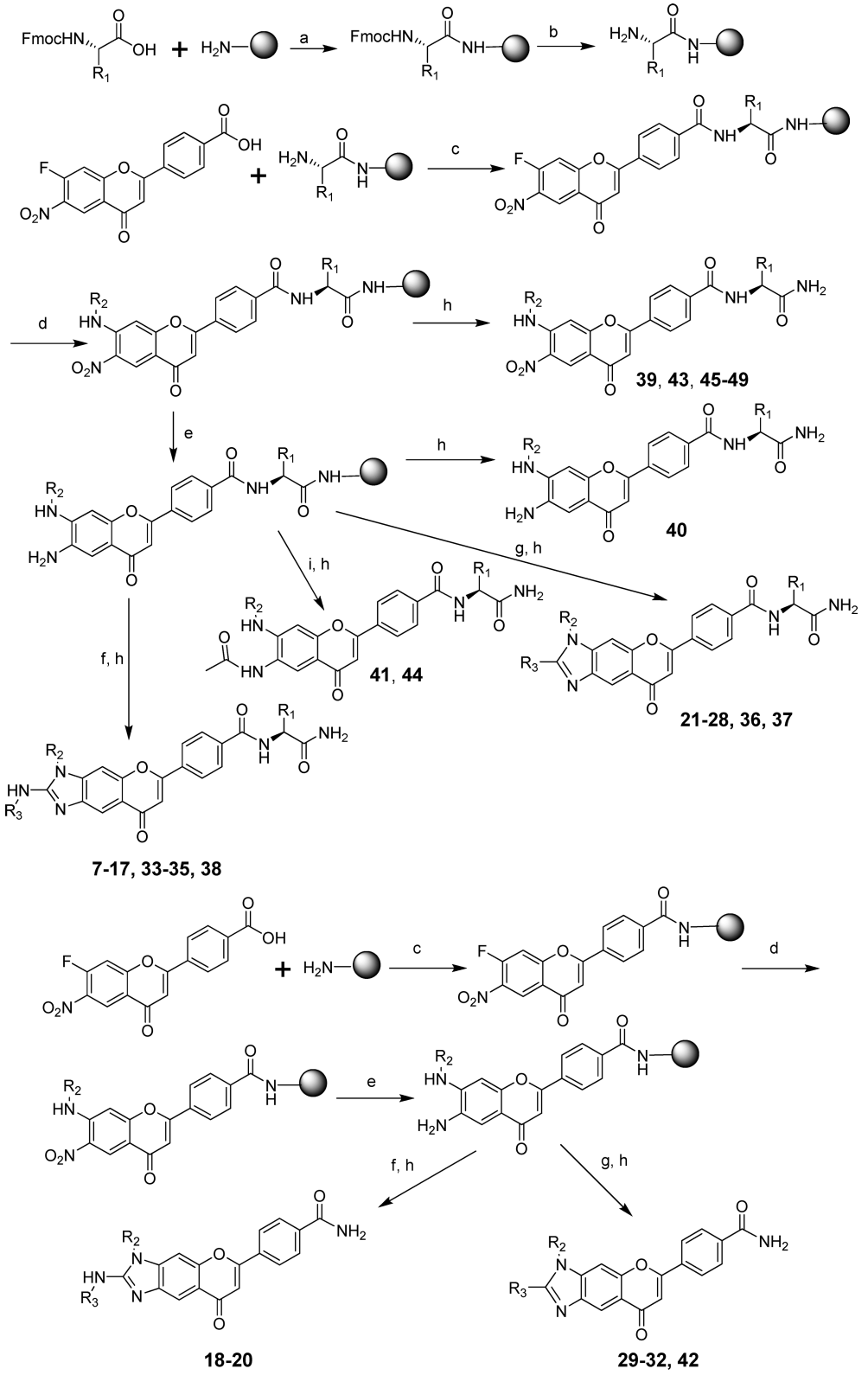


Figure 2. Cytotoxicity of flavone analogues **1**−**6**.

the bound phage clones were analyzed via NCBI blast. In the panning experiment with **10**, 4 out of 12 phage clones encoded DNA matched with known human proteins, two clones encoded eukaryotic elongation factor 1A1 (eEF1A1), one clone encoded apolipoprotein 3 (APOL3), and one encoded tropomysin 2 (TMP2) (sequence shown in Supporting Information). Eukaryotic elongation factor 1A 1 (eEF1A1) was also recovered in the panning experiment with **24**. To verify eEF1A1 as the protein target for **10** and **24**, biotinylated **10** and **24** were synthesized (Supporting Information Scheme 2), and incubated with MDA-MB-231 cell lysate. Neutravidin beads were then used to pull down proteins bound to **10** and **24**. Subsequent Western blot analysis demonstrated that eEF1A did indeed bind to **10** and **24**. (Figure 4)

Structure−Activity Relationships (SAR) and Surface Plasmon Resonance (SPR) Analysis. To discover more active flavone derivatives and to establish preliminary SAR, **10** and **24** were further optimized. Exchange of R1, R2, and R3 groups of **10** and **24**, afforded **33**−**37** (Figure 5). Systematic structural simplification of **10** and **24** by removal of R1 and R3 group,

Scheme 1[a]



[a] Reagents and conditions: (a) Amino Acid (3 equiv), HOBt/DIC (3 equiv), DMF, r.t., 2 h; (b) 20% piperidine/DMF, 15 min, twice; (c) 4-(7-fluoro-6-nitro-4-oxo-4*H*-chromen-2-yl)-benzoic acid (2 equiv), HOBt/DIC (3 equiv), DMF, r.t., 3 h; (d) 1 M amine, 25% DIPEA/DMF, r.t., overnight; (e) 2 M $SnCl_2$/DMF, r.t., overnight; (f) 1 M isothiocyanate, 1 M DIC, DMF, r.t., overnight; (g) aldehyde (10 equiv), 5% HOAc/DMF, r.t. overnight; (h) TFA/TIS/H_2O (95%:2.5%:2.5%), r.t., 3 h. (i) 10% Acetic anhydride/10% pyridine/DMF, 30 min.

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Table 1. Building Blocks of Compounds 7−32

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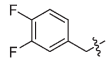
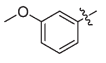
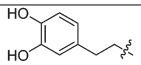
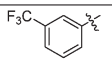
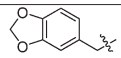
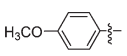
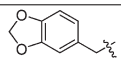
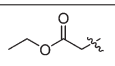
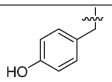
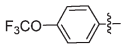
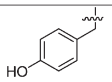
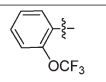
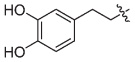
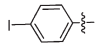
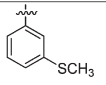
7	H		
8	H		
9			
10			
11			
12			
13			
14			
15			
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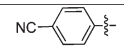
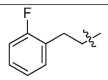
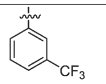
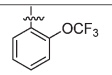
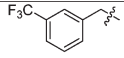
17			
18			
19			

Compounds	R1 (Amino Acid)	R2 (Amine)	R3 (Isothiocyanate or Aldehyde)
20			
21			
22			
23			
24			
25	H		
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27			
28			
29			
30			
31			
32			

Figure 3. Cytotoxicity of flavone analogues 7−32.

reduction of the nitro group, and subsequently capping with acetyl group, yielded **38**−**44** (Figure 5). **33**−**44** were tested for antiproliferation against MDA-MB-231 cells(ER−) (Figure 5). In the case of modification of **10**, replacement of Leu with Phe (**33**), (*R*)-(−)-1-cyclohexylethylamine with benzylamine (**34**), and both Leu with Phe and (*R*)-(−)-1-Cyclohexylethylamine with benzylamine (**35**), resulted in a significant loss of anticancer activity. In contrast, optimization of **24** using the same strategy (**36** and **37**) showed the equal potency as parent compound **24**. Compared to **10** (IC_{50} = 5 μM), **39** in absence of an R3 group showed a 5-fold decrease in IC_{50}. However, reduction of the nitro group to an amino group (**40**) and subsequently capping amino

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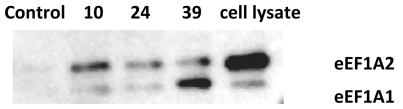

Figure 4. Western blot analysis. Biotinylated **10**, **24**, and **39** were incubated with MDA-MB-231cell lysate and pulled down using neutravidin beads. Linker-Lys-biotin was used as negative control. The membrane was blotted with anti-eEF1A antibody.

group with acetyl group (**41**) resulted in a marked increase in IC_{50}, suggesting that the nitro group may be critical for MDA-MB-231 cell killing. Additionally, removal of the R3 group in **24** (**41**), reduction of the nitro group to an amino group (**42**), and capping the amino group with acetyl group (**43**), all resulted in a significant loss of anticancer activity. Consequently, **39**, (simplified from **10**) with deletion of the R3 group and possession of two chiral centers in Leu(R1) and (R)-(−)-1-cyclohexylethylamine(R2), was found to have higher potency against MDA-MB-231 cells (IC_{50} = 1 μM). Encouraged by this result, we further synthesized the optical isomers of **39** (**45**−**47**), and derivatives of **39** by replacing Leu with Phe (**48**) and D-Phe (**49**) (Figure 5). MTT assay was carried on **45**−**49** using MDA-MB-231 cells (Figure 5). The result, however, indicated that further modifications of **39** led to a reduction in the antiproliferative activity.

In comparison to **10** and **24**, **39** lacked an imidazole ring. To explore whether eEF1A1 was also a binding protein to **39**, the same pull down approach described above was performed on biotinylated **39**. Interestingly, Western blot analysis showed a slight difference between **39**, **10**, and **24**, (Figure 4, Supporting Information, Figure 2) which may be due to the two isomers present in eukaryotic elongation factor (eEF1A1 and eEF1A2), sharing 92% amino acid identity.[23] Therefore, we hypothesize that **39** may selectively bind to one of eukaryotic elongation factors. To test this assumption, we attempted to use surface plasmon resonance (SPR) to evaluate their binding affinity between eukaryotic elongation factor and **10**, **24**, or **39**. Biotionylated **10**, **24**, and **39** were immobilized on the streptavidin (SA) sensor chip *via* biotin. Different concentrations of eEF1A1 and eEF1A2 were each infused over this sensor chip (Supporting Information, Figure 3). It is clear that both eEF1A1 and eEF1A2 did bind to these flavonoids. Unfortunately because of the lack of sufficient amount of target proteins to cover the entire concentration range, the apparent K_Ds obtained from the SPR studies were unreliable. Furthermore, **10**, **24**, and **39** were subjected to MTT assay with immortalized normal breast cells MCF10A. The result showed that **10** and **24** killed normal MCF10A cell in a similar manner as cancerous MCF-7 and MDA-MB-213 cells; whereas, **39** only killed about 65% of normal MCF10A cells at 50 μM. The preferential anticancer effects of **39** could be explained by its selective binds to eEF1A2, which was overexpressed in breast tumor but not normal breast tissue. eEF1A2 might be the therapeutic target for the anticancer activity of **39**.

Validation of Selective Binding of 39 to eEF1A2. eEF1A proteins are one of the key enzymes in protein synthesis, which carries aminoacyl-tRNA into ribosome and catalyzes the first step of peptide elongation.[24] Besides protein synthesis, eEF1A is also involved in several cell regulatory processes, including cytoskeletal remodeling, apoptosis, and ubiquitin mediated protein degradation.[24] Recently, eEF1A2 (eukaryotic elongation factor 1A2), has been reported to be a putative oncoprotein.[25] eEF1A2 has been shown to be overexpressed in 30−60% of ovarian, lung

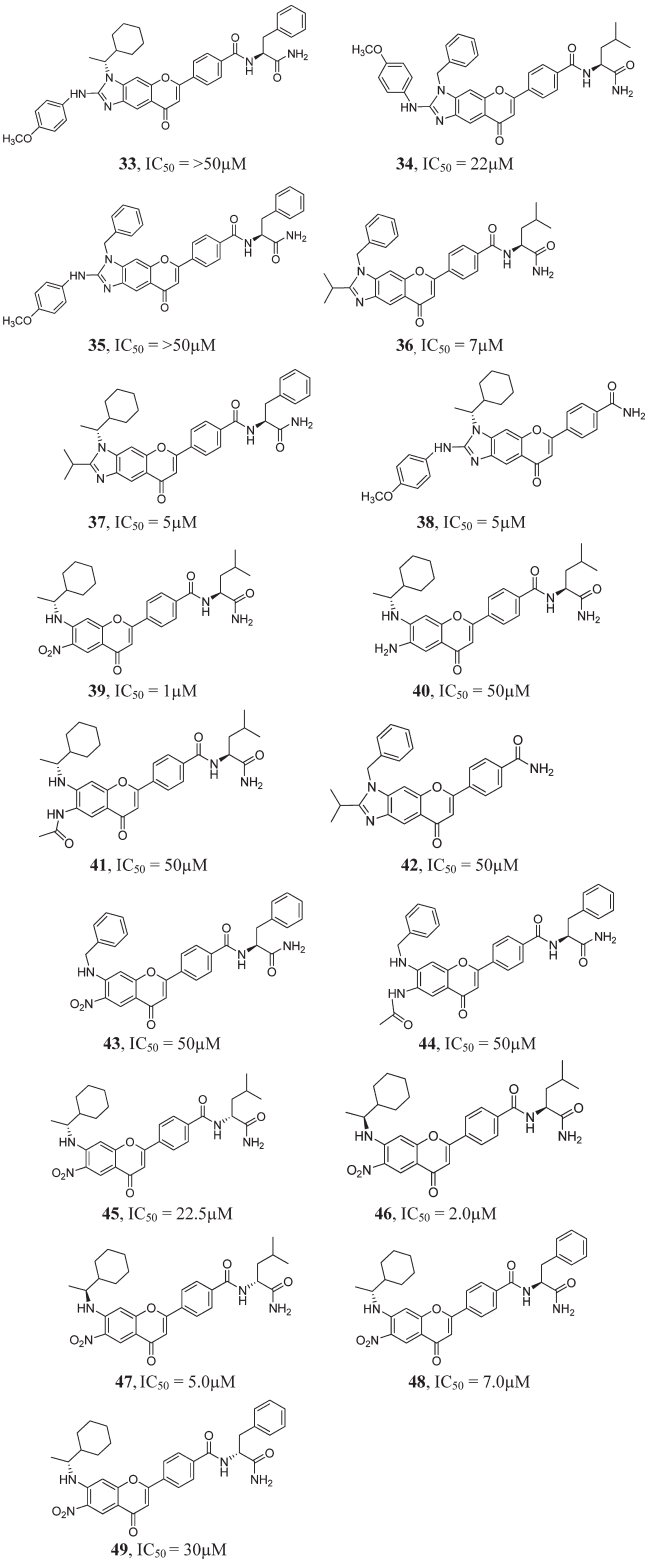

Figure 5. Structure and cytotoxicity of **10** and **24** derivatives.

and breast cancers.[25−28] Expression of eEF1A2 in fibroblast cells show tumorigenicity and increase the growth of xenograft in nude mice.[25]

To investigate the effect of **39** binding to eEF1A1 and eEF1A2, we first tested whether it could inhibit protein translation.

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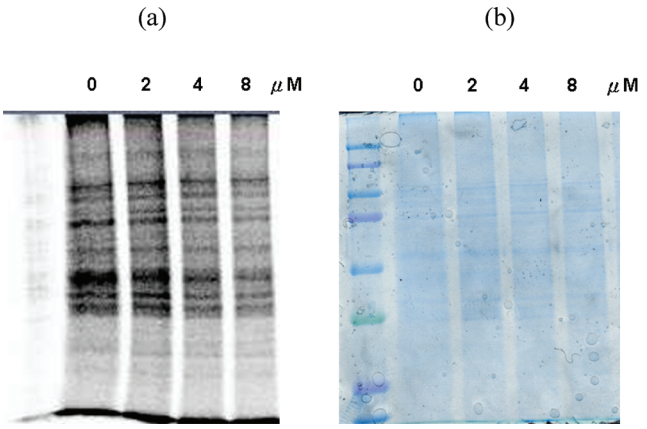

Figure 6. Cells were treated with DMSO, 2, 4, and 8 μM of **39** for 8 h followed by depletion of methionine for 1 h. Before lysing the cells, ^{35}S-labeled methionine were incubated with the cells for 3 h. Cell lysate were resolved by SDS-PAGE. (a) ^{35}S labeled proteins were detected by phosphoimager. A decrease of ^{35}S-methionine incorporation into protein was shown. (b) Commassie blue staining of the same gel was shown as loading control.

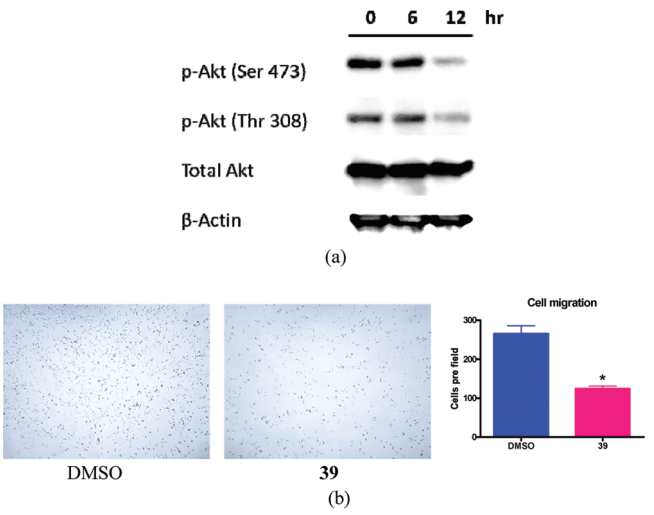

Figure 7. (a) MDA-MB-231 cells were treated with DMSO and 2 μM of **39** for 6 and 12 h, respectively. Total protein was extracted, and subjected to Western blot analysis using antiphospho-Akt (Ser473 and Thr308), and anti-β actin. (b) MDA-MB-231 Cells were treated with DMSO or 2 μM of **39** for 4 h, and then seeded into a transwell. After 48 h, cell migration to the bottom well was monitored, and cells were counted and expressed as numbers of cells per field. The experiments were carried out at least twice with triple counts. * indicate $p < 0.05$.

^{35}S-methionine was used to monitor protein translation in cell culture. Cells treated with **39** showed decreased ^{35}S-methionine incorporation into cellular proteins in a dose-dependent manner, indicating that **39** could indeed inhibit protein biosynthesis (Figure 6). Recently, Amiri A. et al. reported that overexpression of eEF1A2 could lead to Akt activation in phosphoinositide 3-kinase (PI3K)-dependent manner, enhance filopodia formation and increase cell invasion, suggesting that eEF1A2 does play a critical role in tumor development and metastasis.[29] To determine whether **39** could inhibit both eEF1A2 function and Akt activation, MDA-MB-231 cells were treated with **39**. Western blot analysis showed that **39** was able to suppress phosphorylation of serine 473 and threonine 308 in Akt (Figure 7a). Moreover, eEF1A2 is known to involve in actin remodeling and could increase cell migration.[29,30] To test whether **39** inhibits cell migration, cells were treated with **39** for 4 h, and then subjected to a transwell migration assay. **39** was found to show nearly 50% inhibition in cell migration (Figure 7b). Taken together, the inhibitory effects of **39** on cell proliferation, protein translation, Akt phosphorylation and cell migration of MDA-MB-231 breast cancer cells, can be explained by its inhibitory effect on eEF1A2. However, it would be very difficult to prove that the cellular and biochemical effects caused by **39** are mediated entirely through eEF1A2. More studies will be needed to fully elucidate the mechanisms of action of this anticancer flavonoid compound.

CONCLUSION

A series of flavone analogues were synthesized and evaluated against breast cancer cells. The primary compounds (**10** and **24**) had an imidazole ring attached on the flavone and were found to exhibit modest activity against breast cancer cells (MDA-MB-231(ER−) and MCF-7 ER+)). Furthermore, eEF1A1 was demonstrated to be a target protein of **10** and **24** through screening T7 phage display (human breast tumor cDNA expression) proteome library. Importantly, subsequent SAR study indicated that simplification of **10** resulted in **39** with more potent anticancer activity, suggesting that the imidazole ring is not needed for the desired activity; whereas, the original nitro group is necessary for anticancer activity. Additionally, **39** was found to selectively bind to eEF1A2. Overall, our results establish that **39** is an excellent drug lead with novel mechanisms against breast cancer.

EXPERIMENTAL SECTION

Materials and Methods. TentaGel S NH_2 resin (90 μm, 0.26 mmol/g) was purchased from Rapp Polymere GmbH (Tübingen, Germany). Rink amide MBHA resin (0.5 mmol/g), amino acid derivatives, HOBt, and DIC were purchased from GL Biochem (Shanghai, China). 4-(7-Fluoro-6-nitro-4-oxo-4H-chromen-2-yl)-benzoic acid was synthesized in our laboratory.[15] All solvents and other chemical reagents were purchased from Aldrich (Milwaukee, WI) and were analytical grade. Analytical HPLC analyses (Vydac column; 4.6 mm × 250 mm; 5 μm; 300 Å; C18; 1.0 mL/min; 25-min gradient from 100% aqueous H_2O (0.1% TFA) to 100% CH_3CN (0.1% TFA); 214, 220, 254 and 280 nm) were performed on a Beckman System Gold HPLC system (Fullerton, CA), or on Waters 2996 photodiode array Detector, a Waters 2525 Binary Gradient Module, a Waters 2767 Sample Manager equipped with a 4.6 × 150 mm Waters Xterra MS C18 5.0 μm column employing a 20 min gradient from 100% aqueous H_2O (0.1% TFA) to 100% CH_3CN (0.1% TFA) at a flow rate of 1.0 mL/min. NMR was recorded on a Bruker DRX spectrometer (Billerica, MA) in DMSO-d_6 at 25 °C (500 MHz for 1H NMR, 125 MHz for 13C NMR spectra). The purity of all the compounds was assessed by RP-HPLC under 254 nm. All final compounds were confirmed to be ≥95% purity by analysis of their peak area. ESIMS was performed with Finnigan LCQ. The dissociation constants were obtained using Biacore 3000 system (Biacore Inc. Piscataway, NJ). MDA-MB-231 cells and MCF-7 cells were obtained from American Type Culture Collection (Manassas,VA). T7 phage expressing human breast tumor cDNA, T7 up primer, T7 down primer, and NovaTaq Hot Start DNA Polymerase were purchased from Novagen (Madison, WI). ExoSAP-IT PCR cleanup kit was purchased from GE Healthcare Bio-Sciences Corp.(Piscataway, NJ)

Anti-eEF1A antibody was purchased from Cell signaling Technology, Inc.(Danvers, MA). Tagged eEF1A1 and eEF1A2 proteins were purchased for, Abcam, Inc. (Cambridge, MA).

Preparation of Compounds 7−32. *Synthesis of Compounds* ***7−17***. For each compound, 200 mg of Rink-MBHA resin was swollen in DMF for 2 h. The resin was treated with 20% piperidine (v/v) in DMF (2 × 15 min). The resin was then thoroughly washed with DMF, MeOH, DCM, and DMF. A mixture of Fmoc-AA-OH (3equiv), HOBt (3equiv), and DIC (3equiv) was added to the resin, the final mixture was shaken until a negative Kaiser Test was obtained. The resin was washed with DMF, MeOH, DCM and DMF, followed by Fmoc deprotection. A solution of 4-(7-fluoro-6-nitro-4-oxo-4*H*-chromen-2-yl)-benzoic acid (2equiv), HOBt (3equiv), and DIC (3equiv) was added to the resin. The final mixture was shaken until the Kaiser Test was negative. The resin was washed with DMF, MeOH, DCM, and DMF, and then added a solution of various amines (1M) in 25% DIEPA/DMF. The mixture was shaken overnight, and the supernatant was drained off. The resin was washed with DMF, MeOH, and DMF. 2 M $SnCl_2$/DMF (3 mL) was added to the resin. The mixture was shaken overnight. After washing with DMF, MeOH, and DMF, the resin was added a solution of various Isothiocyanates (1 M) and DIC (1 M) in DMF. The mixture was shaken overnight, and the supernatant was removed. The resin was washed with DMF, MeOH, and DCM. After TFA cleavage, the crude product was purified by HPLC.

Compound 7, yellow solid, Purity, 96%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.77(t, J = 5 Hz, 1H), 8.19(d, J = 5 Hz, 2H), 8.05(d, J = 5 Hz, 2H), 7.94(brs, 1H), 7.74(s, 1H), 7.58(brs, 1H), 7.38−7.44 (m, 3H), 7.33(brs, 1H), 7.29(t, J = 10 Hz, 1H), 7.03(s, 2H), 6.94(brs, 1H), 6.67(dd, J = 10, 5 Hz, 1H), 5.64(s, 2H), 3.87(d, J = 5 Hz, 2H), 3.80(s, 3H). ESI-MS m/z: 610.4 $[M + H]^+$.

Compound 8, yellow solid; Purity, 98%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.79(t, J = 5 Hz, 1H), 8.23(d, J = 10 Hz, 2H), 8.08(d, J = 10 Hz, 2H), 7.92(brs, 1H), 7.78(dd, J = 10, 5 Hz, 1H), 7.69(brs, 1H), 7.61(t, J = 10 Hz, 1H), 7.36−7.40(m, 3H), 7.14(s, 1H), 7.03(s, 1H), 6.97(brs, 1H), 6.70(s, 1H), 6.62(d, J = 5 Hz, 1H), 6.55(d, J = 5 Hz, 1H), 4.55(brs, 2H), 3.87(t, J = 5 Hz, 2H), 2.91(t, J = 5 Hz, 2H). ESI-MS m/z: 658.5 $[M + H]^+$.

Compound 9, yellow solid, Purity, 97%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70(d, J = 10 Hz, 1H), 8.20(d, J = 10 Hz, 2H), 8.05(d, J = 10 Hz, 2H), 7.79(s, 1H), 7.73(brs, 1H), 7.32(brs, 1H), 7.06(s, 2H), 6.90(brs, 1H), 6.88(s, 1H), 6.79(d, J = 5 Hz, 1H), 5.98(s, 2H), 5.34(brs, 2H), 4.79(m, 2H), 4.79(m, 1H), 3.45(q, J = 5 Hz, 2H), 2.64−2.85(m, 2H), 1.68(m, J = 5 Hz, 2H), 0.95(t, J = 5 Hz, 3H). ESI-MS m/z: 612.4 $[M + H]^+$.

Compound 10, yellow solid, Purity, 99%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.51(d, J = 10 Hz, 1H), 8.29(d, J = 10 Hz, 2H), 8.09(d, J = 10 Hz, 3H), 7.84(s, 1H), 7.61(d, J = 10 Hz, 2H), 7.36(brs, 1H), 7.09(s, 1H), 7.05(d, J = 10 Hz, 2H), 6.92(brs, 1H), 4.51(m, 2H), 3.81(s, 3H), 2.30(m, 1H), 2.03(m, 1H), 1.83(m, 1H),1.75(m, 1H), 1.70(d, J = 5 Hz, 3H), 1.59−1.62(m, 3H), 1.35(m, 1H), 1.07−1.21(m, 4H), 0.95(d, J = 5 Hz, 3H), 0.93(d, J = 5 Hz, 3H). ESI-MS m/z: 650.5 $[M + H]^+$.

Compound 11, yellow solid, Purity, 96%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.49(d, J = 10 Hz, 1H), 8.18(d, J = 5 Hz, 2H), 8.08(d, J = 5 Hz, 2H), 7.81(s, 1H), 7.76(s, 1H), 7.35(brs, 1H), 7.04(s, 1H), 6.97(s, 1H), 6.90(d, J = 10 Hz, 1H), 6.89(s, 2H), 5.98(s, 2H), 5.40(s, 2H), 4.50(m, 1H), 4.31(brs, 1H), 4.19(q, J = 5 Hz, 2H), 1.69−1.72(m, 2H), 1.59(m, 1H), 1.23(t, J = 5 Hz, 3H), 0.93(d, J = 5 Hz, 3H), 0.91(d, J = 5 Hz, 3H). ESI-MS m/z: 654.4 $[M + H]^+$.

Compound 12, yellow solid, Purity, 98%; ^{1}H NMR (500 MHz, DMSO-d_6): δ8.54(d, J = 10 Hz, 1H), 8.27(d, J = 5 Hz, 2H), 8.03(s, 1H), 7.99(d, J = 5 Hz, 2H), 7.91(brs, 2H), 7.45(brs, 1H), 7.40(d, J = 10 Hz, 2H), 7.14(s, 1H), 7.13(brs, 2H), 7.06(s, 1H), 7.00(brs, 1H), 6.65(d, J = 10 Hz, 2H), 4.80(m, 1H), 4.60(m, 1H), 3.05(m, 1H), 2.91(m, 1H), 2.21(m, 1H), 2.04(m, 1H), 1.69(d, J = 10 Hz, 3H), 0.83(t, J = 5 Hz, 3H). ESI-MS m/z: 700.5 $[M + H]^+$.

Compound 13, yellow solid, Purity, 97%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.53(d, J = 10 Hz, 1H), 8.24(d, J = 5 Hz, 2H), 7.98(d, J = 5 Hz, 2H), 7.86(brs, 1H), 7.59(brs, 1H), 7.41−7.45(m, 4H), 7.21(brs, 1H), 7.13(d, J = 10 Hz, 2H), 7.00(s, 1H), 6.97(brs, 1H), 6.65(d, J = 10 Hz, 2H), 4.92(m, 1H), 4.61(m, 1H), 3.05(m, 1H), 2.91(m, 1H), 2.25(m, 1H), 1.72(m, 1H), 1.60(d, J = 5 Hz, 3H), 1.48(m, 1H), 0.98(d, J = 5 Hz, 3H), 0.87(d, J = 5 Hz, 3H). ESI-MS m/z: 728.5 $[M + H]^+$.

Compound 14, yellow solid, Purity, 96%; ^{1}H NMR (500 MHz, DMSO-d_6) δ8.52(d, J = 5 Hz, 1H), 8.23(d, J = 10 Hz, 2H), 8.10(d, J = 10 Hz, 2H), 7.87(s, 1H), 7.73(s, 1H), 7.70(d, J = 5 Hz, 2H), 7.64(brs, 2H), 7.37(s, 1H), 7.05(s, 1H), 7.00(brs, 1H), 6.68(d, J = 5 Hz 1H), 6.61(d, J = 10 Hz, 1H), 6.53(dd, J = 10, 5 Hz, 1H), 4.52(t, J = 5 Hz, 2H), 4.44(m, 1H), 2.90(t, J = 5 Hz, 2H), 2.35(t, J = 10 Hz, 2H), 2.10(m, 1H), 1.97(m, 1H). ESI-MS m/z: 788.4 $[M + H]^+$.

Compound 15, yellow solid, Purity, 98%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.53(d, J = 10 Hz, 1H), 8.29(d, J = 5 Hz, 2H), 8.10(d, J = 5 Hz, 2H), 8.06(s, 1H), 7.90(s, 1H), 7.70(s, 1H), 7.53(brs, 1H), 7.34−7.38(m, 2H), 7.09(s, 1H), 7.00−7.04(m, 2H), 4.94(m, 1H), 4.45(m, 1H), 2.52(s, 3H), 2.35(t, J = 5 Hz, 2H), 2.24(m, 1H), 2.11(m, 1H), 1.98(m, 1H), 1.80(m, 1H), 1.69(d, J = 10 Hz, 3H), 1.34(m, 1H), 0.93(t, J = 5 Hz, 3H), 0.82(t, J = 5 Hz, 3H). ESI-MS m/z: 656.5 $[M + H]^+$.

Compound 16, yellow solid, Purity, 99%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.31(d, J = 5 Hz, 2H), 8.10(d, J = 5 Hz, 2H), 8.07(d, J = 5 Hz, 1H), 8.02(s, 1H), 7.94(s, 1H), 7.83(brs, 1H), 7.57(d, J = 10 Hz, 1H), 7.41(d, J = 5 Hz, 1H), 7.37(brs, 1H), 7.07(s, 1H), 7.04(brs, 1H), 6.88(td, J = 10, 5 Hz, 1H), 4.80(m, 1H), 4.40(m, 1H), 4.13(m, 1H), 2.21(m, 1H), 2.03(m, 1H), 1.68(d, J = 5 Hz, 3H), 1.15(d, J = 5 Hz, 3H), 0.82(t, J = 5 Hz, 3H). ESI-MS m/z: 572.4 $[M + H]^+$.

Compound 17, yellow solid, Purity, 97%; ^{1}H NMR (500 MHz, DMSO-d_6) δ8.26(d, J = 10 Hz, 2H), 8.10(d, J = 10 Hz, 2H), 8.06(d, J = 10 Hz, 1H), 7.99(brs, 1H), 7.82(s, 1H), 7.81(d, J = 10 Hz, 2H), 7.37(brs, 1H), 7.07(s, 1H), 7.05(brs, 1H), 4.40(m, 2H), 4.13(m, 1H), 1.64−1.68(m, 3H), 1.15(d, J = 5 Hz, 3H), 0.98(d, J = 5 Hz, 6H). ESI-MS m/z: 593.5 $[M + H]^+$.

Synthesis of Compounds ***18−20***. Two hundred milligrams of Rink-MBHA resin was swollen in DMF for 2 h. The resin was treated with 20% piperidine (v/v) in DMF (2 × 15 min). After thorough washing with DMF, MeOH, DCM, and DMF, a mixture of 4-(7-fluoro-6-nitro-4-oxo-4*H*-chromen-2-yl)-benzoic acid (2equiv), HOBt (3equiv), and DIC (3equiv) was added to the resin. The final mixture was shaken until a negative Kaiser Test was obtained. The resin was washed with DMF, MeOH, and DCM, and a solution of various amines (1 M) in 25% DIEPA/DMF was added. The mixture was shaken overnight, and the supernatant was drained off. The resin was washed with DMF, MeOH, and DMF. 2 M $SnCl_2$/DMF (3 mL) was added to the resin. The mixture was shaken overnight. The resin was added a solution of various isothiocyanates (1 M) and DIC (1 M) in DMF followed by washing with DMF, MeOH, and DMF. The mixture was shaken overnight, and the supernatant was removed. The resin was washed with DMF, MeOH, and DCM. After TFA cleavage, the crude product was purified by HPLC.

Compound 18, yellow solid, Purity, 99%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.21(d, J = 10 Hz, 2H), 8.17(s, 1H), 8.08(d, J = 10 Hz, 2H), 7.86(s, 1H), 7.74(s, 1H), 7.65(brs, 2H), 7.57(s, 1H), 7.44(t, J = 5 Hz, 2H), 7.33(t, J = 5 Hz, 1H), 7.18(m, 1H), 7.10(m, 2H), 7.09(s, 1H), 4.67(t, J = 7.5 Hz, 2H), 3.16(t, J = 7.5 Hz, 2H). ESI-MS m/z: 519.5 $[M + H]^+$.

Compound 19, yellow solid, Purity, 96%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.29(brs, 2H), 8.23(d, J = 10 Hz, 2H), 8.17(s, 1H), 8.07(d, J = 10 Hz, 2H), 7.92(s, 1H), 7.88(s, 1H), 7.64(t, J = 10 Hz, 1H), 7.56(s, 1H), 7.40(d, J = 10 Hz, 1H), 7.09(s, 1H), 4.57(t, J = 7.5 Hz, 2H), 3.75(t, J = 7.5 Hz, 2H), 3.26(s, 3H). ESI-MS m/z: 523.5 $[M + H]^+$.

Compound 20, yellow solid, Purity, 98%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.22(d, J = 10 Hz, 2H), 8.17(s, 1H), 8.07(d, J = 10 Hz,

2H), 7.56(s, 1H), 7.47(m, 2H), 7.25(m, 1H), 7.10(s, 1H), 4.42(t, J = 7.5 Hz, 2H), 2.90(t, J = 7.5 Hz, 2H). ESI-MS m/z: 553.5 $[M + H]^+$.

Synthesis of Compounds ***21***−***28***. Two hundred milligrams of Rink-MBHA resin was swollen in DMF for 2 h. The resin was incubated with 20% 4-methylpiperidine(v/v) in DMF (2 × 15 min). The resin was then thoroughly washed with DMF, MeOH, DCM and DMF. A mixture of Fmoc-AA-OH (3equiv), HOBt (3equiv), and DIC (3equiv) was added to the resin, the final mixture was shaken until the Kaiser Test was negative. The resin was washed with DMF, MeOH, DCM and DMF, followed by Fmoc deprotection. A solution of 4-(7-Fluoro-6-nitro-4-oxo-4*H*-chromen-2-yl)-benzoic acid(2equiv), HOBt(3equiv) and DIC-(3equiv) was added to the resin. The final mixture was shaken until the Kaiser Test was negative. A solution of amines (1M) in 25% DIEPA/DMF was added to the resin. The mixture was shaken overnight, and the supernatant was drained off. The resin was washed with DMF, MeOH, and DMF. 2 M $SnCl_2$/DMF (3 mL) was added to the resin. The mixture was shaken overnight. The resin was then washed with DMF, MeOH, and DMF. A solution of aldehydes (10 equiv) in 5%HOAc/DMF (3 mL) was added to the resin. The mixture was shaken overnight, and the supernatant was removed. The resin was washed with DMF, MeOH, and DCM. After TFA cleavage, the crude product was purified by HPLC.

Compound 21, yellow solid, Purity, 98%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.89(d, J = 10 Hz, 1H), 8.74(d, J = 5 Hz, 1H), 8.37 (s, 1H), 8.31(d, J = 5 Hz, 1H), 8.28(d, J = 5 Hz, 1H), 8.22(s, 1H), 8.19 (s, 1H), 8.18(d, J = 5 Hz, 1H), 8.09(d, J = 10 Hz, 1H), 8.07(d, J = 5 Hz, 1H), 8.01(d, J = 5 Hz, 1H), 7.89(t, J = 5 Hz, 1H), 7.16(brs, 1H), 7.13 (s, 1H), 6.95(brs, 1H), 4.80(m, 1H), 4.40(t, J = 10 Hz, 2H), 2.82(m, 1H), 2.64(m, 1H), 1.66(q, J = 5 Hz, 2H), 1.54(m, 1H), 0.81(d, J = 5 Hz, 6H). ESI-MS m/z: 635.5 $[M + H]^+$.

Compound 22, yellow solid, Purity, 96%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.69(d, J = 5 Hz, 1H), 8.42(s, 1H), 8.33(d, J = 10 Hz, 2H), 8.33(s, 1H), 8.00(d, J = 10 Hz, 2H), 7.94(d, J = 10 Hz, 2H), 7.82(d, J = 10 Hz, 2H), 7.80(d, J = 10 Hz, 2H), 7.59(brs, 1H), 7.54(t, J = 10 Hz, 2H), 7.45(m, 1H), 7.19(s, 1H), 7.15(d, J = 10 Hz, 2H), 7.11(brs, 1H), 6.67(d, J = 10 Hz, 2H), 4.51−4.68(m, 2H), 3.05(m, 1H), 2.90(m, 1H), 2.25(m, 1H), 1.99(m, 1H), 1.78(d, J = 10 Hz, 3H), 0.58(t, J = 5 Hz, 3H). ESI-MS m/z: 677.5 $[M + H]^+$.

Compound 23, yellow solid, Purity, 97%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.68(d, J = 5 Hz, 1H), 8.39(s, 1H), 8.33(d, J = 10 Hz, 2H), 8.28(s, 1H), 8.00(d, J = 10 Hz, 2H), 7.69(d, J = 10 Hz, 2H), 7.57(brs, 1H), 7.15(d, J = 10 Hz, 2H), 7.15(s, 1H), 7.10(brs, 1H), 6.64(d, J = 10 Hz, 2H), 4.71(m, 1H), 4.60(m, 1H), 3.88(s, 3H), 3.02(m, 1H), 2.90(m, 1H), 2.18(m, 1H), 1.78(d, J = 5 Hz, 3H), 1.73(m, 1H), 1.10(m, 1H), 0.65(d, J = 5 Hz, 3H), 0.58(d, J = 5 Hz, 3H). ESI-MS m/z: 659.5 $[M + H]^+$.

Compound 24, yellow solid, Purity, 99%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72(d, J = 10 Hz, 1H), 8.24(s, 1H), 8.19(d, J = 10 Hz, 2H), 8.09(s, 1H), 7.97(d, J = 10 Hz, 2H), 7.60(brs, 1H), 7.35(d, J = 10 Hz, 4H), 7.25(t, J = 10 Hz, 2H), 7.13(m, 4H), 7.10(s, 1H), 5.67 (s, 2H), 4.66(m, 1H), 3.36(m, 1H), 3.13(dd, 1H), 3.00(dd, J = 10, 5 Hz, 1H), 1.78(dd, J = 10, 10 Hz, 1H), 1.28(d, J = 5 Hz, 6H). ESI-MS m/z: 585.5 $[M + H]^+$.

Compound 25, yellow solid, Purity, 96%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.91(t, J = 5 Hz, 1H), 8.30(d, J = 5 Hz, 1H), 8.29(s, 1H), 8.27(s, 1H), 8.09(d, J = 10 Hz, 2H), 7.85(d, J = 10 Hz, 2H), 7.44(brs, 1H), 7.18(d, J = 5 Hz, 2H), 7.15(s, 1H), 7.08(brs, 1H), 4.36(d, J = 5 Hz, 2H), 3.88(s, 3H), 3.86(t, J = 5 Hz, 2H), 1.11(m, 1H), 0.41(m, 2H), 0.24(m, 2H). ESI-MS m/z: 523.4 $[M + H]^+$.

Compound 26, yellow solid, Purity, 98%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.92(t, J = 5 Hz, 1H), 8.37(s, 1H), 8.36(d, J = 5 Hz, 2H), 8.34(s, 1H), 8.09(d, J = 5 Hz, 2H), 7.85(m, 2H), 7.69(d, J = 5 Hz, 1H), 7.60(t, J = 5 Hz, 1H), 7.44(brs, 1H), 7.17(s, 1H), 7.08(brs, 1H), 4.20(m, 1H), 3.87(d, J = 5 Hz, 2H), 2.24(m, 2H), 2.02(m, 2H), 0.68(t, J = 5 Hz, 6H). ESI-MS m/z: 587.4 $[M + H]^+$.

Compound 27, yellow solid, Purity, 98%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25(d, J = 5 Hz, 2H), 8.23(s, 1H), 8.14(d, J = 5 Hz, 1H), 8.00(d, J = 5 Hz, 2H), 8.02(s, 1H), 7.44(brs, 1H), 7.36(m, 1H), 7.24(m, 1H), 7.07(s, 1H), 7.07(brs, 1H), 6.77(brs, 1H), 5.70(s, 2H), 4.36(dd, J = 10, 5 Hz, 1H), 4.10(m, 1H), 2.52(m, 1H), 1.28(d, J = 10 Hz, 6H), 1.09(d, J = 10 Hz, 3H). ESI-MS m/z: 575.5 $[M + H]^+$.

Compound 28, yellow solid, Purity, 97%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35(s, 1H), 8.33(d, J = 5 Hz, 2H), 8.24(s, 1H), 8.17(d, J = 5 Hz, 1H), 8.12(d, J = 5 Hz, 2H), 7.46(brs, 1H), 4.60(t, J = 5 Hz, 2H), 4.37(dd, J = 10, 5 Hz, 1H), 4.11(m, 1H), 3.88(s, 9H), 3.85(t, J = 5 Hz, 2H), 3.78(d, J = 10 Hz, 6H), 1.13(d, J = 5 Hz, 3H). ESI-MS m/z: 631.6 $[M + H]^+$.

Synthesis of Compounds ***29***−***32***. Two hundred milligrams of Rink-MBHA resin was swollen in DMF for 2 h. The resin was retreated with 20% piperidine (v/v) in DMF (2 × 15 min). The resin was then thoroughly washed with DMF, MeOH, DCM, and DMF. A mixture of 4-(7-fluoro-6-nitro-4-oxo-4*H*-chromen-2-yl)-benzoic acid (2equiv), HOBt (3equiv), and DIC (3equiv) was added to the resin. The final mixture was shaken until the Kaiser Test was negative. The resin was washed with DMF, MeOH, and DCM, and added a solution of various amines (1 M) in 25% DIEPA/DMF. The mixture was shaken overnight, and the supernatant was drained off. The resin was washed with DMF, MeOH, and DMF. 2 M $SnCl_2$/DMF (3 mL) was added to the resin. The mixture was shaken overnight. After washed with DMF, MeOH, and DMF, the resin was added a solution of aldehyde (10 equiv) in 5% HOAc/DMF (3 mL). The mixture was shaken overnight, and the supernatant was removed. The resin was washed with DMF, MeOH, and DCM. After TFA cleavage, the crude product was purified by HPLC.

Compound 29, yellow solid, Purity, 99%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.32(s, 1H), 8.27(d, J = 10 Hz, 2H), 8.26(s, 1H), 8.18(brs, 1H), 8.09(d, J = 10 Hz, 2H), 8.06(d, J = 10 Hz, 2H), 7.91(d, J = 10 Hz, 2H), 7.81(d, J = 10 Hz, 2H), 7.58(brs, 1H), 7.54(t, J = 10 Hz, 2H), 7.45(m, 1H), 7.15(s, 1H), 4.59(t, J = 5 Hz, 2H), 3.90(t, J = 5 Hz, 2H), 3.39(t, J = 5 Hz, 2H), 3.33(t, J = 5 Hz, 2H). ESI-MS m/z: 546.5 $[M + H]^+$.

Compound 30, yellow solid, Purity, 96%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.26(d, J = 10 Hz, 2H), 8.18(s, 2H), 8.12(brs, 1H), 8.06(d, J = 10 Hz, 2H), 7.57(brs, 1H), 7.11(s, 1H), 4.18(d, J = 5 Hz, 2H), 1.88(m, 1H), 1.68(m, 2H), 1.62(m, 1H), 1.54(m, 2H), 1.37(d, J = 5 Hz, 6H), 1.16(m, 6H). ESI-MS m/z: 444.4 $[M + H]^+$.

Compound 31, yellow solid, Purity, 98%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.40(s, 1H), 8.35(d, J = 10 Hz, 2H), 8.29(s, 1H), 8.20 (s, 1H), 8.08(d, J = 10 Hz, 2H), 7.58(brs, 1H), 7.17(s, 1H), 7.08(s, 1H), 6.91(s, 1H), 4.14(m, 1H), 3.93(s, 3H), 3.84(s, 3H), 3.74(s, 3H), 2.15(m, 1H), 1.93(m, 1H), 1.68(d, J = 10 Hz, 3H), 0.57(m, 3H). ESI-MS m/z: 528.4 $[M + H]^+$.

Compound 32, yellow solid, Purity, 97%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.57(s, 2H), 8.43(s, 1H), 8.39(s, 1H), 8.32(s, 1H), 8.29(d, J = 10 Hz, 2H), 8.19(brs, 1H), 8.09(d, J = 10 Hz, 2H), 7.58(brs, 1H), 7.17(s, 1H), 4.40(t, J = 5 Hz, 2H), 1.70(m, 2H), 1.59(m, 1H), 0.82(d, J = 5 Hz, 6H). ESI-MS m/z: 588.5 $[M + H]^+$.

MTT Assay. Cells (MDA-MB-231 and MCF-7) were seeded in a 96-well plate (5000 cells/well) for 24 h. Cells were then treated with 10 μM of compounds or varying concentrations of compounds for 72 h. Each compound or each concentration was tested as a triplicate. After 72 h incubation, cells were washed with PBS for three times and then MTT was added for 4hours. Purple crystal formed was then dissolved in 0.04N HCl in isopropanol. The plates were read under 570 nm.

Phage Panning. TentaGel resins bound compound **10** and **24** were swelled in PBSTB (8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 137 mM NaCl, 27 mM KCl, 0.1%(V/V) Tween 20, 0.1% (W/V) BSA, and 0.05% (W/V) NaN_3, pH 7.5 overnight, respectively. 10 μL of each beads were incubated with 10^5 of phage in 1 mL PBSTB for 2 h. The beads were then washed extensively with PBSTB for 5 times and 50 μL of log phase *E.* coli

(strain BLT5403) were added and shaken for 30 min at 37 °C. The infected *E. coli* were then added to 1 mL log phase *E.* coli, and shaken for 3 h at 37 °C until all the *E.* coli were lysed. The *E.* coli debris was then spun down at 14,000 rpm for 10 min. The amplified phage was used for a second round of panning. The panning was repeated three more times. Finally, the phage was mixed with log phase *E.* coli in soft agar for single colony on ampicilin plate. Single colony were picked and subjected to PCR for DNA sequencing. 1 μL of amplified phage were used for PCR with NovaTaq Hot Start DNA Polymerase. T7 down primer (5′-AACCCCTCAAGACCCGTTTA-3′), and T7 up primer (5′-GGA-GCTGTCGTATTCCAGTC-3′) were used as primer pair. The PCR conditions were 95 °C for 7 min and then repeat 34 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min. The last cycle was extended for 1 more minute at 72 °C. The PCR product were cleaned up using ExoSAP-IT kit, and finally subjected to DNA sequencing.

Pull Down Assay. MDA-MB-231 were grown to 85% confluence and lysed with lysis buffer (PBS, protease inhibitor cocktail, DNase, RNase, and 0.1% TritonX-100). 1 mM of biotinylated **10** and **24** were added to the cell lysate overnight in 4 °C. Then the compounds were pulled down by incubating with avidin beads for 2 h at 4 °C. Beads were washed with PBS for 3 times and boiled with sampler buffer for 10 min at 95 °C. Samples were then run on SDS-PAGE and transferred on PVDF membrane following blotting with anti-eEF1A antibody (1:1000 dilution) and antirabbit HRP conjugate (Cell Signaling Technology, MA).

Western Blot Analysis. MDA-MB-231 were grown to 80% confluence and treated with compound for 16 h. Cells were then lysed with lysis buffer (25 mM tris HCl, 10 mM $MgCl_2$, 1 mM Na_3VO_4, 50 mM β-glycerol phosphate, 0.1% Triton-X 100, and protease inhibitor cocktail). The cell lysate was then separated using SDS-PAGE and transferred to PVDF membrane. The membrane was blotted using anti-phosohoAkt (Ser 437 and Thr 308) (1:1000 dilution) and antirabbit HRP conjugate (Cell Signaling Technology, MA).

Surface Plasmon Resonance (SPR) Analysis. One micromolar of biotinylated compounds **10**, **24**, and **39** in Biacore HBS-EP buffer were individually immobilized on 3 of the 4 flow cells on a streptavidin sensor chip (Biacore SA Chip) at 5uL/min for 10 min each. The remaining one empty flow cell was assigned as a reference flow cell and was immobilized with Linker-Lysin-Biotin only. eEF1A1 and GST were 2-fold serial diluted in HBS-EP buffer from 20 nM to 1.25 nM. eEF1A2 was 2-fold serial diluted in HBS-EP buffer from 100 nm to 6.25 nM. The protein at each concentration was then injected at 30uL/min for 3 min and dissociated for 5 min. K_{on}, K_{off}, and K_D were evaluated by using BiaEvaluation software to fit binding curves with proper binding models.

Cell Migration Assay. MDA-MB-231 cells were grown to 80% confluence and treated with compound **39** (2 μM) for 4 h; 0.2 mL of cells (5×10^5cell/mL) in serum free medium were seeded into the top chambers of 6.5-mm Corning Costar transwells (Corning, NY, New York, USA), and the bottom wells were loaded with complete medium. After 48 h incubation, the images of cells in the bottom wells were captured and the cell number was counted.

^{35}S-Methionine Labeled Protein Translation Assay. Cells were treated with DMSO or different concentration of **39** for 8 h. Cell culture medium was then changed to methionine free medium for 1 h. To start the translation labeling, 5 μL of ^{35}S-methionine (specific activity >1000 Ci/mMole) (PerkinElmer, Waltham, MA) were added to the cells and incubated for 3 h. Cells were then harvested and lysed. Proteins were separated on SDS-PAGE and stained with commassie blue. The same gel was then dried and the ^{35}S-methionine was detected using phosphoimager.

Preparation of Compounds 33−51. Compounds **33**−**51** were synthesized in the same manner as above.

Compound 33, yellow solid, Purity, 96%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72(d, J = 10 Hz, 1H), 8.19(d, J = 10 Hz, 2H), 7.96(d, J = 10 Hz, 2H), 7.86(s, 1H), 7.82(brs, 1H), 7.67(d, J = 10 Hz, 2H), 7.60(s, 1H), 7.39(d, J = 10 Hz, 2H), 7.30−7.36(m, 4H), 7.25(t, J = 10 Hz, 2H), 7.13−7.19(m, 3H), 7.09(s, 1H), 7.06(d, J = 10 Hz, 2H), 5.62(s, 2H), 4.67(m, 1H), 3.79(s, 3H), 3.14(dd, J = 10, 10 Hz, 1H), 2.99(dd, J = 10, 5 Hz, 1H). ESI-MS m/z: 664.7 $[M + H]^+$.

Compound 34, yellow solid, Purity, 98%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.58(d, J = 10 Hz, 1H), 8.20(d, J = 10 Hz, 2H), 8.07(d, J = 10 Hz, 2H), 7.87(s, 1H), 7.83(brs, 1H), 7.66(d, J = 5 Hz, 2H), 7.44(brs, 1H), 7.39(d, J = 10 Hz, 2H), 7.34(m, 3H), 7.11(s, 1H), 7.06(d, J = 5 Hz, 2H), 7.00(s, 1H), 5.66(s, 2H), 4.48(m, 1H), 3.79(s, 3H), 1.61−1.73(m. 2H), 1.55(m, 1H), 0.92(d, J = 10 Hz, 3H), 0.89(d, J = 10 Hz, 3H). ESI-MS m/z: 630.7 $[M + H]^+$.

Compound 35, yellow solid, Purity, 98%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.76(d, J = 10 Hz, 1H), 8.29(d, J = 10 Hz, 2H), 8.24(s, 1H), 7.98(d, J = 10 Hz, 2H), 7.83(s, 1H), 7.63(brs, 1H), 7.56(d, J = 5 Hz, 2H), 7.37(d, J = 10 Hz, 2H), 7.26(t, J = 10 Hz, 2H), 7.19(t, J = 10 Hz, 1H), 7.15(brs, 2H), 7.11(d, J = 5 Hz, 2H), 4.68(m, 1H), 4.55(m, 1H), 3.82(s, 3H), 3.13(dd, J = 10, 10 Hz, 1H), 3.00(dd, J = 10, 5 Hz, 1H), 2.29(m, 1H), 2.04(m, 1H), 1.83(m, 1H), 1.72(d, J = 5 Hz, 3H), 1.62(m, 2H), 1.00−1.36(m, 6H). ESI-MS m/z: 684.6 $[M + H]^+$.

Compound 36, yellow solid, Purity, 97%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.59(d, J = 10 Hz, 1H), 8.32(s, 1H), 8.23(d, J = 10 Hz, 2H), 8.09(s, 1H), 8.08(d, J = 10 Hz, 2H), 7.45(brs, 1H), 7.34(t, J = 10 Hz, 2H), 7.31(s, 1H), 7.29(t, J = 10 Hz, 1H), 7.15(d, J = 10 Hz, 2H), 7.14(s, 1H), 7.00(s, 1H), 5.84(s, 2H), 4.48(m, 1H), 3.39(m, 1H), 1.63−1.74(m. 2H), 1.55(m, 1H), 1.29(d, J = 5 Hz, 6H), 0.92(d, J = 10 Hz, 3H), 0.89(d, J = 10 Hz, 3H). ESI-MS m/z: 551.7 $[M + H]^+$.

Compound 37, yellow solid, Purity, 97%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.76(d, J = 10 Hz, 1H), 8.37(brs, 1H), 8.31(d, J = 10 Hz, 2H), 8.22(s, 1H), 7.98(d, J = 10 Hz, 2H), 7.62(s, 1H), 7.36(d, J = 5 Hz, 2H), 7.26(t, J = 5 Hz, 2H), 7.18(t, J = 5 Hz, 1H), 7.17(s, 1H), 4.68(m, 1H), 4.41(m, 1H), 3.54(m, 1H), 3.15(dd, J = 10, 10 Hz, 1H), 3.08(dd, J = 10, 5 Hz, 1H), 2.29(m, 1H), 2.06(m, 1H), 1.84(m, 1H), 1.69(d, J = 5 Hz, 3H), 1.62(m, 2H), 1.52(1, 1H), 1.45(d, J = 10 Hz, 3H), 1.33(d, J = 10 Hz, 3H), 0.88−1.22(m, 6H). ESI-MS m/z: 605.7 $[M + H]^+$.

Compound 38, yellow solid, Purity, 99%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.31(d, J = 10 Hz, 2H), 8.26(brs, 1H), 8.20(brs, 1H), 8.07(d, J = 10 Hz, 2H), 7.83(s, 1H), 7.59(s, 1H), 7.54(d, J = 10 Hz, 2H), 7.16(s, 1H), 7.11(d, J = 10 Hz, 2H), 4.55(m, 1H), 3.82(s, 3H), 2.29(m, 1H), 2.04(m, 1H), 1.83(m, 1H), 1.72(d, J = 5 Hz, 3H), 1.62(m, 2H), 0.99−1.36(m, 6H). ESI-MS m/z: 537.6 $[M + H]^+$.

Compound 39, yellow solid, Purity, 98%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70(s, 1H), 8.61(d, J = 5 Hz, 1H), 8.23(d, J = 10 Hz, 2H), 8.08(d, J = 10 Hz, 2H), 7.45(brs, 1H), 7.36(s, 1H), 7.06(s, 1H), 7.00(s, 1H), 4.48(m, 1H), 3.81(m, 1H), 1.87(m, 1H), 1.55−1.75(m, 10H), 1.24(d, J = 10 Hz, 3H), 1.03−1.15(m, 3H), 0.93(d, J = 10 Hz, 3H), 0.89(d, J = 10 Hz, 3H). ESI-MS m/z: 549.7 $[M + H]^+$.

Compound 40, yellow solid, Purity, 99%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70(s, 1H), 8.23(d, J = 10 Hz, 2H), 8.14(brs, 1H), 8.10(d, J = 10 Hz, 1H), 8.05(d, J = 10 Hz, 2H), 7.58(brs, 1H), 7.37(s, 1H), 7.05(s, 1H), 3.80(m, 1H), 1.59−1.89(m, 7H), 1.23(d, J = 5 Hz, 3H), 1.00−1.18(m, 4H). ESI-MS m/z: 436.6 $[M + H]^+$.

Compound 41, yellow solid, Purity, 96%; ^{1}H NMR (500 MHz, DMSO-d_6): δ9.22(s, 1H), 8.57(d, J = 10 Hz, 1H), 8.17(d, J = 10 Hz, 2H), 8.06(d, J = 10 Hz, 2H), 7.80(s, 1H), 7.45(brs, 1H), 6.99(brs, 1H), 6.92(s, 1H), 6.85(s, 1H), 5.63(d, J = 10 Hz, 1H), 4.48(m, 1H), 3.52(m, 1H), 2.11(s, 3H), 1.87(m, 1H), 1.54−1.74(m, 8H), 1.16(d, J = 10 Hz, 3H), 0.98−1.23(m, 5H), 0.91(d, J = 10 Hz, 3H), 0.89(d, J = 10 Hz, 3H). ESI-MS m/z: 561.8 $[M + H]^+$.

Compound 42, yellow solid, Purity, 97%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25(s, 1H), 8.21(d, J = 10 Hz, 2H), 8.16(brs, 1H), 8.06(s, 1H), 8.05(d, J = 10 Hz, 2H), 7.55(brs, 1H), 7.36(t, J = 10 Hz, 2H), 7.29(t, J = 5 Hz, 1H), 7.19(t, J = 5 Hz, 1H), 7.15(d, J = 5 Hz, 2H), 7.12(s, 1H), 5.68(s, 2H), 3.37(m, 1H), 1.29(d, J = 5 Hz, 6H). ESI-MS m/z: 438.5 $[M + H]^+$.

Compound 43, orange solid, Purity, 99%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.88(t, J = 5 Hz, 1H), 8.72(s, 1H), 8.71(d, J = 5 Hz, 1H), 8.12(d, J = 10 Hz, 2H), 7.94(d, J = 10 Hz, 2H), 7.60(brs, 1H), 7.45(d, J = 10 Hz, 2H), 7.39(d, J = 10 Hz, 2H), 7.35(t, J = 10 Hz, 2H), 7.28(t, J = 10 Hz, 1H), 7.25(t, J = 10 Hz, 2H), 7.16(t, J = 10 Hz, 1H), 7.14(s, 1H), 7.13(brs, 1H), 7.14(s, 1H), 7.02(s, 1H), 4.74(d, J = 5 Hz, 2H), 4.66(m, 1H), 3.13(dd, J = 10, 10 Hz, 1H), 2.99(dd, J = 10, 5 Hz, 1H). ESI-MS m/z: 563.7 $[M + H]^+$.

Compound 44, yellow solid, Purity, 97%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.33(s, 1H), 8.68(d, J = 5 Hz, 1H), 8.06(d, J = 10 Hz, 2H), 7.91(d, J = 10 Hz, 2H), 7.76(s, 1H), 7.58(brs, 1H), 7.43(d, J = 10 Hz, 2H), 7.36(d, J = 10 Hz, 2H), 7.34(t, J = 10 Hz, 2H), 7.23−7.27(m, 3H), 7.07−7.17(m, 3H), 6.88(s, 1H), 6.66(s, 1H), 4.65(m, 1H), 4.51(d, J = 5 Hz, 2H), 3.13(dd, J = 10, 10 Hz, 1H), 2.99(dd, J = 10, 5 Hz, 1H), 2.11(s, 3H). ESI-MS m/z: 575.7 $[M + H]^+$.

Compound 45, orange solid, Purity, 96%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.71(s, 1H), 8.60(d, J = 10 Hz, 1H), 8.23(d, J = 10 Hz, 2H), 8.11(d, J = 5 Hz, 1H), 8.07(d, J = 10 Hz, 2H), 7.45(brs, 1H), 7.37(s, 1H), 7.06(s, 1H), 7.00(s, 1H), 4.47(m, 1H), 3.83(m, 1H), 1.55−1.90(m, 10H), 1.23(d, J = 5 Hz, 3H), 1.03−1.15(m, 4H), 0.93(d, J = 5 Hz, 3H), 0.89(d, J = 5 Hz, 3H). ESI-MS m/z: 549.7 $[M + H]^+$.

Compound 46, orange solid, Purity, 98%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.71(s, 1H), 8.60(d, J = 10 Hz, 1H), 8.23(d, J = 10 Hz, 2H), 8.10(d, J = 5 Hz, 1H), 8.07(d, J = 10 Hz, 2H), 7.45(brs, 1H), 7.37(s, 1H), 7.06(s, 1H), 7.00(s, 1H), 4.47(m, 1H), 3.82(m, 1H), 1.55−1.90(m, 10H), 1.23(d, J = 5 Hz, 3H), 1.03−1.15(m, 4H), 0.93(d, J = 5 Hz, 3H), 0.89(d, J = 5 Hz, 3H). ESI-MS m/z: 549.7 $[M + H]^+$.

Compound 47, orange solid, Purity, 96%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70(s, 1H), 8.60(d, J = 10 Hz, 1H), 8.23(d, J = 10 Hz, 2H), 8.10(d, J = 5 Hz, 1H), 8.07(d, J = 10 Hz, 2H), 7.45(brs, 1H), 7.36(s, 1H), 7.06(s, 1H), 7.00(s, 1H), 4.47(m, 1H), 3.82(m, 1H), 1.54−1.89(m, 10H), 1.23(d, J = 5 Hz, 3H), 1.03−1.15(m, 4H), 0.93(d, J = 5 Hz, 3H), 0.89(d, J = 5 Hz, 3H). ESI-MS m/z: 549.7 $[M + H]^+$.

Compound 48, orange solid, Purity, 99%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.74(d, J = 10 Hz, 1H), 8.70(s, 1H), 8.22(d, J = 10 Hz, 2H), 8.11(d, J = 10 Hz, 1H), 7.96(d, J = 10 Hz, 2H), 7.61(s, 1H), 7.36(s, 1H), 7.35(d, J = 5 Hz, 2H), 7.26(t, J = 5 Hz, 2H), 7.17(t, J = 10 Hz, 1H), 7.13(brs, 1H), 7.03(s, 1H), 4.66(m, 1H), 3.80(m, 1H), 3.14(dd, J = 10, 5 Hz, 1H), 3.00(dd, J = 10, 10 Hz, 1H), 1.54−1.89(m, 7H), 1.23(d, J = 5 Hz, 3H), 1.00−1.15(m, 4H). ESI-MS m/z: 583.7 $[M + H]^+$.

Compound 49, orange solid, Purity, 97%; ^{1}H NMR (500 MHz, DMSO-d_6): δ8.74(d, J = 10 Hz, 1H), 8.70(s, 1H), 8.21(d, J = 10 Hz, 2H), 8.10(d, J = 10 Hz, 1H), 7.96(d, J = 10 Hz, 2H), 7.61(s, 1H), 7.36(s, 1H), 7.35(d, J = 5 Hz, 2H), 7.26(t, J = 5 Hz, 2H), 7.16(t, J = 10 Hz, 1H), 7.13(brs, 1H), 7.03(s, 1H), 4.66(m, 1H), 3.80(m, 1H), 3.14(dd, J = 10, 5 Hz, 1H), 3.00(dd, J = 10, 10 Hz, 1H), 1.59−1.89(m, 7H), 1.23(d, J = 5 Hz, 3H), 1.00−1.15(m, 4H). ESI-MS m/z: 583.7 $[M + H]^+$.

■ ASSOCIATED CONTENT

Supporting Information. Synthetic procedures, Surface plasmon resonance (SPR) analysis, and Biological data.This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

Fmoc, fluorenylmethoxycarbonyl; HOBt, *N*-hydroxybenzotriazole; DIC, *N*,*N*′-diisopropylcarbodiimide; DIEPA, *N*,*N*-diisopropylethylamine; TFA, trifluoroacetic acid; TIS, triisopropylsilane; HOAc, acetic acid; DMF, *N*,*N*-dimethylformamide; DCM, dichloromethane; eEF1A, eukaryotic elongation factor 1 alpha; ESI, electrospray ionization; NMR, nuclear magnetic resonance; RP-HPLC, reverse-phase high performance liquid chromatography

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