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Synthesis and Biological Evaluation of Analogues of AKT (Protein Kinase B) Inhibitor-IV

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Abstract

Inhibitors of the PI3-kinase/AKT (protein kinase B) pathway are under investigation as anticancer and antiviral agents. The benzimidazole derivative AKT inhibitor-IV (ChemBridge 5233705) affects this pathway and exhibits potent anticancer and antiviral activity. To probe its biological activity, we synthesized AKT inhibitor-IV and 21 analogues using a novel six-step route based on $ZrCl_4$-catalyzed cyclization of 1,2-arylenediamines with α,β-unsaturated aldehydes. We examined effects on viability of HeLa carcinoma cells, viability of normal human cells (NHBE), replication of recombinant parainfluenza virus 5 (PIV5) in HeLa cells, and replication of the intracellular bacterium *Mycobacterium fortuitum* in HeLa cells. Replacement of the benzimidazole *N*-ethyl substitutent of AKT inhibitor-IV with *N*-hexyl and *N*-dodecyl groups enhanced antiviral activity and cytotoxicity against the cancer cell line, but these compounds showed substantially lower toxicity (from 6-fold to >20-fold) against NHBE cells, and no effect on *M. fortuitum*, suggesting inhibition of one or more host protein(s) required for proliferation of cancer cells and PIV5. The key structural elements identified here may facilitate identification of targets of this highly biologically active scaffold.

Introduction

In higher eukaryotes, the phosphatidylinositol-3-kinase (PI3K) pathway regulates a number of critical physiological functions including vesicular trafficking, cellular growth, survival, and proliferation. When activated, receptor tyrosine kinases, such as the epidermal growth factor receptor, recruit PI3K to the plasma membrane, resulting in the conversion of membrane-bound phosphatidylinositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-triphosphate. This lipid second messenger in turn recruits the serine-threonine kinase AKT (also known as protein kinase B) to the plasma membrane by binding the pleckstrin homology (PH) domain of this enzyme. At the plasma membrane, AKT is fully activated upon phosphorylation by 3-phosphoinositide-dependent kinase (PDK1) and the rapamycin-insensitive mammalian target of rapamycin complex (mTORC2). Phosphorylation of substrates by activated AKT subsequently results in a cascade of downstream signaling

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Supporting Information Available: NMR spectra and analytical HPLC data for synthetic compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

events. Mutations in PI3K that result in constitutive growth-factor-independent activation of AKT are frequently observed in human cancers.[1, 2]

In addition to its established contribution to cancer progression, the PI3K/AKT pathway also plays key roles in the infection and replication of certain viral pathogens. This pathway is hijacked to promote host cell survival in cells infected by Influenza A virus[3] and HIV-1,[4] it plays a critical role in the cellular entry of Ebola virus,5 and it has been hypothesized in some studies,6, 7 but not others,[8] to be involved in the replication of nonsegmented negative stranded RNA viruses such as parainfluenza virus 5 (PIV5). Among the numerous small molecules known to block the PI3K/AKT pathway, many inhibit AKT through interactions with the ATP-binding site, allosteric sites, or the PH domain.[9] Other inhibitors of this pathway bind PI3K, other upstream components, kinases between PI3K and AKT, or downstream proteins.10 AKT inhibitor-IV (**1**, also known as ChemBridge 5233705, Figure 1) was initially proposed to target the ATP binding site of unknown kinases upstream of AKT but downstream of PI3K.[11] In 786-O cells, **1** blocks AKT-mediated nuclear export of the transcription factor FOXO1a (IC_{50} = 625 nM)[11] and cell proliferation (IC_{50} < 1.25 μM).[12–15] More recent studies indicate that a low (1 μM) concentration of **1** inhibits phosphorylation of substrates downstream of AKT, but the antiviral effects observed at this concentration do not result from direct inhibition of kinase activity of the AKT1 or AKT2 subtypes or other known kinases of the PI3K pathway.[8] Consequently, the mechanism of antiviral and antiproliferative activities of **1** remains poorly understood, and molecular probes that facilitate the identification of specific protein targets of this compound are needed.

To better define the structural basis of its antiviral/antiproliferative activities, we report here a new synthetic approach that allowed preparation of **1** and 21 related analogues. This route is based on a $ZrCl_4$-mediated cyclization reaction of substituted *N*-aryl 1,2-arylenediamines with α,β-unsaturated aldehydes. We evaluated the *in vitro* antiviral activity of these compounds against PIV5 in infected HeLa cells, examined the cytotoxic effects of the most potent compounds against HeLa cells and normal human bronchial/tracheal epithelial (NHBE) cells, and assessed effects on replication of the intracellular bacterium *M. fortuitum* in HeLa cells. These studies identified two analogues with greater biological activity than the parent compound and provide strategies for structural modification that may be valuable for future target identification studies.

Results and discussion

Benzimidazole represents an important scaffold in natural products and is considered a privileged structure in medicinal chemistry.[16–18] Although numerous routes to benzimidazoles have been reported,[19] an efficient route for preparation of the commercially available benzimidazole derivative AKT inhibitor-IV (**1**) has not been previously described. For the synthesis of simpler benzimidazoles, unconjugated aldehydes can often be condensed with 1,2-phenylenediamines to generate benzimidazoline intermediates[20–22] that can be further oxidized with mild oxidants such as potassium peroxymonosulfate,[20] MnO_2,[23] and DDQ,[24] to afford the desired products. However, the use of this approach with sensitive α,β-unsaturated aldehyde substrates as building blocks for the preparation of more complex benzimidazole derivatives such as **1** is known[20, 23, 24] to be problematic. To develop an improved route to these more sensitive derivatives, we initially synthesized the novel 1,2-arylenediamine **7** (Scheme 1) in three steps from 4-chloro-3-nitrobenzaldehyde (**2**). Compound **2** was condensed with 2-aminothiophenol (**3**) to generate benzothiazole **4** using conditions described by Mortimer for similar substrates.[25] Treatment of **4** with freshly distilled aniline (**5**) afforded **6**, which was reduced with hydrazine in the presence of Pd(0)[26] to yield **7**. However, when **7** and the known[27] α,β-unsaturated-γ-amino aldehyde **10** were

subjected to well-precedented conditions for generating benzimidazolines, such as refluxing in ethanol, the reaction was extremely sluggish. Moreover, subsequent addition of potassium peroxymonosulfate,[20] MnO_2,[23] or DDQ,[24] resulted in decomposition, and complex reaction mixtures were obtained. To provide better methodology for the preparation of benzimidazoles using α,β-unsaturated aldehyde coupling partners, we alternatively executed the cyclization and oxidation steps separately. We found that in the presence of 3Å molecular sieves, the benzimidazoline intermediate **11** was generated as a major byproduct in refluxing ethanol, and MnO_2 was sufficiently mild to afford **12**, but unfortunately this reaction generated only low yields of the desired product **12**. To improve this outcome, we examined the use of Lewis acids such as $ZrOCl_2$, $ZrCl_4$, $CuSO_4$, and $FeCl_3$ previously reported[28–31] for the preparation of benzimidazoles, benzothiazoles, and purines. By screening a variety of Lewis acids, we found that addition of 0.5 equivalents of $ZrCl_4$ to **7** and **10** in refluxing ethanol afforded **11**, which could be oxidized *in situ* with MnO_2 to afford **12** in a remarkably high 75% yield. As shown in Scheme 1, alkylation of **12** with excess ethyl iodide followed by purification by flash column chromatography afforded AKT inhibitor-IV (**1**). Analogues **13**-**32**, shown in Figure 2, were prepared by this zirconium-mediated cyclization route or similar methods as illustrated in Schemes 2 and 3.

To examine the antiviral activity of compounds **12**-**32**, we constructed a recombinant parainfluenza virus 5 minigenome system (rPIV5-RL). As shown in Figure 3, this system is analogous to a previously reported[32] recombinant PIV5 that expresses GFP, but we replaced the gene encoding GFP with renilla luciferase (RL), which was inserted into the viral genome between the HN and L genes. To validate the utility of rPIV5-RL for testing compounds related to **1**, we quantified luciferase activity in infected HeLa cells, a human cervical carcinoma line suitable for virus replication, as a function of multiplicity of infection (MOI). The strong correlation of MOI with luciferase activity, as shown in Figure 3, confirmed the utility of rPIV5-RL for evaluation of antiviral activity of synthetic compounds. Assessment of antiviral activity initially involved infection of HeLa cells with rPIV5-RL at 1 MOI followed by treatment with compounds **1** and **12**-**32** at 1 μM to generate singleton data points. As shown in Figure 4, compounds comparable in activity to **1** at this concentration were further studied at 0.5 μM as singletons. Dose-response curves against rPIV5-RL and cytotoxic effects towards HeLa cells were used to generate IC_{50} values for **1** and the three most potent analogues (**29**-**31**, Figure 5). Importantly, two compounds were identified (**30**, **31**) that exhibit greater antiviral activity than the parent compound **1**. However, the cytotoxicity of all of these compounds towards HeLa cancer cells, assessed by quantifying cellular ATP using a firefly luciferase-based assay, generally paralleled their antiviral activity. Interestingly, 6-fold (for **1**) to >20-fold (for **31**) higher concentrations of these compounds were required for comparable cytotoxic effects against NHBE cells, suggesting the possibility of inhibition of one or more host protein(s) required for proliferation of cancer cells and PIV5. Control experiments examining inhibition of both renilla luciferase and firefly luciferase present in cell extracts confirmed that the observed biological activity does not result from inhibition of either these reporter enzymes (data provided in the supporting information). To further confirm that the biological activity of analogues **29**-**31** does not relate to non-specific detergent-like action, we evaluated the effect of these compounds on replication of intracellular *M. fortuitum* bacteria in infected HeLa cells. Whereas the non-ionic detergent Triton X-100 (1%) reduced bacterial survival by 18-fold compared with DMSO vehicle alone, no deleterious effects of **1** or **29**-**31** (0.5 μM) on bacterial replication was observed. Together, these results suggest that **1** and the potent analogues **29**-**31** selectively inhibit one or more mammalian host proteins necessary for both proliferation of HeLa cervical carcinoma cells and replication of PIV5.

How AKT inhibitor-IV (**1**) affects the PI3K/AKT pathway is not well understood. In studies of the relatively high concentration of 10 μM, **1** was originally proposed by Silver[11] to target

the ATP binding site of unknown kinases upstream of AKT but downstream of PI3K. However, a more recent investigation[8] by Connor of cells treated with 1 μM of **1** indicates that this compound exhibits potent antiviral and antiproliferative effects through a unique mechanism that does not involve inhibition of the AKT1 or AKT2 subtypes or inhibition of other known kinases within the PI3K/AKT pathway. At 1 μM, **1** is known to promote hyperphosphorylation of AKT in multiple mammalian cell lines, a process that requires PI3K activity, and could potentially involve activation of the PDK1 kinase directly upstream of AKT.[8] Additionally, in a high-throughput screening assay against 84 kinases, **1** (1 μM) showed no inhibitory activity against 82 of these enzymes, and only slight inhibition of the AGC kinases SGK1 and MKK1 was observed.[8] However, treatment of cells with **1** is known to decrease phosphorylation of AKT substrates such as 4E-BP-1, suggesting that inhibition occurs downstream of AKT kinase, possibly implicating mTORC2 as a target.[8] Inhibition or activation of other aspects of normal cellular function outside of the PI3K/AKT pathway may also play key roles in the potent biological activities of **1**.[8]

In conclusion, we synthesized the potent antiviral/antiproliferative agent AKT inhibitor-IV (**1**) by developing a zirconium-mediated condensation approach that allows coupling of the sensitive α,β-unsaturated aldehydes with the novel 1,2-arylenediamine **7**. This methodology allowed the preparation of a collection of 21 analogues. Evaluation of these analogues identified two compounds (**30**, **31**) with greater activity than **1** against recombinant PIV5 expressing luciferase in HeLa cells. Although these compounds were found to exhibit potent cytotoxic activity against HeLa carcinoma cells, this toxicity was 6-fold to >20-fold lower against normal NHBE cells, and no effect against replication of intracellular *M. fortuitum* bacteria was observed, suggesting the possibility of selective inhibition of unidentified host protein(s) involved in proliferation of cancer cells and PIV5. The ability to substitute AKT inhibitor-IV (**1**) with long side chains at the N3 position without deleteriously affecting its antiviral and cytotoxic activity may be useful for future affinity chromatography[33] or related methods directed at identification of protein target(s) of AKT inhibitor-IV (**1**).

Experimental Section

General

Chemical reagents and solvents were obtained from Acros, Aldrich, EMD Biosciences and Combi-Blocks. Commercial grade reagents were used without further purification unless otherwise noted. Compounds **10**[27] and **33**[34] were prepared using previously described methods. Compounds **35** and **36** were prepared by converting 4-chloro-3-nitro-benzoic acid to the acid chloride with thionyl chloride followed by reaction with methanol and aniline, respectively. Anhydrous solvents were obtained after passage through a drying column of a solvent purification system from GlassContour (Laguna Beach, CA). All reactions were performed under an atmosphere of dry nitrogen. Reactions were monitored by analytical thin-layer chromatography on plates coated with 0.25 mm silica gel 60 F254 (EMD Chemicals). TLC plates were visualized by UV irradiation (254 nm) or stained with 20% phosphomolybdic acid in ethanol. Flash column chromatography employed silica gel (ICN SiliTech, 32–63 μm). Melting points were measured with a Thomas Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were obtained with a Perkin Elmer 1600 Series FTIR. NMR spectra were obtained with Bruker CDPX-300 and AV-400 instruments with chemical shifts reported in parts per million (ppm, δ). High-resolution mass spectra were obtained from the proteomics and mass spectrometry core facility at Penn State University, University Park, PA or the mass spectrometry facility at the University of Kansas. Peaks are reported as *m/z*. Analytical purity of compounds was determined by analytical reverse-phase HPLC with a PRP-1 (polystyrene-divinylbenzene) reverse-phase column (4.1 × 250 mm, 7 μm; Hamilton) running a gradient of 10% to 99.9% CH_3CN in nanopure water (containing 0.1% TFA) over 20 min at a flow rate of 0.8 mL/min. All

compounds subjected to biological assays were ≥98% pure by analytical reverse phase HPLC.

2-(4-Chloro-3-nitro-phenyl)-benzothiazole (4)

To a solution of compound **2 (**5.5 g, 30 mmol) in anhydrous ethanol (250 mL) was added 2-aminothiophenol (**3**, 3.3 mL, 30 mmol). The reaction was refluxed for 16 h and cooled to 22 °C. A white precipitate was collected by filtration with a fritted funnel, and the solid crude product was washed with cold ethanol (10 mL × 3). Recrystallization from toluene afforded the product (6.26 g, 72%) as an off white solid; mp 163–164 °C; R_f = *0*.51 (hexane/ethyl acetate, 3:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (s, 1H), 8.35 (d, 1H, *J* = 8.4 Hz), 8.21 (d, 1H, *J* = 8.0 Hz), 8.12 (d, 1H, *J* = 8.0 Hz), 7.96 (d, 1H, *J* = 8.4 Hz), 7.58 (dd, 1H, *J* = 8.4 Hz), 7.52 (dd, 1H, *J* = 8.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.3, 153.7, 148.6, 135.4, 133.4, 133.2, 132.3, 127.8, 127.6, 126.8, 124.1, 123.8, 123.2; IR (film) ν max 1604, 1566, 1538, 1456, 1430, 1339, 1127, 1050, 990, 887, 835, 764, 730 cm^{-1}; HRMS (ESI-) *m/z* 288.9831 (M-H$^-$, $C_{13}H_6ClN_2O_2S$ requires 288.9839).

(4-Benzothiazol-2-yl-2-nitrophenyl)phenylamine (6)

To a solution of compound **4** (2.9 g, 10 mmol) in DMSO (50 mL) was added freshly distilled aniline (**5**, 4.6 mL, 50 mmol). The reaction was heated to 70 °C and stirred for 8 h. The reaction was cooled to 22 °C and poured into water (200 mL). The aqueous phase was extracted with diethyl ether (100 mL × 4). The organic extracts were combined and washed with saturated aqueous NaCl (200 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (CH_2Cl_2 eluent) to afford the product (2.46 g, 71%) as a orange solid; mp 160–162 °C; R_f = *0*.56 (hexane/ethyl acetate, 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.75 (s, 1H), 8.86 (d, 1H, *J* = 2.1 Hz), 8.07 (dd, 1H, J_1 = 2.1 Hz, J_2 = 9.0 Hz), 8.00 (d, 1H, J = 8.1 Hz), 7.86 (d, 1H, *J* = 9 Hz), 7.49-7.44 (m, 3H), 7.36-7.25 (m, 5H); ^{13}C NMR (75 MHz, CDCl3) δ 165.6, 156.6, 144.5, 137.8, 134.7, 134.0, 132.7, 129.9 (× 2), 126.5, 126.4, 126.0, 125.2, 124.8 (× 2), 123.3, 123.0, 121,6, 116.4; IR (film) ν max 3333, 1624, 1594, 1567, 1531, 1496, 1353, 1266, 1210, 753 cm^{-1}; HRMS (ESI +) *m/z* 348.0833 (M+H$^+$, $C_{19}H_{14}N_3O_2S$ requires 348.0807).

4-Benzothiazol-2-yl-*N*^{1}-phenylbenzene-1,2-diamine (7)

To a slurry of compound **6** (1.74 g, 5 mmol) in ethanol (50 mL) was added palladium on carbon (10%, 530 mg, 0.5 mmol) and anhydrous hydrazine (0.5 mL, 16 mmol). The reaction was heated to 80 °C and refluxed for 30 min. The reaction was cooled to 22 °C and filtered through a fritted funnel. The solid Pd/C was washed with ethanol (10 mL), and the combined filtrate was concentrated *in vacuo* to afford the crude product. Recrystallization from CH_2Cl_2/hexane afforded the product (1.52 g, 95%) as a light yellow solid; mp 154–156 °C; R_f = *0*.13 (hexane/ethyl acetate, 3:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.06 (d, 1H, *J* = 7.5 Hz), 7.97 (d, 1H, *J* = 7.7 Hz), 7.55 (d, 1H, *J* = 2.0 Hz), 7.52-7.46 (m, 1H) 7.44 (s, 1H) 7.41-7.36 (m, 1H) 7.29-7.19 (m, 4H), 7.01 (d, 2H, *J* = 8 Hz) 6.83 (t, 1H, *J* = 7.3 Hz), 5.17 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.0, 153.8, 143.7, 140.2, 134.1, 132.3, 129.1 (× 2), 126.4, 126.3, 124.8, 122.2, 122.1, 119.7, 118.4, 117.0 (× 2), 116.4, 113.2; IR (film) ν max 3360, 3037, 1595, 1496, 1434, 1313, 1194, 1000, 810, 749, 728, 693 cm^{-1}; HRMS (ESI+) *m/z* 318.1066 (M+H$^+$, $C_{19}H_{16}N_3S$ requires 318.1065).

[(*E*)-2-(5-Benzothiazol-2-yl-1-phenyl-1*H*-benzoimidazol-2-yl)ethenyl]methyl phenylamine (12)

To a solution of compound **7** (64 mg, 0.2 mmol) and **10** (32 mg, 0.2 mmol) in anhydrous ethanol (15 mL) was added $ZrCl_4$ (24 mg, 0.1 mmol). The reaction was heated to 80 °C and refluxed for 30 min. When the starting material was consumed as evidenced by TLC, MnO_2

(70 mg, 0.8 mmol) was added and the reaction was stirred at room temperature (22 °C) for 5 min. The reaction was cooled and filtered through a fritted funnel. The solid MnO_2 was washed with ethanol (10 mL), and the combined filtrate was concentrated *in vacuo*. Flash column chromatography (hexane/ethyl acetate, 4:1) afforded the product (68 mg, 75%) as a yellow solid; mp 215–217 °C; R_f = *0*.13 (hexane/ethyl acetate, 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.31 (d, 1H, *J* = 0.9 Hz), 8.05 (d, 1H, *J* = 6.0 Hz), 7.99 (dd, 1H, J_1 = 1.2 Hz, J_2 = 6.3 Hz), 7.89 (d, 1H, *J* = 5.8 Hz), 7.64-7.44 (m, 5H), 7.34 (dd, 3H, J_1 = 5.6 Hz, J_2 = 6.0 Hz), 7.18 (d, 2H, *J* = 5.8 Hz), 7.13 (d, 1H, *J* = 6.3 Hz), 7.08 (t, 1H, *J* = 5.5 Hz), 5.22 (d, 1H, *J* = 9.9 Hz), 3.19 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.8, 155.9, 154.8, 147.0, 143.2 (× 2), 139.1, 136.2, 135.5, 130.4 (× 2), 129.8 (× 2), 129.2, 128.7, 128.0 (× 2), 126.4, 125.1, 123.9, 121.9, 121.3, 119.8, 117.7, 114.9, 111.7, 109.9 87.5, 36.5; IR (film) ν max 3059, 2944, 2901, 1627, 1594, 1492, 1464, 1435, 1347, 1298, 1270, 1127, 978, 910, 758, 730, 696 cm^{-1}; HRMS (ESI+) *m/z* 459.1663 ($M+H^+$, $C_{29}H_{23}N_4S$ requires 459.1643).

5-Benzothiazol-2-yl-3-ethyl-2-[(*E*)-2-(methylphenylamino)ethenyl]-1-phenyl-3*H*-benzo-imidazol-1-ium, iodide (1)

A slurry of **12** (35 mg, 0.076 mmol) in ethyl iodide (**76**, 3 mL) was heated to 75 °C and refluxed for 24 h. The solvent was removed *in vacuo*. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 20:1) to afford the product (41 mg, 88%) as a yellow solid; mp 165–167 °C; R_f = *0*.18 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.43 (s, 1H), 8.10-8.00 (m, 3H), 7.83-7.69 (m, 5H), 7.53-7.14 (m, 7H), 6.74 (d, *J* = 6.5 Hz, 2H), 5.52 (d, *J* = 12.3 Hz, 1H), 4.61 (q, *J* = 7.1 Hz, 2H), 3.41 (s, 3H), 1.61 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 167.2, 154.1, 152.1, 150.7 (× 2), 135.9, 135.3, 135.0, 132.1, 131.9 (× 2), 131.5, 131.2, 129.7 (× 2), 128.1 (× 2), 127.0, 126.3, 126.0, 125.5, 123.0, 122.1, 120.4 (× 2), 112.0 (× 2), 109.6, 78.2, 40.3, 12.9; IR (film) ν max 3290, 3058, 2924, 1688, 1618, 1507, 1537, 1494, 1465, 1371, 1308, 1203, 1132, 1029, 800, 763, 697 cm^{-1}; HRMS (ESI+) *m/z* 487.1949, ($M+H^+$, $C_{31}H_{27}N_4S$ requires 487.1956).

(4-Benzooxazol-2-yl-2-nitrophenyl)phenylamine (40)

2-(4-Chloro-3-nitro-phenyl)benzooxazole (**33**, 1.2 g, 4.5 mmol) and freshly distilled aniline (1.8 mL, 20 mmol) were used to synthesize **40** using the procedure described for preparation of **6**. Column chromatography (CH_2Cl_2) afforded the product (2.24 g, 84%) as a orange solid, mp 164–166 °C; R_f = *0*.15 (hexane/ethyl acetate, 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.82 (s, 1H), 9.09 (s, 1H), 8.20 (d, *J* = 9.0 Hz, 1H), 7.73 (m, 1H), 7.57 (m, 1H), 7.52-7.47 (m, 2H), 7.36-7.29 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.3, 150.6, 144.9, 142.0, 137.6, 134.0, 132.6, 130.0 (× 2), 126.7, 126.4, 125.1, 125.0 (× 2), 124.7, 119.7, 116.4 (× 2), 110.5; IR (film) ν max 3339, 1628, 1596, 1507, 1452, 1352, 1264, 1242, 1152, 1077 cm^{-1}; HRMS (ESI+) *m/z* 332.1035 ($M+H^+$, $C_{19}H_{14}N_3O_3$ requires 332.1035).

(2-Nitrophenyl)phenylamine (41)

1-Chloro-2-nitrobenzene (**34**, 1.57 g, 10 mmol) and freshly distilled aniline (2.7 mL, 30 mmol) were used to synthesize **41** using the procedure described for preparation of **6**. Column chromatography (CH_2Cl_2) afforded the product (1.58 g, 74%) as a orange solid, mp 73–75 °C; R_f = 0.18 (hexane/ethyl acetate, 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.50 (s, br, 1H), 8.20 (dd, J_1 = 7.6 Hz, J_2 = 1.4 Hz, 1H), 7.46-7.21 (m, 7H) 6.78 (m, 1H); ^{13}C NMR (75 MHz, CDC_{l3}) δ 143.1, 138.7, 135.7, 133.2, 129.7, 126.7, 125.6, 124.4, 117.5, 116.0; IR (film) ν max 3389, 1630, 1591, 1576, 1508, 1452, 1415, 1351, 1262, 1241, 1153, 1076 cm^{-1}. HRMS (ESI+) *m/z* 215.0822 ($M+H^+$, $C_{12}H_{11}N_2O_2$ requires 215.0821).

3-Nitro-4-phenylaminobenzoic acid methyl ester (42)

4-Chloro-3-nitrobenzoic acid methyl ester (**35**, 2.15 g, 10 mmol) and freshly distilled aniline (2.7 mL, 30 mmol) were used to synthesize **42** using the procedure described for preparation of **6**. Column chromatography (CH_2Cl_2) afforded the product (2.37 g, 87%) as a yellow solid, mp 121–123 °C; R_f = 0.48 (hexane/ethyl acetate, 3:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.81 (s, br, 1H), 8.93 (d, J = 2.0 Hz, 1H), 7.97 (dd, J_1 = 8.9 Hz, J_2 = 2.1 Hz, 1H), 7.48 (dd, $J_1 = J_2$ = 7.6 Hz, 2H), 7.32 (m, J_1 = 8.9 Hz, J_2 = 2.1 Hz, 3H), 7.17 (d, J = 9.0 Hz, 1H), 3.92 (s, 3H); ^{13}C NMR (100 MHz, CDC_{l3}) δ 165.4, 146.0, 137.6, 135.9, 132.2, 129.8 (× 2), 129.3, 126.8, 125.1 (× 2), 119.2, 115.6; IR (film) ν max 3337, 2951, 1718, 1624, 1596, 1567, 1524, 1497, 1436, 1354, 1312, 1281, 1212, 1151, 1119, 1071, 987, 918, 756, 692 cm^{-1}; HRMS (ESI+) *m/z* 273.0851 ($M+H^+$, $C_{14}H_{13}N_2O_4$ requires 273.0875).

3-Nitro-*N*-phenyl-4-phenylaminobenzamide (43)

4-Chloro-3-nitro-*N*-phenylbenzamide (2.2 g, 8 mmol) and freshly distilled aniline (3.7 mL, 40 mmol) were used to synthesize **43** using the procedure described for preparation of **6**. Flash column chromatography (hexane/ethyl acetate, 8:1) afforded the product (2.24 g, 84%) as a orange solid, mp 212–215 °C; R_f = 0.16 (hexane/ethyl acetate, 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.81 (s, 1H), 8.74 (d, J = 1.5 Hz, 1H), 7.98 (dd, J_1 = 1.3 Hz, J_2 = 6.7 Hz, 1H), 7.79 (s, 1H), 7.66 (d, J = 5.9 Hz, 2H), 7.50 (t, J = 5.9 Hz, 2H), 7.43 (t, J = 5.6 Hz, 2H), 7.34-7.27 (m, 3H), 7.19 (t, J = 5.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.8, 144.1, 139.8, 139.6, 131.9, 129.0 (× 2), 128.8, 128.5 (× 2), 123.1, 120.1 (× 2), 119.3, 118.3, 116.5 (× 2), 116.3, 114.7; IR (film) ν max 3331, 1648, 1623, 1596, 1440, 1319, 1267, 1214, 1152, 753, 690 cm^{-1}; HRMS (ESI+) *m/z* 334.1181 ($M+H^+$, $C_{19}H_{16}N_3O_3$ requires 334.1192).

4-Benzooxazol-2-yl-N^1-phenylbenzene-1,2-diamine (47)

Compound **40** (900 mg, 2.7 mmol) was treated with anhydrous hydrazine (0.5 mL, 16 mmol) and Pd/C (318 mg, 0.3 mmol) using the procedure described for preparation of **7**. Flash column chromatography (hexane/ethyl acetate, 6:1) afforded the product (758 mg, 93%) as a yellow solid; R_f = *0*.53 (hexane/ethyl acetate, 1:1); mp 142–143 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7,79-7.69 (m, 3H), 7.60-7.57 (m, 1 H), 7.37-7,29 (m, 5H), 7.01-6.98 (m, 3H), 5,60 (s, 1H), 3.65 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.4, 150.6, 142.9, 142.2, 138.7, 134.2, 129.4 (× 2), 124.6, 124.4, 121.6, 121.1, 119.7, 119.7, 119.5, 117.6 (× 2), 115.6, 110.5; IR (film) ν max 3364, 3048, 1614, 1594, 1557, 1497, 1454, 1313, 1244, 744 cm^{-1}; HRMS (ESI+) *m/z* 302.1302 ($M+H^+$, $C_{19}H_{16}N_3O$ requires 302.1293).

N-Phenylbenzene-1,2-diamine (48)

Compound **41** (1.07 g, 5.0 mmol) was treated with anhydrous hydrazine (0.75 mL, 24 mmol) and Pd/C (530 mg, 0.5 mmol) using the procedure described for preparation of **7**. Flash column chromatography (hexane/ethyl acetate, 8:1) afforded the product (840 mg, 91%) as a yellow solid; R_f = *0*.65 (hexane/ethyl acetate, 1:1); mp 77–79 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (dd, J_1 = 7.6 Hz, J_2 = 8.5 Hz, 2H), 7.10 (dd, J_1 = 7.9 Hz, J_2 = 1.5 Hz, 1H), 7.00 (dd, $J_1 = J_2$ = 7.2 Hz, 1H), 6.81-6.71 (m, 5H), 5,15 (s, br, 1H), 3.70 (s, br, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ145.3, 141.9, 129.2 (× 2), 128.5, 125.6, 124.9, 119.2, 119.0, 116.1, 115.1 (× 2); IR (film) ν max 3445, 3388, 3360, 3048, 1604, 1594, 1502, 1461, 1323, 1265, 1254 cm^{-1}; HRMS (ESI+) *m/z* 185.1075 ($M+H^+$, $C_{12}H_{12}N_2$ requires 185.1073).

3-Amino-4-phenylaminobenzoic acid methyl ester (49)

Compound **42** (816 mg, 3.0 mmol) was treated with anhydrous hydrazine (0.5 mL, 16 mmol) and Pd/C (318 mg, 0.3 mmol) using the procedure described for preparation of **7**.

Flash column chromatography (hexane/ethyl acetate, 6:1) afforded the product (675 mg, 93%) as a white solid; R_f = 0.45 (hexane/ethyl acetate, 1:1); mp 89–90 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.39 (s, 1H), 7.35 (d, *J* = 2.0 Hz, 1H), 7.24 (dd, $J_1 = J_2$ = 7.6 Hz, 2H), 7.15 (dd, J_1 = 2.0 Hz, J_2 = 7.6 Hz, 1H), 7.07 (d, J_1 = 7.6 Hz, 1H), 6.99 (d, *J* = 7.6 Hz, 2H), 6.85 (dd, *J* = 7.6 Hz, 1H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.0, 143.6, 139.2, 134.5, 129.6 (× 2), 122.5, 120.6, 119.1, 118.1 (× 2), 116.8, 116.1, 52.0; IR (film) ν max 3368, 3049, 2949, 1698, 1594, 1518, 1497, 1447, 1311, 1252, 1112, 1079, 993, 887, 763, 749, 695 cm^{-1}; HRMS (ESI+) *m/z* 243.1136 (M+H$^+$, $C_{14}H_{15}N_2O_2$ requires 243.1134).

3-Amino-*N*-phenyl-4-phenylaminobenzamide (50)

Compound **43** (1.0 g, 3.0 mmol) was treated with anhydrous hydrazine (0.5 mL, 16 mmol) and Pd/C (318 mg, 0.3 mmol) using the procedure described for preparation of **7**. Flash column chromatography (hexane/ethyl acetate, 6:1) afforded the product (875 mg, 96%) as a white solid; R_f = *0*.53 (hexane/ethyl acetate, 1:1); mp 176–178 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.34-7.29 (m, 4H), 7.23-7.10 (m, 4H), 7.05 (t, *J* = 7.4 Hz, 1H), 6.93 (d, *J* = 7.6 Hz, 2H), 6.79 (t, *J* = 7.2 Hz, 1H), 5.0 (s, 2H), 7.19 (t, *J* = 5.6 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.8, 144.1, 139.8, 139.6, 131.9, 129.1 (× 2), 128.8, 128.5 (× 2), 123.1, 120.1 (× 2), 119.3, 118.3, 116.5 (× 2), 116.4, 114.7; IR (film) ν max 3324, 1643, 1594, 1497, 1434, 1316, 1241, 886, 774, 691 cm^{-1}; HRMS (ESI+) *m/z* 304.1457 (M+H$^+$, $C_{19}H_{18}N_3O$ requires 304.1450).

[(*E*)-2-(5-Benzooxazol-2-yl-1-phenyl-1*H*-benzoimidazol-2-yl)ethenyl]methylphenylamine (61)

Compound **47** (60 mg, 0.2 mmol), **10** (32 mg, 0.2 mmol), and $ZrCl_4$ (24 mg, 0.1 mmol) were used to synthesize **61** using the procedure described for preparation of **12**. Flash column chromatography (hexane/ethyl acetate, 5:1) afforded the product (48 mg, 54%) as a light yellow solid; mp 194–196 °C; R_f = *0*.54 (hexane/ethyl acetate, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, *J* = 1.1 Hz, 1H), 8.35 (d, *J* = 13.2 Hz, 1H), 8.09 (dd, J_1 = 1.5 Hz, J_2 = 8.4 Hz, 1H), 7.78 (m, 1H), 7.68-7.54 (m, 4 H), 7.51-7.49 (m, 2H), 7.39-7.31 (m, 4H), 7.21-7.02 (m, 4H), 5.36 (d, *J* = 13.2 Hz, 1H), 3.15 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.3, 155.6, 150.8, 146.6, 142.8, 142.4, 140.0, 135.7, 130.0 (× 2), 129.3 (× 3), 128.9, 127.6 (× 2), 124.4, 124.3, 123.5, 121.4, 121.4, 119.6, 119.4 (× 2), 117.1, 110.4, 109.5, 87.0, 36.1; IR (film) ν max 3060, 1627, 1594, 1582, 1492, 1453, 1347, 1297, 1244, 1127, 746, 696 cm^{-1}; HRMS (ESI+) *m/z* 443.1875 (M+H$^+$, $C_{29}H_{23}N_4O$ requires 443.1872).

Methyl-phenyl-[(*E*)-2-(1-phenyl-1*H*-benzoimidazol-2-yl)ethenyl]amine (62)

N-Phenylbenzene-1,2-diamine (**48**, 37 mg, 0.2 mmol), **10** (32 mg, 0.2 mmol), and $ZrCl_4$ (24 mg, 0.1 mmol) were used to synthesize **62** using the procedure described for preparation of **12**. Flash column chromatography (hexane/ethyl acetate, 5:1) afforded the product (39 mg, 61%) as a yellow solid; mp 118–121 °C; R_f = *0*.54 (hexane/ethyl acetate, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (d, *J* = 13.3 Hz, 1H), 7.71(d, *J* = 7.9 Hz, 1H), 7.64-7.46 (m, 5H), 7.34 (t, *J* = 7.9 Hz, 2H), 7.25-7.05 (m, 6H), 5.26 (d, *J* = 13.3 Hz, 1H), 3.20 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.8, 146.7, 142.0, 136.5, 136.3, 129.8 (× 2), 129.3 (× 2), 128.5, 127.6 (× 2), 123.1, 122.4, 121.1, 119.2 (× 2), 117.6, 109.2 (× 2), 87.6, 35.9; IR (film) ν max 3060, 1629, 1594, 1492, 1454, 1347, 1301, 1286, 1267, 1127, 757, 695 cm^{-1}; HRMS (ESI +) *m/z* 326.1651 (M+H$^+$, $C_{22}H_{20}N_3$ requires 326.1657).

2-[(*E*)-2-(Methylphenylamino)ethenyl]-1-phenyl-1*H*-benzoimidazole-5-carboxylic acid methyl ester (63)

3-Amino-4-phenylaminobenzoic acid methyl ester (**49**, 48 mg, 0.2 mmol), **10** (32 mg, 0.2 mmol), and $ZrCl_4$ (24 mg, 0.1 mmol) were used to synthesize **63** using the procedure described for preparation of **12**. Flash column chromatography (hexane/ethyl acetate, 5:1)

afforded the product (50 mg, 65%) as a light yellow solid; mp 169–170 °C; R_f = 0.64 (hexane/ethyl acetate, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (d, *J* = 1.3 Hz, 1H), 8.28 (d, *J* = 13.3 Hz, 1H), 7.82 (dd, J_1 = 1.5 Hz, J_2 = 8.4 Hz, 1H), 7.63-7.53 (m, 3H), 7.45-7.43 (m, 2H), 7.33 (t, *J* = 7.4 Hz, 2H), 7.15 (d, *J* = 7.7 Hz, 2H), 7.07 (m, 2H), 5.20 (d, *J* = 13.3 Hz, 1H), 3.93 (s, 3H), 3.17 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.9, 155.7, 146.6, 143.3, 142.7, 139.9, 135.8, 130.0 (× 2), 129.3 (× 2), 128.8, 127.5 (× 2), 124.4, 123.5, 123.0, 119.6, 119.3 (× 2), 108.6, 87.1, 52.0, 36.1; IR (film) ν max 1712, 1628, 1492, 1438, 1348, 1297, 1224, 1127, 1085, 751, 696 cm^{-1}; HRMS (ESI+) *m/z* 384.1731 ($M+H^+$, $C_{24}H_{22}N_3O_2$ requires 384.1712).

2-[(*E*)-2-(Methylphenylamino)ethenyl]-1-phenyl-1*H*-benzoimidazole-5-carboxylic acid phenylamide (64)

Compound **50** (61 mg, 0.2 mmol), **10** (32 mg, 0.2 mmol), and $ZrCl_4$ (24 mg, 0.1 mmol) were used to synthesize **64** using the procedure described for preparation of **12**. Flash column chromatography (hexane/ethyl acetate, 5:1) afforded the product (50 mg, 56%) as a yellow solid; mp 121–123 °C; R_f = 0.50 (hexane/ethyl acetate, 1:1); ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.28 (d, *J* = 17.3 Hz, 1H), 8.12(s, 1H), 7.72-7.07 (m, 18H), 5.21 (d, *J* = 13.4 Hz, 1H), 3.16 (s, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 169.3, 158.3, 157.5, 148.0, 144.5, 143.7, 140.1, 140.0, 136.8, 131.4 (× 2), 130.8, 130.6 (× 2), 130.5, 129.8 (× 2), 128.7, 125.4, 124.8, 122.6, 122.4 (× 2), 120.3 (× 2), 117.4, 110.2, 87.1, 36.3; IR (film) ν max 3290, 3059, 1625, 1595, 1540, 1498, 1433, 1316, 1297, 1127, 751 cm^{-1}; HRMS (ESI+) *m/z* 445.2021 ($M+H^+$, $C_{29}H_{25}N_4O$ requires 445.2028).

5-Benzooxazol-2-yl-3-ethyl-2-[(*E*)-2-(methylphenylamino)ethenyl]-1-phenyl-3*H*-benzoimidazol-1-ium, iodide (16)

Compound **61** (35 mg, 0.079 mmol) and ethyl iodide (3 mL) were used to synthesize **16** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 20:1) afforded the product (40 mg, 86%) as a yellow solid, mp 181–184 °C; R_f = 0.22 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.75 (s, 1H), 8.44 (d, *J* = 12.2 Hz, 1H), 7.71-7.56 (m, 7H), 7.31-7.03 (m, 7H), 6.52 (m, 2H), 5.41 (d, *J* = 11.6 Hz, 1H), 4.50 (q, *J* = 6.7 Hz, 2H), 3.17 (s, 3H), 1.50 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.4, 153.3, 152.2, 151.8, 142.9, 137.3, 136.0, 132.9 (× 3), 132.6, 130.7 (× 3), 129.2 (× 3), 127.3, 127.0, 126.3, 126.2, 125.4, 120.8, 113.1, 111.9, 111.3, 79.1, 41.1, 36.9, 13.9; IR (film) ν max 3425, 1681, 1617, 1586, 1537, 1494, 1453, 1365, 1298, 1197, 1136, 761, 697 cm^{-1}; HRMS (ESI+) *m/z* 471.2168 (M^+, $C_{31}H_{27}N_4O$ requires 471.2185).

1-Ethyl-2-[(*E*)-2-(methylphenylamino)ethenyl]-3-phenyl-3*H*-benzoimidazol-1-ium, iodide (15)

Compound **62** (30 mg, 0.092 mmol) and ethyl iodide (3 mL) were used to synthesize **15** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 20:1) afforded the product (37 mg, 85%) as a yellow solid; mp 43–45 °C; R_f = 0.28 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, MeOH-d_4) δ 7.83-7.77 (m, 4H), 7.69-7.66(m, 2H), 7.52 (t, *J* = 7.2, 1H), 7.44 (t, *J* = 7.3 Hz, 1H), 7.31-7.08 (m, 5H), 6.74 (d, *J* = 7.9 Hz, 2H), 5.56 (d, *J* = 11.3 Hz, 1H), 4.58 (d, *J* = 7.3 Hz, 2H), 3.41 (s, 3H), 1.58 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 152.1, 151.6, 151.5, 147.6, 136.8, 135.5, 133.2 (× 2), 132.9, 132.7, 131.1 (× 2), 129.7 (× 2), 127.4, 127.3, 127.2, 121.8, 112.9, 112.8, 79.5, 41.6, 37.5, 14.4; IR (film) ν max 3360, 1688, 1623, 1585, 1533, 1494, 1361, 1310, 1201, 1177, 1129, 759, 697 cm^{-1}; HRMS (ESI+) *m/z* 354.1949 (M^+, $C_{24}H_{24}N_3$ requires 354.1970).

1-Ethyl-6-methoxycarbonyl-2-[(*E*)-2-(methylphenylamino)ethenyl]-3-phenyl-3*H*-benzoimidazol-1-ium, iodide (14)

Compound **63** (30 mg, 0.078 mmol) and ethyl iodide (3 mL) were used to synthesize **14** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 20:1) afforded the product (39 mg, 93%) as a yellow solid, mp 43–46 °C; R_f = 0.25 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.47 (s, 1H), 8.02 (dd, J_1 = 8.6 Hz, J_2 = 1.2 Hz, 1H), 7.84-8.63 (m, 5H), 7.29 (t, J = 8.1 Hz, 2H), 7.21-7.14 (m, 3H), 6.70 (d, J = 7.9 Hz, 2H), 5.76 (d, J = 13.5 Hz, 1H), 4.70 (q, J = 7.1 Hz, 2H), 3.92 (s, 3H), 3.41 (s, 3H), 1.46 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.6, 152.0, 149.5, 145.2, 136.6, 134.5, 131.5 (× 2), 131.1, 130.9, 129.5 (× 3), 128.0 (× 2), 126.5, 126.4, 125.6, 119.6, 112.7, 111.3, 79.1, 52.5, 39.4, 36.4, 13.7; IR (film) ν max 3412, 1716, 1692, 1622, 1588, 1538, 1621, 1588, 1538, 1494, 1463, 1373, 1312, 1290, 1266, 1198, 1134, 764, 698 cm^{-1}; HRMS (ESI+) *m/z* 412.2000 (M^+, $C_{26}H_{26}N_3O_2$ requires 412.2028).

3-Ethyl-2-[(*E*)-2-(methylphenylamino)ethenyl]-1-phenyl-5-phenylcarbamoyl-3*H*-benzoimidazol-1-ium, iodide (18)

Compound **64** (35 mg, 0.079 mmol) and ethyl iodide (3 mL) were used to synthesize **18** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 20:1) afforded the product (40 mg, 84%) as a yellow solid, mp 285–287 °C (decomp.); R_f = 0.44 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.4 (s, 1H), 8.52(s, 1H), 8.06 (d, J = 8.4 Hz, 1H), 7.82 (m, 7H), 7.40 (t, J = 7.4 Hz, 2H), 7.32 (t, J = 7.2 Hz, 2H), 7.24-7.15 (m, 4H), 6.73 (d, J = 7.6 Hz, 2H), 5.77 (d, J = 12.8 Hz), 4.69 (q, J = 6.5 Hz, 2H), 3.43 (s, 3H), 1.53 (t, J = 6.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.8, 152.0, 149.7, 145.6, 139.3, 135.8, 134.9, 132.2, 131.9 (× 2), 131.4, 131.1, 129.9 (× 2), 129.1 (× 2), 128.4 (× 2), 125.9, 125.7, 124.4, 121.0 (× 2), 119.9 (× 2), 111.6, 111.3, 79.6, 40.9, 36.8, 14.2; IR (film) ν max 3260, 1662, 1620, 1587, 1525, 1493, 1463, 1440, 1369, 1306, 1251, 1128, 1209, 758 cm^{-1}; HRMS (ESI+) *m/z* 473.2335 (M^+, $C_{31}H_{29}N_4O$ requires 473.2341).

2-(1,2-Diphenyl-1*H*-benzoimidazol-5-yl)benzothiazole (68)

Compound **7** (64 mg, 0.2 mmol) and benzaldehyde (20 mL, 0.2 mmol) were used to synthesize **68** using the procedure described for preparation of **12**. Flash column chromatography (hexane/ethyl acetate, 6:1) afforded the product (42 mg, 52%) as a light yellow solid; mp 184–186 °C; R_f = 0.28 (hexane/ethyl acetate, 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, J = 1.3 Hz, 1H), 8.14 (dd, J_1 = 1.7 Hz, J_2 = 8.6 Hz, 1H), 8.07 (d, J = 8.0 Hz, 1H), 7.90 (d, J = 8.3 Hz, 1H), 7.60-7.43 (m, 6H), 7.38-7.29 (m, 7H); ^{13}C NMR (75 MHz, $CDCl_3$) δ168.8, 154.3, 153.9, 143.2, 139.2, 136.6, 135.1, 130.0 (× 2), 129.8, 129.5 (× 2), 129.0, 128.9, 128.4 (× 2), 127.3 (× 2), 126.6, 126.2, 123.0, 122.9 (× 2), 121.5, 119.7, 111.0 cm^{-1}; HRMS (ESI+) *m/z* 404.1249 ($M+H^+$, $C_{26}H_{18}N_3S$ requires 404.1221).

2-(2-Phenethyl-1-phenyl-1*H*-benzoimidazol-5-yl)benzothiazole (71)

Compound **7** (64 mg, 0.2 mmol) and 3-phenylpropionaldehyde (26 mL, 0.2 mmol) were used to synthesize **71** using the procedure described for preparation of **12**. Flash column chromatography (hexane/ethyl acetate, 3:1) afforded the product (41 mg, 48%) as a light yellow solid; mp 56–58 °C; R_f = 0.10 (hexane/ethyl acetate, 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (s, 1H), 8.07 (dd, 2H, J_1 = 3.2 Hz, J_2 = 4.8 Hz), 7.91 (d, 1H, J = 7.9 Hz), 7.55-7.31 (m, 5H), 7.25-7.14 (m, 6H), 7.08 (d, 2H, J = 7.0 Hz), 3.21 (t, 4H, J = 6.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 156.0, 154.3, 142.8, 140.6, 138.4, 135.3, 135.1, 130.0 (× 2), 129.3, 128.5 (× 2), 128.4 (× 2), 127.3 (× 2), 126.3, 126.2, 124.9, 123.0 (× 2), 122.4, 121.5, 119.1, 110.6, 34.0, 29.8; IR (film) ν max 3052, 3028, 2921, 2862, 1596, 1514, 1498,

1462, 1434, 1393, 1314, 1274, 758, 728, 699 cm^{-1}; HRMS (ESI+) *m/z* 432.1542 ($M+H^+$, $C_{28}H_{22}N_3S$ requires 432.1534).

2-[2-((*E*)-2-Furan-2-yl-ethenyl)-1-phenyl-1*H*-benzoimidazol-5-yl]benzothiazole (69)

To a solution of compound **7** (64 mg, 0.2 mmol) and *trans*-3-(2-furyl)acrolein (24 mg, 0.2 mmol) in ethanol (15 mL) was added $ZrCl_4$ (24 mg, 0.1 mmol). The reaction was heated to 80 °C and refluxed for 30 min. The reaction was cooled and poured into aqueous ammonium hydroxide (5%, 50 mL). The product was extracted with CH_2Cl_2 (50 mL × 2). The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography (hexane/ethyl acetate, 5:1) afforded the product (69 mg, 82%) as a yellow solid; mp 97–99 °C; R_f = 0.18 (hexane/ethyl acetate, 3:1); ^1H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, 1H, *J* = 1.1 Hz), 8.11 (dd, 1H, J_1 = 1.5 Hz, J_2 = 8.5 Hz), 8.07 (d, 1H, *J* = 7.9 Hz), 7.91 (d, 1H, *J* = 7.8 Hz), 7.83 (d, 1H, *J* = 15.7 Hz), 7.68-7.58 (m, 3H), 7.50-7.46 (m, 3H), 7.40-7.37 (m, 2H), 7.26 (d, 1H, *J* = 8.5 Hz), 6.71 (d, 1H, *J* = 15.7 Hz), 6.57 (d, 1H, *J* = 3.3 Hz), 6.45 (dd, 1H, J_1 = 1.8 Hz, J_2 = 3.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.7, 154.3, 152.3, 152.0, 144.2, 138.4, 135.2, 134.9, 130.3 (× 2), 130.1, 129.5, 127.7 (× 2), 127.4, 125.7, 123.1, 123.0, 122.9 (× 2), 118.8, 118.7, 113.5, 112.5, 112.3, 110.9, 110.7; IR (film) ν max 3284, 3060, 2931, 2860, 1652, 1608, 1595, 1498, 1434, 1380, 1314, 1273, 1224, 1016, 757, 695 cm^{-1}; HRMS (ESI+) *m/z* 420.1166 ($M+H^+$, $C_{26}H_{18}N_3OS$ requires 420.1171).

2-(1-Phenyl-2(*E*)-styryl-1*H*-benzoimidazol-5-yl)benzothiazole (70)

Compound **7** (64 mg, 0.2 mmol) and *trans*-cinnamaldehyde (26 mL, 0.2 mmol) were used to synthesize **70** using the procedure described for preparation of **69**. Flash column chromatography (hexane/ethyl acetate, 5:1) afforded the product (73 mg, 85%) as a yellow solid; mp 218–220 °C; R_f = 0.20 (hexane/ethyl acetate, 3:1); ^1H NMR (300 MHz, $CDCl_3$) δ 8.48 (d, 1H, *J* = 1.4 Hz), 8.11 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.5 Hz), 8.07 (d, 1H, *J* = 8.1 Hz), 8.01 (d, 1H, *J* = 16.0 Hz), 7.90 (d, 1H, *J* = 7.6 Hz), 7.66-7.55 (m, 3H), 7.50-7.45 (m, 5H), 7.37-7.32 (m, 5H), 6.85 (d, 1H, *J* = 16.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.8, 154.3, 152.4, 143.4, 138.5, 138.1, 135.8, 130.14 (× 2), 129.3 (× 2), 129.2 (× 2), 128.8 (× 2), 127.4, 126.6 (× 2), 126.2, 123.0 (× 2), 122.7, 121.5, 119.2 (× 2), 113.5, 110.7 (× 2); IR (film) ν max 3059, 2217, 1633, 1596, 1499, 1433, 1386, 1340,1274, 1216, 909, 756, 729 cm^{-1}; HRMS (ESI+) *m/z* 430.1393 ($M+H^+$, $C_{28}H_{20}N_3S$ requires 430.1378).

2-{2-[(*E*)-2-(4-Methoxyphenyl)ethenyl]-1-phenyl-1*H*-benzoimidazol-5-yl} benzothiazole (72)

Compound **7** (64 mg, 0.2 mmol) and *trans*-4-methoxycinnamaldehyde (33 mg, 0.2 mmol) were used to synthesize **72** using the procedure described for preparation of **69**. Flash column chromatography (hexane/ethyl acetate, 5:1) afforded the product (79 mg, 86%) as a yellow solid; mp 177–179 °C; R_f = 0.17 (hexane/ethyl acetate, 3:1); ^1H NMR (300 MHz, $CDCl_3$) δ 8.46 (d, 1H, *J* = 1.4 Hz), 8.10 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.6 Hz), 8.07 (d, 1H, *J* = 7.2 Hz), 7.98 (d, 1H, *J* = 16.0 Hz), 7.91 (d, 1H, *J* = 7.9 Hz), 7.66-7.59 (m, 3H), 7.49-7.34 (m, 6H), 7.25 (d, 1H, *J* = 8.5 Hz), 6.88 (d, 2H, *J* = 8.7 Hz), 6.69 (d, 1H, *J* = 16.0 Hz), 3.81 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 160.6, 154.3, 152.9, 143.3, 138.5, 137.9, 135.2, 135.1, 130.2 (× 2), 129.3, 129.1 (× 2), 129.0, 128.6 (× 2), 127.5, 126.2, 124.9, 123.0, 122.5, 121.5, 118.9, 114.3 (× 2), 111.0, 110.5, 55.3; IR (film) ν max 3061, 2954, 2929, 2837, 1603, 1514, 1500, 1434, 1304, 1254, 1173, 759 cm^{-1}; HRMS (ESI+) *m/z* 460.1473 ($M+H^+$, $C_{29}H_{22}N_3OS$ requires 460.1484).

2-(2-Nona-1(*E*),3(*E*)-dienyl-1-phenyl-1*H*-benzoimidazol-5-yl)benzothiazole (73)

Compound **7** (64 mg, 0.2 mmol) and *trans*,*trans*-2,4-decadienal (35 mL, 0.2 mmol) were used to synthesize **73** using the procedure described for preparation of **69**. Flash column

chromatography (hexane/ethyl acetate, 8:1) afforded the product (63 mg, 67%) as a yellow glassy solid; R_f = 0.40 (hexane/ethyl acetate, 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (d, *J* = 1.5 Hz, 1H), 8.07 (m, 2H), 7.89 (d, *J* = 7.9 Hz, 1H), 7.64-7.56 (m, 4H), 7.50-7.33 (m, 4H), 7.23 (d, *J* = 19.0 Hz, 1H), 6.24-6.04 (m, 3H), 2.18-2.11 (m, 2H), 1.47-1.23 (m, 6H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 154.4, 152.9, 143.5, 142.0, 139.1, 138.5, 135.3, 135.1, 130.0 (× 2), 129.5, 129.2, 129.0 (× 2), 127.5, 126.2, 124.8, 123.0, 122.4, 121.5, 119.0, 114.4, 110.4, 33.0, 31.4, 28.6, 22.5, 14.0; IR (film) ν max 3048, 2925, 2855, 1638, 1614, 1596, 1498, 1455, 1434, 1388, 1314, 1291, 993, 757 cm^{-1}; HRMS (ESI +) *m/z* 450.2025 (M+H$^+$, $C_{29}H_{28}N_3S$ requires 450.2004).

2-(1-Phenyl-1*H*-benzoimidazol-5-yl)benzothiazole (74)

To a solution of **7** (130 mg, 0.4 mmol) and **10** (65 mg, 0.4 mmol) in anhydrous ethanol (15 mL) was added activated 3Å molecular sieves (0.5 g). The reaction was heated to 80 °C and refluxed for 48 h. The reaction was cooled and filtered through a fritted funnel. The molecular sieves were washed with ethanol (10 mL), and the combined filtrate was concentrated *in vacuo*. Flash column chromatography (hexane/ethyl acetate, 2:1) afforded the product (20 mg, 31%) as a white solid; mp 138–140 °C; R_f = 0.17 (hexane/ethyl acetate, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, *J* = 1.1 Hz, 1H), 8.15 (s, 1H), 8.14 (dd, J_1 = 1.4 Hz, J_2 = 9.9 Hz, 1H), 8.06 (d, *J* = 8.1 Hz, 1H), 7.87 (d, *J* = 7.9 Hz, 1H), 7.58-7.54 (m, 3H), 7.49-7.44 (m, 4H), 7.34 (t, *J* = 7.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 154.2, 144.3, 143.7, 135.8, 135.5, 135.0, 130.2 (× 2), 128.8, 128.4, 126.9, 126.2, 124.9, 124.0 (× 2), 123.2, 123.0, 121.5, 120.3, 111.0; IR (film) ν max 3382, 3064, 2954, 2360, 1617, 1599, 1509, 1461, 1435, 1314, 1284, 1240, 1216, 1167, 750, 719, 692 cm^{-1}; HRMS (ESI+) *m/z* 328.0897 (M+H$^+$, $C_{20}H_{14}N_3S$ requires 328.0908).

3-(5-Benzothiazol-2-yl-1-phenyl-1*H*-benzoimidazol-2-yl)chromen-2-one (75)

To coumarin-3-carboxylic acid (40 mg, 0.2 mmol) in a round bottom flask (25 mL) fitted with a condenser was added thionyl chloride (2 mL, 10 mmol). The reaction was heated to 80 °C and refluxed for 2 h. Excess thionyl chloride was removed by distillation, and the residue was further dried by applying high vacuum for 30 min. The residue was dissolved in anhydrous toluene (5 mL), compound **7** (64 mg, 0.2 mmol) was added, and the reaction was stirred at 22 °C for 30 min. The reaction was subsequently heated to 110 °C and refluxed for 12 h. The reaction was cooled and concentrated *in vacuo*. Flash column chromatography (hexane/ethyl acetate, 5:1) afforded the product (36 mg, 38%) as a off white solid; mp 164–166 °C; R_f = 0.20 (hexane/ethyl acetate, 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, *J* = 1.4 Hz, 1H), 8.40 (s, 1H), 8.21 (dd, J_1 = 1.6 Hz, J_2 = 8.5 Hz, 1H), 8.11 (d, *J* = 8.1 Hz, 1H), 7.94 (d, *J* = 7.9 Hz, 1H), 7.64-7.35 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 157.8, 154.5, 154.3, 149.0, 146.4, 142.9, 138.6, 136.1, 135.1, 133.1, 129.8 (× 2), 129.2, 128.9, 128.8, 126.2 (× 2), 126.1,125.0, 124.9, 123.7, 124.9, 123.7, 123.0, 121.6, 119.9, 119.3, 118.5, 116.8, 111.2; IR (film) ν max 3060, 2966, 1735, 1608, 1499, 1456, 1434, 1386, 1325, 1282, 1242, 1215, 756 cm^{-1}; HRMS (ESI+) *m/z* 472.1113 (M+H$^+$, $C_{29}H_{18}N_3O_2S$ requires 472.1120).

5-Benzothiazol-2-yl-3-ethyl-1,2-diphenyl-3*H*-benzoimidazol-1-ium, iodide (19)

Compound **68** (30 mg, 0.074 mmol) and ethyl iodide (3 mL) were used to synthesize **19** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 20:1) afforded the product (37 mg, 90%) as a white solid; mp 72–74 °C; R_f = 0.33 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.88 (s, 1H), 8.43 (d, *J* = 8.6 Hz, 1H), 8.09 (t, *J* = 8.2 Hz, 2H), 7.73-7.47 (m, 13H), 4.65 (q, *J* = 7.1 Hz, 2H), 1.64 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 166.6, 154.1, 152.5, 135.6, 135.0, 133.4, 133.3, 132.9, 132.0, 131.2, 130.7 (× 2), 130.5 (× 2), 129.7 (× 2), 127.7 (× 2), 127.4, 127.1, 126.3, 123.3, 122.2, 121.4, 114.6, 112.2, 42.6, 14.0; IR (film) ν max 3425, 3060, 2990, 1778, 1737,

1688, 1502, 1454, 1434, 1198, 1151, 1138, 760, 703 cm^{-1}; HRMS (ESI+) *m/z* 432.1518 (M^+, $C_{28}H_{22}N_3S$ requires 432.1534).

5-Benzothiazol-2-yl-3-ethyl-2-phenethyl-1-phenyl-3*H*-benzoimidazol-1-ium, iodide (22)

Compound **71** (35 mg, 0.08 mmol) and ethyl iodide (3 mL) were used to synthesize **22** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 20:1) afforded the product (44 mg, 93%) as a white solid; mp 131–133 °C; R_f = 0.30 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.73 (s, 1H), 8.30 (dd, J_1 = 1.4 Hz, J_2 = 8.6 Hz, 1H), 8.04 (t, *J* = 8.1 Hz, 2H), 7.83-7.72 (m, 3H), 7.58-7.44 (m, 5H), 7.24-7.21 (m, 3H), 6.99-6.96 (m, 2H), 4.70 (q, *J* = 7.2 Hz, 2H), 3.52 (t, *J* = 7.6 Hz, 2H), 2.98 (t, *J* = 7.6 Hz, 2H), 1.65 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 167.5, 155.8, 155.0, 139.4, 136.6, 135.8, 133.9, 133.2, 132.9, 132.5, 132.0 (× 2), 130.2 (× 2), 129.4 (× 2), 128.5 (× 2), 128.4, 128.1, 128.0, 127.2, 124.2, 123.1, 115.1, 112.8, 42.9, 33.8, 27.8, 14.9; IR (film) ν max 3416, 3064, 1733, 1688, 1506, 1471, 1455, 1199, 1135, 762, 701 cm^{-1}; HRMS (ESI+) *m/z* 460.1833 (M^+, $C_{30}H_{26}N_3S$ requires 460.1847).

5-Benzothiazol-2-yl-3-ethyl-2-((*E*)-2-furan-2-yl-ethenyl)-1-phenyl-3*H*-benzo imidazol-1-ium, iodide (20)

Compound **69** (30 mg, 0.07 mmol) and ethyl iodide (3 mL) were used to synthesize **20** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 20:1) afforded the product (35 mg, 88%) as a yellow solid; mp 83–85 °C; R_f = 0.30 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.60 (d, 1H, *J* = 1.0 Hz), 8.19 (dd, J_1 = 1.5 Hz, J_2 = 8.7 Hz, 1H), 7.95 (t, *J* = 7.3 Hz, 2H), 7.72-7.62 (m, 6H), 7.47-7.35 (m, 3H), 6.85 (d, *J* = 1.4 Hz, 2H), 6.73 (d, *J* = 3.5 Hz, 1H), 6.51 (q, *J* = 1.8 Hz, 1H), 4.73 (q, *J* = 7.3 Hz, 2H), 1.61 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 168.0, 155.5, 152.0, 150.4, 149.0, 136.9, 136.8, 134.8, 134.7, 134.3, 133.6, 133.4, 132.7, 131.8, 129.2 (× 2), 128.5 (× 2), 127.6, 120.2, 115.1, 114.9, 112.7, 104.4, 43.2, 15.2; IR (film) ν max 3429, 3063, 2342, 1688, 1626, 1447, 1199, 1128, 762 cm^{-1}; HRMS (ESI+) *m/z* 448.1454 (M^+, $C_{28}H_{22}N_3OS$ requires 448.1484).

5-Benzothiazol-2-yl-3-ethyl-1-phenyl-2(*E*)-styryl-3*H*-benzoimidazol-1-ium, iodide (21)

Compound **70** (35 mg, 0.08 mmol) and ethyl iodide (3 mL) were used to synthesize **21** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 20:1) afforded the product (43 mg, 92%) as a light yellow solid; mp 199–202 °C; R_f = 0.33 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.76 (d, *J* = 0.9 Hz, 1H), 8.34 (dd, J_1 = 1.5 Hz, J_2 = 8.7 Hz, 1H), 8.06 (t, *J* = 7.7 Hz, 2H), 7.82-7.76 (m, 6H), 7.57-7.42 (m, 9H), 7.21 (q, *J* = 7.2 Hz, 2H), 1.73 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 166.6, 154.1, 149.5, 148.9, 135.6, 135.4, 134.1, 133.6, 133.0, 132.1, 132.0, 131.8, 131.2 (× 2), 129.4 (× 2), 128.5 (× 2), 127.8 (× 2), 127.2, 127.1, 126.3, 123.3, 122.2, 114.0, 111.6, 106.6, 42.0, 13.9; IR (film) ν max 3416, 3061, 2988, 1688, 1632, 1503, 1470, 1441, 1199, 1135, 760 cm^{-1}; HRMS (ESI+) m/z 458.1684 (M^+, $C_{30}H_{24}N_3S$ requires 458.1691).

5-Benzothiazol-2-yl-3-ethyl-2-[(*E*)-2-(4-methoxyphenyl)ethenyl]-1-phenyl-3*H*-benzoimidazol-1-ium, iodide (23)

Compound **72** (35 mg, 0.076 mmol) and ethyl iodide (3 mL) were used to synthesize **23** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 20:1) afforded the product (42 mg, 90%) as a yellow solid; mp 188–192 °C; R_f = 0.33 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.51 (d, *J* = 1.0 Hz, 1H), 8.09 (d, *J* = 1.4 Hz, 1H), 7.88 (t, *J* = 7.5 Hz, 1H), 7.68-7.66 (m, 5H), 7.60-7.33 (m, 5H), 6.90 (d, *J* = 3.1 Hz, 2H), 6.81 (d, *J* = 8.8 Hz, 2H), 4.67 (q, *J* = 7.2 Hz, 2H), 3.68 (s, 3H), 1.55(t, *J*

= 7.2 Hz, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 170.4, 167.2, 157.8, 153.4, 152.3, 139.2, 139.0, 137.4, 136.4, 135.7, 135.5, 135.0 (× 2), 134.4 (× 2), 131.6 (× 2), 130.9, 130.8, 130.7, 130.0 126.9, 125.9, 118.5 (× 2), 117.4, 115.0, 107.0, 58.8, 46.2, 17.6; IR (film) ν max 3404, 3064, 1688, 1628, 1599, 1573, 1520, 1470, 1256, 1199, 1176, 1116, 763 cm^{-1}; HRMS (ESI +) *m/z* 488.1770 (M^+, $C_{31}H_{26}N_3OS$ requires 488.1797).

5-Benzothiazol-2-yl-3-ethyl-2-nona-1(*E*),3(*E*)-dienyl-1-phenyl-3*H*-benzoimidazol-1-ium, iodide (24)

Compound **73** (30 mg, 0.067 mmol) and ethyl iodide (3 mL) were used to synthesize **24** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 20:1) afforded the product (36 mg, 89%) as a yellow solid; mp 66–68 °C; R_f = 0.33 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.68 (d, J = 1.0 Hz, 1H), 8.26 (dd, J_1 = 1.5 Hz, J_2 = 8.7 Hz, 1H), 8.04 (t, J = 6.4 Hz, 2H), 7.81-7.30 (m, 9H), 6.73-6.62 (m, 1H), 6.42-6.34 (m, 1H), 6.11-6.01 (m, 1H), 4.73 (q, J = 7.2 Hz, 2H), 2.22-2.16 (m, 2H), 1.65 (t, J = 7.2 Hz, 3H), 1.44-1.29 (m, 6H), 0.89 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 166.7, 154.1, 149.6, 149.4, 135.6, 135.4, 134.0, 133.5, 132.8, 132.0, 131.9, 131.2 (× 2), 129.6, 127.8 (× 2), 127.1, 127.0, 126.2, 123.2, 122.2, 113.7, 111.3, 107.4, 41.7, 33.2, 31.5, 28.3, 22.5, 13.8, 13.6; IR (film) ν max 3416, 3051, 2950, 2923, 2862, 1687, 1632, 1613, 1501, 1469, 1198, 1166, 1126, 762 cm^{-1}; HRMS (ESI+) *m/z* 478.2298 (M^+, $C_{31}H_{32}N_3S$ requires 478.2317).

5-Benzothiazol-2-yl-3-ethyl-1-phenyl-3*H*-benzoimidazol-1-ium, iodide (13)

Compound **74** (35 mg, 0.11 mmol) and ethyl iodide (3 mL) were used to synthesize **13** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 20:1) afforded the product (49 mg, 93%) as a white solid; mp 122–124 °C; R_f = 0.30 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, MeOH-d_4) δ 9.95 (s, 1H), 8.71 (d, J = 1.0 Hz, 1H), 8.34 (dd, J_1 = 1.5 Hz, J_2 = 8.8 Hz, 1H), 7.98 (t, J = 7.1 Hz, 2H), 7.87 (d, J = 8.8 Hz, 1H), 7.76-7.68 (m, 5H), 7.49-7.32 (m, 2H), 4.70 (q, J = 7.3 Hz, 2H), 1.72 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 167.4, 155.1, 144.4, 136.6, 134.7, 134.5, 134.2, 133.5, 132.2, 131.8 (× 2), 128.3, 128.1, 127.3, 126.3 (× 2), 124.3, 123.1, 115.7, 113.4, 44.4, 14.5; IR (film) ν max 3434, 3131, 3064, 1780, 1736, 1686, 1561, 1499, 1478, 1446, 1416, 1314, 1200, 1143, 758, 706 cm^{-1}; HRMS (ESI+) *m/z* 356.1200 (M^+, $C_{22}H_{18}N_3S$ requires 356.1221).

5-Benzothiazol-2-yl-3-ethyl-2-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-3*H*-benzoimidazol-1-ium, iodide (25)

Compound **75** (20 mg, 0.042 mmol) and ethyl iodide (3 mL) were used to synthesize **25** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 20:1) afforded the product (21 mg, 81%) as a light yellow solid; mp 206–208 °C; R_f = 0.25 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.88 (d, J = 1.0 Hz, 1H), 8.63 (s, 1H), 8.43 (dd, J_1 = 1.5 Hz, J_2 = 8.8 Hz, 1H), 8.06 (dd, J_1 = 1.0 Hz, J_2 = 8.1 Hz, 2H), 7.78-7.38 (m, 12H), 4.76 (q, J = 7.2 Hz, 2H), 1.60 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.7, 158.2, 155.8, 154.7, 153.5, 147.2, 136.7, 136.2, 135.5, 134.1, 133.0, 132.5, 132.4, 131.4 (× 2), 131.3, 128.4, 128.0 (× 2), 127.9, 127.0, 123.1, 118.3, 117.8, 115.6, 113.3, 110.8, 43.7, 15.0; IR (film) ν max 3412, 3060, 1725, 1687, 1609, 1576, 1503, 1437, 1255, 1201, 1174, 1128, 761 cm^{-1}; HRMS (ESI+) *m/z* 500.1440 (M^+, $C_{31}H_{22}N_3O_2S$ requires 500.1433).

(4-Benzothiazol-2-yl-2-nitrophenyl)cyclohexylamine (44)

To a solution of **4** (580 mg, 2.0 mmol) in DMSO (5 mL) was added cyclohexylamine (1.16 mL, 10.0 mmol). The reaction was stirred at 22 °C for 48 h. The reaction was poured into

aqueous HCl (1.0 M, 50 mL), and the product was extracted with diethyl ether (50 mL × 3). The combined organic phase was washed with saturated aqueous NaCl (100 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Column chromatography (CH_2Cl_2) afforded the product (609 mg, 87%) as a orange solid, mp 138–140 °C; R_f = 0.47 (hexane/ethyl acetate, 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.28 (d, *J* = 7.2 Hz,, 1H), 8.13 (d, *J* = 9.0 Hz, 1H), 7.98 (d, *J* = 8.1 Hz, 1H), 7.83 (d, *J* = 7.9 Hz, 1H), 7.45 (dd, J_1 = 7.4 Hz, J_2 = 7.9 Hz, 1H), 7.36 (dd, J_1 = 7.6 Hz, J_2 = 7.6 Hz, 1H), 6.92 (d, *J* = 9.1 Hz, 1H), 3.56 (m, 1H), 2.05 (m, 2H), 1.81 (m, 2H), 1.69-1.21 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.1, 154.0, 145.8, 135.1, 134.6, 134.3, 131.2, 126.5, 126.3, 124.9, 122.7, 121.5, 120.8, 114.7, 51.3, 32.6 (× 2), 25.5, 24.5 (× 2); IR (film) ν max 3556, 2931, 2854, 1624, 1567, 1534, 1488, 1436, 1361, 1266, 1217, 1155, 757 cm^{-1}; HRMS (ESI+) *m/z* 354.1280 ($M+H^+$, $C_{19}H_{20}N_3O_2S$ requires 354.1276).

(4-Benzothiazol-2-yl-2-nitrophenyl)hexylamine (45)

Compound **4** (580 mg, 2.0 mmol) and *n*-hexylamine (1.3 mL, 10.0 mmol) were used to synthesize **45** using the procedure described for preparation of the synthesis of **44**. The reaction was stirred at 22 °C for 48 h. The reaction was poured into aqueous HCl (1.0 M, 50 mL), and the product was extracted with diethyl ether (50 mL × 3). The combined organic phase was washed with saturated aqueous NaCl (100 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with CH_2Cl_2 to afford the product (625 mg, 88%) as a orange solid, mp 98–100 °C; R_f = 0.50 (hexane/ethyl acetate, 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.78 (t, *J* = 2.0 Hz, 1H), 8.28 (s, br, 1H), 8.15 (d, *J* = 6.9 Hz, 1H), 7.98 (d, *J* = 8.1 Hz, 1H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.45 (dd, J_1 = 7.2 Hz, J_2 = 8.1 Hz, 1H), 7.34 (dd, J_1 = 7.3 Hz, J_2 = 7.8 Hz, 1H), 6.89 (dd, J_1 = 2.4 Hz, J_2 = 9.1 Hz, 1H), 3.31 (m, 2H), 1.73 (m, 2H), 1.45 (m, 2H), 1.35 (m, 4H), 0.91 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0, 154.0, 146.7, 134.6, 134.4, 131.3, 126.3, 126.2, 125.0, 122.8, 121.5, 121.1, 114.3, 43.3, 31.4, 28.8, 26.7, 22.5, 14.0; IR (film) ν max 3372, 2955, 2931, 2858, 1626, 1566, 1488, 1360, 1269, 1214, 1159, 758 cm^{-1}; HRMS (ESI+) *m/z* 356.1413 ($M+H^+$, $C_{19}H_{22}N_3O_2S$, requires 356.1433).

(4-Benzothiazol-2-yl-2-nitrophenyl)-(4-methoxyphenyl)amine (46)

Compound **4** (580 mg, 2.0 mmol) and *p*-anisidine (1.23 g, 10.0 mmol) was used to synthesize **46** using the procedure described for preparation of the synthesis of **44**. Column chromatography (CH_2Cl_2) afforded the product (560 mg, 74%) as a orange solid, mp 188–190 °C; R_f = 0.40 (hexane/ethyl acetate, 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.65 (s, 1H), 8.84 (s, 1H), 8.02 (m, 2H), 7.85 (d, *J* = 9.0 Hz, 1H), 7.48-6.96 (m, 7H), 3.85 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.7, 158.4, 154.0, 145.7, 134.7, 134.0, 132.1, 130.3, 127.2 (× 2), 126.4, 126.0, 125.1, 122.9, 122.7, 121.6, 116.3, 115.1 (× 2); IR (film) ν max 3337, 1626, 1593, 1568, 1510, 1486, 1352, 1247, 1208, 1147, 753 cm^{-1}; HRMS (ESI+) *m/z* 378.0919 ($M+H^+$, $C_{20}H_{16}N_3O_3S$ requires 378.0912).

4-Benzothiazol-2-yl-N^1-cyclohexylbenzene-1,2-diamine (51)

Compound **44** (354 mg, 1.0 mmol) was treated with anhydrous hydrazine (0.2 mL, 6.4 mmol) and Pd/C (10%, 106 mg, 0.1 mmol) using the procedure described for preparation of **7**. Flash column chromatography (hexane/ethyl acetate, 8:1) afforded the product (291 mg, 90%) as a yellow solid, mp 168–170 °C; R_f = 0.37 (hexane/ethyl acetate, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, *J* = 7.9 Hz,, 1H), 7.97 (d, *J* = 8.1 Hz, 1H), 7.51 (m, 2H), 7.42 (dd, J_1 = 7.7 Hz, J_2 = 7.7 Hz, 1H), 7.29 (dd, J_1 = 7.6 Hz, J_2 = 7.6 Hz, 1H), 6.65 (d, *J* = 8.7 Hz, 1H), 3.48 (br, 3H), 3.32 (m, 1H), 2.08 (m, 2H), 1.78 (m, 2H), 1.66 (m, 1H), 1.29 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.1, 154.3, 140.6, 134.6, 133.1, 126.0, 124.2, 122.5, 121.9, 121.3, 115.9, 110.6, 51.4, 33.3 (× 2), 25.9, 24.9 (× 2); IR (film) ν max 3369, 3215,

3050, 2930, 2862, 1604, 1462, 1431, 1300, 1256, 1147, 756 cm^{-1}; HRMS (ESI+) *m/z* 324.1506 ($M+H^+$, $C_{19}H_{22}N_3S$ requires 324.1534).

4-Benzothiazol-2-yl-*N*[1]-hexylbenzene-1,2-diamine (53)

Compound **45** (356 mg, 1.0 mmol) was treated with anhydrous hydrazine (0.2 mL, 6.4 mmol) and Pd/C (10%, 106 mg, 0.1 mmol) according to the procedure described for **7**. Flash column chromatography (hexane/ethyl acetate, 8:1) afforded the product (282 mg, 87%) as a yellow solid, mp 151–153 °C; R_f = 0.40 (hexane/ethyl acetate, 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8.1 Hz, 1H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.53 (m, 1H), 7.42 (dd, J_1 = 7.3 Hz, J_2 = 7.8 Hz, 1H), 7.29 (dd, J_1 = 7.3 Hz, J_2 = 7.8 Hz, 1H), 6.64 (d, *J* = 8.8 Hz, 1H), 3.77 (br, 1H) 3.38 (br, 2H), 3.14 (t, *J* =7.1 Hz, 2H), 1.65 (m, 2H), 1.39 (m, 2H), 1.32 (m, 4H), 0.90 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 154.3, 141.7, 134.6, 133.2, 126.0, 124.3, 122.9, 122.3, 121.8, 121.4, 115.4, 110.2, 43.9, 31.6, 29.4, 26.9, 22.6, 14.0; IR (film) ν max 3379.8, 3212.9, 3018.9, 2952.1, 2916.7, 2848.5, 1647.9, 1594.3, 1459.8, 1438.6, 1364.1, 1307.4, 1215.5, 1160.7 769.8, 753.6, 726.3 cm^{-1}; HRMS (ESI+) *m/z* 326.1666 ($M+H^+$, $C_{19}H_{24}N_3S$, requires 326.1691).

4-Benzothiazol-2-yl-*N*[1]-cyclohexylbenzene-1,2-diamine (53)

Compound **46** (378 mg, 1.0 mmol) was treated with anhydrous hydrazine (0.2 mL, 6.4 mmol) and Pd/C (10%, 106 mg, 0.1 mmol) using the procedure described for preparation of **7**. Flash column chromatography (hexane/ethyl acetate, 6:1) afforded the product (295 mg, 85%) as a yellow solid, mp 155–157 °C; R_f = 0.33 (hexane/ethyl acetate, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.0 Hz, 1H), 7.85 (d, *J* = 7.7 Hz, 1H), 7.58 (d, *J* = 1.9 Hz, 1H), 7.47-7.30 (m, 3H), 7.04 (d, *J* = 8.2 Hz, 1H), 6.95 (d, *J* = 8.9 Hz, 1H), 6.86 (d, *J* = 8.9 Hz, 1H), 5.40 (s, 1H), 3.79 (s, 3H), 3.68 (br, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 155.1, 154.2, 137.3, 136.2, 135.7, 134.8, 127.1, 126.1, 124.6, 122.6, 121.5, 121.0 (× 2), 120.2, 117.3, 115.3, 114.8 (× 2), 55.6; IR (film) ν max 3361, 3260, 3058, 3003, 2953, 2833, 1600, 1509, 1477, 1437, 1311, 1243, 1034, 822, 758 cm^{-1}; HRMS (ESI+) *m/z* 348.1156 ($M+H^+$, $C_{20}H_{18}N_3OS$ requires 348.1156).

[(*E*)-2-(5-Benzothiazol-2-yl-1-cyclohexyl-1*H*-benzoimidazol-2-yl)ethenyl] methylphenylamine (65)

Compound **51** (65 mg, 0.2 mmol), **10** (32 mg, 0.2 mmol), and $ZrCl_4$ (24 mg, 0.1 mmol) were used to synthesize **65** using the procedure described for preparation of **12**. Flash column chromatography (hexane/ethyl acetate, 6:1) afforded the product (64 mg, 69%) as a yellow solid; mp 114–116 °C; R_f = 0.53 (hexane/ethyl acetate, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (d, *J* = 13.1 Hz,, 1H), 8.25 (d, *J* = 1.5 Hz,, 1H), 8.02 (d, *J* = 9.2 Hz, 1H), 7.95 (dd, J_1 = 8.1 Hz, J_2 = 1.5 Hz, 1H), 7.84 (d, *J* = 8.2 Hz, 1H), 7.51 (d, *J* = 8.2 Hz, 1H), 7.45 (dd, J_1 = J_2 = 7.7 Hz, 1H), 7.36-7.20 (m, 5H), 7.06 (t, *J* = 7.3 Hz, 1H), 5.46 (d, *J* = 13.2 Hz, 1H), 4.26 (m, 1H), 3.39 (s, 3H), 2.25 (m, 2H), 2.02 (m, 4H), 1.84 (m, 1H), 1.45 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.6, 155.1, 154.4, 146.8, 144.2, 143.1, 136.5, 135.1, 129.4 (× 2), 127.2, 126.0, 124.6, 123.4, 122.8, 121.5, 119.8, 119.5 (× 2), 117.6, 111.2, 87.0, 56.0, 36.4, 30.1 (× 2), 26.2 (× 2), 25.4; IR (film) ν max 3060, 2931, 2849, 1625, 1593, 1490, 1466, 1436, 1301, 1127, 755 cm^{-1}; HRMS (ESI+) *m/z* 465.2129 ($M+H^+$, $C_{29}H_{29}N_4S$ requires 465.2113).

[(*E*)-2-(5-Benzothiazol-2-yl-1-hexyl-1*H*-benzoimidazol-2-yl)ethenyl]methyl phenylamine (66)

Compound **52** (65 mg, 0.2 mmol), **10** (32 mg, 0.2 mmol), and $ZrCl_4$ (24 mg, 0.1 mmol) were used to synthesize **66** using the procedure described for preparation of **12**. Flash column chromatography (hexane/ethyl acetate, 6:1) afforded the product (66 mg, 71%) as a yellow solid; mp 60–62 °C; R_f = 0.53 (hexane/ethyl acetate, 1:1); ^{1}H NMR (300 MHz,

$CDCl_3$) δ 8.34 (d, *J* = 13.1 Hz, 1H), 8.24 (d, *J* = 1.5 Hz, 1H), 8.03 (m, 2H) 7.87 (d, *J* = 7.9 Hz, 1H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.38-7.23 (m, 6H), 7.09 (t, *J* = 8.2 Hz, 1H), 5.36 (d, *J* = 13.1 Hz, 1H), 4.10 (t, *J* = 7.1 Hz, 2H) 3.37 (s, 3H), 1.82 (m, 2H), 1.33 (m, 6H), 0.90 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.7, 155.2, 154.4, 146.7, 143.7, 142.9, 137.8, 135.1, 129.4 (× 2), 127.6, 126.0, 124.6, 123.5, 122.8, 121.5, 120.2, 119.5 (× 2), 117.4, 108.7, 86.3, 43.3, 36.4, 31.4, 29.8, 22.5, 14.0; IR (film) ν max 3059, 2953, 2928, 2856, 1628, 1593, 1491, 1467, 1437, 1325, 1302, 1265, 1127, 756 cm^{-1}; HRMS (ESI+) *m/z* 467.2257 ($M+H^+$, $C_{29}H_{31}N_4S$ requires 467.2269).

{(*E*)-2-[5-Benzothiazol-2-yl-1-(4-methoxyphenyl)-1*H*-benzoimidazol-2-yl]ethenyl}methylphenylamine (67)

Compound **53** (70 mg, 0.2 mmol), **10** (32 mg, 0.2 mmol), and $ZrCl_4$ (24 mg, 0.1 mmol) were used to synthesize **67** using the procedure described for preparation of **12**. Flash column chromatography (hexane/ethyl acetate, 5:1) afforded the product (70 mg, 72%) as a yellow solid; mp 182–184 °C; R_f = 0.53 (hexane/ethyl acetate, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31-8.27 (m, 2H), 8.04 (d, *J* = 8.2 Hz,, 1H), 7.96 (dd, J_1 = 8.3 Hz, J_2 = 1.6 Hz, 1H), 7.87 (d, *J* = 7.9 Hz, 1H), 7.45-7.30 (m, 6H), 7.17-6.92 (m, 6H), 5.18 (d, *J* = 13.3 Hz, 1H), 3.90 (s, 3H), 3.16 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.5, 159.7, 155.9, 154.4, 146.6, 143.8, 142.4, 139.2, 135.1, 129.3 (× 2), 128.8 (× 2), 128.3, 128.1, 126.0, 124.6, 123.4, 122.8, 121.5, 120.7, 119.2 (× 2), 117.7, 115.1 (× 2), 109.5, 87.3, 55.6, 36.0; IR (film) ν max 3060, 2007, 2934, 2836, 1627, 1592, 1514, 1491, 1465, 1435, 1296, 1251, 1127, 755 cm^{-1}; HRMS (ESI+) *m/z* 489.1754 ($M+H^+$, $C_{30}H_{25}N_4OS$ requires 489.1749).

5-Benzothiazol-2-yl-1-cyclohexyl-3-ethyl-2-[(*E*)-2-(methylphenylamino)ethenyl]-3*H*benzoimidazol-1-ium, iodide (26)

Compound **65** (30 mg, 0.065 mmol) and ethyl iodide (3 mL) were used to synthesize **26** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 20:1) afforded the product (36 mg, 90%) as a yellow solid; mp 112–114 °C; R_f = 0.28 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.40 (s, 1H), 8.11 (m, 2H), 7.98 (m, 2H), 7.66 (dd, J_1 = 7.3 Hz, J_2 = 6.3 Hz, 1H), 7.52-7.31 (m, 6H), 7.23 (dd, J_1 = J_2 = 7.3 Hz, 1H), 5.50 (d, *J* = 13.5 Hz, 1H), 4.63 (m, 1H), 4.49 (q, *J* = 7.2 Hz, 2H), 3.53 (s, 3H), 2.35 (m, 2H), 2.02 (m, 4H), 1.78 (m 1H), 1.54 (t, *J* = 7.2 Hz, 3H), 1.56-1.37 (m, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 168.6, 155.5, 154.4, 154.3 (× 2), 151.3 (× 2), 148.0, 136.8, 134.5, 134.0, 132.3, 131.4 (× 2), 128.4, 127.5, 126.3, 124.4, 123.5, 122.4, 116.8, 111.9, 79.4, 61.6, 43.1, 38.3, 32.0 (× 2), 27.3 (× 2), 26.3, 14.8; IR (film) ν max 3342 (br), 3060, 2931, 2948, 1688, 1616, 1588, 1528, 1494, 1461, 1363, 1305, 1199, 1172, 1130, 761 cm^{-1}; HRMS (ESI+) *m/z* 493.2415 (M^+, $C_{31}H_{33}N_4S$ requires 493.2426).

5-Benzothiazol-2-yl-3-ethyl-1-hexyl-2-[(*E*)-2-(methylphenylamino)ethenyl]-3*H*-benzoimidazol-1-ium, iodide (27)

Compound **66** (30 mg, 0.064 mmol) and ethyl iodide (3 mL) were used to synthesize **27** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 20:1) afforded the product (36 mg, 91%) as a yellow solid; mp 128–131 °C; R_f = 0.25 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.33 (d, *J* = 1.1 Hz, 1H), 8.13 (m, 1H), 7.98 (m, 2H), 7.80 (m, 2H), 7.49-7.17 (m, 7H), 5.45 (d, *J* = 13.2 Hz, 1H), 4.50 (q, *J* = 7.3 Hz, 2H) 4.32 (t, *J* = 7.7 Hz, 2H), 3.53 (s, 3H), 1.87 (m, 2H), 1.53 (t, *J* = 7.2 Hz, 1H) 1.33 (m, 2H), 1.27 (m, 4H), 0.83 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 168.7, 155.5, 153.5, 151.4, 136.8, 135.6, 133.8, 132.5, 131.5 (× 3), 128.4, 127.9, 127.4, 126.7, 124.4, 123.5, 122.9, 122.8, 113.9, 111.4, 79.8, 47.5, 42.7, 32.9 (× 2), 30.2, 27.8, 24.0, 14.7, 14.6; IR (film) ν max 3412.8, 3060.3, 2942.7, 2919.2, 2860.5, 1689.5, 1620.5, 1587.7, 1535.6, 1493.0, 1357.9, 1199.8, 1130.2, 760.2 cm^{-1}; HRMS (ESI+) *m/z* 495.2572 (M^+, $C_{31}H_{35}N_4S$ requires 495.2582).

5-Benzothiazol-2-yl-3-ethyl-1-(4-methoxyphenyl)-2-[(*E*)-2-(methylphenylamino)ethenyl]-3*H*benzoimidazol-1-ium, iodide (28)

Compound **67** (35 mg, 0.072 mmol) and ethyl iodide (3 mL) were used to synthesize **28** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 20:1) afforded the product (42 mg, 92%) as a yellow solid; mp 174-176 °C; R_f = 0.22 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.34 (s, 1H), 7.92 (m, 2H), 7.48 (d, *J* = 8.8 Hz, 2H), 7.41-7.03 (m, 9H), 6.70 (d, *J* = 7.5 Hz, 2H), 5.36 (d, *J* = 14.0 Hz, 1H), 4.47 (q, *J* = 6.9 Hz, 2H), 3.85 (s, 3H), 3.30 (s, 3H), 1.49 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 168.6, 163.7, 155.5, 153.5, 151.9, 147.6, 137.6, 136.7, 133.3, 132.4, 131.1 (× 2), 130.8 (× 2), 128.4, 127.8, 127.4, 124.4, 123.5, 121.8, 118.3 (× 2), 113.4, 110.9, 79.8, 57.0, 41.7, 37.3, 14.4; IR (film) ν max 3412 (br), 3050, 2932, 2840, 1682, 1613, 1588, 1504, 1410, 1369, 1256, 1201, 1126, 762 cm^{-1}; HRMS (ESI+) *m/z* 517.2045 (M^+, $C_{32}H_{29}N_4OS$ requires 517.2062).

5-Benzothiazol-2-yl-2-[(*E*)-2-(methylphenylamino)ethenyl]-3-phenethyl-1-phenyl-3*H*benzoimidazol-1-ium, iodide (29)

Compound **12** (30 mg, 0.066 mmol) and (2-iodoethyl)benzene (3 mL) were used to synthesize **29** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 30:1) afford the product (43 mg, 87%) as a yellow solid; mp 83–85 °C; R_f = 0.41 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.31 (d, *J* = 1.3 Hz, 1H), 8.18-8.03 (m, 3H), 7.78-7.75 (m, 3H), 7.62-7.42 (m, 4H), 7.31-7.02 (m, 9H), 6.83 (d, *J* = 13.5 Hz, 1H), 6.65 (br, 2H), 5.03-4.84 (m, 5H), 4.52 (m, 2H), 3.23 (s, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 168.5, 155.5, 154.1, 151.4, 139.2, 136.9, 136.8, 136.3, 133.5, 133.3 (× 2), 132.9, 132.7, 131.1(× 2), 130.9 (× 2), 130.4 (× 2), 129.4 (× 2), 128.9, 128.4, 127.7, 127. 4, 126.9, 124.4, 123.5, 121.6, 113.4, 111.6, 79.8, 48.0, 37.0, 35.9; IR (film) ν max 3428, 3061, 2943, 2860, 1689, 1616, 1586, 1535, 1494, 1464, 1370, 1307, 1200, 1167, 1127, 799, 760, 697 cm^{-1}; HRMS (ESI+) *m/z* 563.2261 (M^+, $C_{37}H_{31}N_4S$ requires 563.2269).

5-Benzthiazol-2-yl-3-hexyl-2-[(*E*)-2-(methylphenylamino)ethenyl]-1-phenyl-3*H*-benzoimidazol-1-ium, iodide (30)

Compound **12** (35 mg, 0.076 mmol) and 1-iodo-hexane (3 mL) were used to synthesize **30** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 30:1) afforded **30** (47 mg, 92%) as a yellow solid, mp 86–88 °C; R_f = 0.37 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.40 (d, *J* = 1.1 Hz, 1H), 8.06-7.95 (m, 3H), 7.78-7.74 (m, 3H), 7.67-7.64 (m, 2H), 7.48-7.38 (m, 2H), 7.26 (m, 2H), 7.23-7.12 (m, 3H), 6.72 (br, 2H), 5.47 (d, *J* = 11.9 Hz, 1H), 4.50 (m, 2H), 3.36 (s, 3H), 1.95 (m, 2H), 1.51 (m, 2H), 1.36 (m, 4H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 168.6, 155.5, 153.8, 152.1, 137.2, 136.7, 136.4, 134.0, 133.3 (× 2), 133.0, 132.6, 131.1 (× 2), 129.6 (× 2), 128.4, 127.4, 126.9, 124.4, 123.5, 121.9, 113.5, 111.2, 79.9, 46.5, 36.8, 33.0, 30.0, 27.9, 24.1, 14.8; IR (film) ν max 3331, 3060, 2943, 2931, 2849, 1688, 1617, 1586, 1537, 1494, 1464, 1369, 1310, 1120, 1167, 1127, 799, 761, 697 cm^{-1}; HRMS (ESI+) *m/z* 543.2548 (M^+, $C_{35}H_{35}N_4S$ requires 543.2582).

5-Benzothiazol-2-yl-3-dodecyl-2-[(*E*)-2-(methylphenylamino)ethenyl]-1-phenyl-3*H*-benzoimidazol-1-ium, iodide (31)

Compound **12** (30 mg, 0.066 mmol) and 1-iodo-dodecane (3 mL) were used to synthesize **31** using the procedure described for preparation of **1**. Flash column chromatography (CH_2Cl_2/MeOH, 30:1) afforded the product (43 mg, 87%) as a yellow glassy solid; R_f = 0.44 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.42 (s, 1H), 8.07-7.95 (m, 3H), 7.79-7.64 (m, 5H), 7.51-7.36 (m, 2H), 7.27-7.15 (m, 5H), 6.69 (br, 2H), 5.53 (d, *J* = 12.3

Hz, 1H), 4.52 (m, 2H), 3.36 (s, 3H), 1.97 (m, 2H), 1.50-1.00 (m, 20H), 0.78 (t, J = 6.1 Hz, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 168.3, 155.1, 153.4, 151.7, 136.8, 136.4, 136.1, 133.6, 132.9 (× 2), 132.6, 132.3, 130.1 (× 2), 129.2 (× 2), 128.0, 127.1, 126.6, 124.0, 123.1, 121.4, 113.1, 110.9, 79.5, 46.1, 37.1, 33.0, 30.8, 30.7, 30.6, 30.5, 30.3, 29.5, 27.7, 23.7, 14.4; IR (film) ν max 3425, 3060, 2919, 2849, 1689, 1620, 1586, 1537, 1494, 1465, 1370, 1310, 1200, 1131, 799, 762, 697 cm^{-1}; HRMS (ESI+) *m/z* 627.3523 (M^+, $C_{41}H_{47}N_4S$ requires 627.3521).

2-(3-Chloro-4-nitrophenyl)benzooxazole (82)

To a slurry of 3-chloro-4-nitro-benzoic acid (**80**, 1.0 g, 5.0 mmol) in DMF (15 mL) was added HATU (1.9 g, 5.0 mmol) and DIEA (1.7 mL, 10 mmol). The reaction was stirred at 22 °C for 5 min prior to addition of 2-aminophenol (**81**, 550 mg, 5.0 mmol). The reaction was stirred at 22 °C for 4 h followed by addition of saturated aqueous NaCl (150 mL). The aqueous phase was extracted with diethyl ether (100 mL × 3). The organic phases were combined and washed with saturated aqueous NaCl (100 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. To the slurry of resulting residue in xylene (30 mL) was added *p*-toluenesulfonic acid (3.8 g, 20 mmol). The reaction was refluxed for 1 h, cooled, and poured into aqueous NaOH (1.0 M, 100 mL). The aqueous phase was extracted with diethyl ether (100 mL × 3). The organic phases were combined and washed with saturated aqueous NaCl (100 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography (hexane/ethyl acetate, 10:1) afforded the product (1.02 g, 71%) as a off white solid; mp 162–164 °C; R_f = 0.58 (hexane/ethyl acetate, 3:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, J = 1.7 Hz, 1H), 8.23 (dd, J_1 = 1.7 Hz, J_2 = 8.5 Hz, 2H), 7.99 (d, J = 8.5 Hz, 1H), 7.79 (m, 1H), 7.61 (m, 1H), 7.41 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.4, 151.0, 149.0, 141.7, 131.8, 130.6, 128.0, 126.6, 126.2 (× 2), 125.4, 120.7, 111.0; IR (film) ν max 3093, 1610, 1580, 1547, 1523, 1468, 1446, 1331, 1067, 762, 750 cm^{-1}; HRMS (ESI+) *m/z* 275.0238 ($M+H^+$, $C_{13}H_8N_2O_3Cl$ requires 275.0224).

(5-Benzooxazol-2-yl-2-nitrophenyl)ethylamine (85)

To a slurry of **82** (200 mg, 0.73 mmol) in DMSO (2 mL) was added ethylamine (**83**, 2.0 M in THF, 4 mL). The reaction was stirred at 22 °C for 48 h. The reaction was poured into aqueous HCl (1.0 M, 30 mL). The aqueous phase was extracted with diethyl ether (50 mL × 3). The organic phases were combined and washed with saturated aqueous NaCl (50 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Recrystallization of the crude product from CH_2Cl_2/MeOH afforded the product (190 mg, 92%) as a orange solid; mp 168–170 °C; R_f = 0.55 (hexane/ethyl acetate, 3:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, J = 8.9 Hz, 1H), 8.01 (br, 1H), 7.80 (m, 1H), 7.70 (d, J = 1.6 Hz, 1H), 7.59 (m, 1H), 7.46 (d, J_1 = 1.6 Hz, J_2 = 8.9 Hz, 1H), 7.39 (m, 2H), 3.48 (m, 2H), 1.43 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.2, 150.9, 145,3, 141,9, 133.6, 132.8, 127.7, 126.1, 125.1, 120.1, 113.6,112.8, 110.8, 38.0, 14.4; IR (film) ν max 3379, 2969, 2912, 2850, 1627, 1621, 1580, 1553, 1523, 1490, 1402, 1339, 1320, 1281, 1215, 1188, 1048, 743 cm^{-1}; HRMS (ESI +) *m/z* 284.1036 ($M+H^+$, $C_{15}H_{14}N_3O_3$ requires 284.1035).

(5-Benzooxazol-2-yl-2-nitrophenyl)hexylamine (84)

Compound **82** (200 mg, 0.73 mmol) was added to hexylamine (**38**, 1.0 mL, 7.7 mmol) in DMSO/THF (2 mL/2 mL) and stirred at 22 °C for 48 h. The reaction was worked up as described for preparation of **85**. Recrystallization of the crude product from CH_2Cl_2/MeOH afforded the product (233 mg, 94%) as a orange solid; mp 134–135 °C; R_f = 0.68 (hexane/ethyl acetate, 3:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, J = 8.9 Hz, 1H), 8.09 (br, 1H), 7.81 (m, 1H), 7.72 (d, J = 0.9 Hz, 1H), 7.60 (m, 1H), 7.46 (d, J_1 = 0.9 Hz, J_2 = 8.9 Hz, 1H), 7.40 (m, 2H), 3.42 (q, J = 5.2 Hz, 2H), 1.80 (m, 2H), 1.49 (m, 2H), 1.38 (m, 4H), 0.93 (t, J = 5.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.3, 150.9, 145.4, 141.9, 133.6, 132.8, 127.7,

126.1, 125.1, 120.5, 113.5, 112.9, 110.8, 43.3, 31.5, 28.9, 26.7, 22.6, 14.0; IR (film) ν max 3368, 3077, 2950, 2924, 2857, 1630, 1622, 1582, 1556, 1523, 1491, 1470, 1451, 1407, 1318, 1284, 1260, 1244, 1211, 1191, 1180,1050, 760, 744 cm^{-1}; HRMS (ESI+) *m/z* 340.1646 ($M+H^+$, $C_{19}H_{22}N_3O_3$ requires 340.1661).

4-Benzooxazol-2-yl-*N*^{2}-ethylbenzene-1,2-diamine (87)

Compound **85** (160 mg, 0.56 mmol) was treated with anhydrous hydrazine (0.1 mL, 3.2 mmol) and Pd/C (10%, 64 mg, 0.06 mmol) using the procedure described for preparation of **7**. Recrystallization from CH_2Cl_2/hexane afforded the product (132 mg, 93%) as a light yellow solid; mp 172–173 °C; R_f = 0.30 (hexane/ethyl acetate, 1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65 (br, 2H), 7.36-7.18 (m, 4H), 6.66 (d, *J* = 7.6 Hz, 1H), 5.44 (s, 2H), 4.70 (br, 1H), 3.15 (m, 2H), 1.27 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.7, 150.4, 142.7, 140.4, 135.8, 124.7, 124.4, 119.1, 118.3, 114.7, 113.3, 110.7, 108.0, 38.4, 14.8; IR (film) ν max 3429, 3379, 3335, 3225, 2973, 2956, 2923, 2852, 1643, 1607, 1586, 1495, 1451, 1036, 1278, 1240, 1150, 1053, 856, 793, 759, 746 cm^{-1}; HRMS (ESI+) *m/z* 254.1287 ($M+H^+$, $C_{15}H_{16}N_3O$ requires 254.1293).

4-Benzooxazol-2-yl-*N*^{2}-hexylbenzene-1,2-diamine (86)

Compound **84** (160 mg, 0.47 mmol) was treated with anhydrous hydrazine (0.1 mL, 3.2 mmol) and Pd/C (10%, 53 mg, 0.05 mmol) using the procedure described for preparation of **7**. Recrystallization from CH_2Cl_2/hexane afforded the product (136 mg, 94%) as a light yellow solid; mp 126–127 °C; R_f = 0.24 (hexane/ethyl acetate, 3:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (m, 2H), 7.62 (dd, J_1 = 1.8 Hz, J_2 = 8.0 Hz, 1H), 7.53 (m, 2H), 7.28 (m, 2H), 6.77 (d, *J* = 8.0 Hz, 1H), 3.6 (br, 3H), 3.20 (t, *J* = 7.2 Hz, 2H), 1.69 (m, 2H), 1.44 (m, 2H), 1.34 (m, 4H), 0.91 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3,150.6, 142.4, 138.5, 137.5,124.2 (× 2), 119.3 (× 2), 118.7, 115.4, 110.8, 110.2, 44.4, 31.7, 29.7, 27.0, 22.6, 14.1; IR (film) ν max 3410, 3360, 3220, 2950, 2927, 2846, 1646, 1591, 1582, 1557, 1505, 1484, 1455, 1443, 1318, 1292, 1158, 955, 851, 797, 758, 743 cm^{-1}; HRMS (ESI+) *m/z* 310.1899 ($M+H^+$, $C_{19}H_{24}N_3O$ requires 310.1919).

[(*E*)-2-(6-Benzooxazol-2-yl-1-ethyl-1*H*-benzoimidazol-2-yl)ethenyl]methyl phenylamine (17)

To a solution of **87** (51 mg, 0.2 mmol) and **10** (32 mg, 0.2 mmol) in ethanol (10 mL), $ZrOCl_2 \cdot 8H_2O$ (32 mg, 0.1 mmol) was added. The reaction was stirred at 22 °C for 30 min. The reaction was heated to 80 °C and refluxed for 5 min before the addition of MnO_2 (86 mg, 1.0 mmol). After 10 min, the solution was cooled to 22 °C and filtered through a fritted funnel. The MnO_2 was washed with ethanol (10 mL). The combined filtrate was concentrated *in vacuo*. Flash column chromatography (hexane/ethyl acetate, 3:1) afforded the product (53 mg, 68%) as a yellow solid; mp 171–173 °C; R_f = 0.15 (hexane/ethyl acetate, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (d, *J* = 13.0 Hz, 1H), 8.12 (m, 2H),7.72 (m, 2H), 7.58 (m, 1H), 7.39-7.22 (m, 6H), 7.12 (t, *J* = 6.7 Hz, 1H), 5.38 (d, *J* = 13.0 Hz, 1H), 4.23 (q, *J* = 6.7 Hz, 2H), 3.38 (s, 3H), 1.47 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.5, 156.0, 150.8, 146.8, 146.6, 143.5 (× 2), 142.5, 135.3, 129.4(× 2), 124.4, 124.3, 123.7, 121.9, 119.7 (× 2), 119.4, 119.0, 117.6, 110.3, 107.8, 85.9, 38.0, 36.5, 15.0; IR (film) ν max 3379, 3060, 2978, 2934, 1628, 1594, 1558, 1491, 1453, 1410, 1347, 1326, 1291, 1242, 1128, 809, 747 cm^{-1}; HRMS (ESI+) *m/z* 395.1849 ($M+H^+$, $C_{25}H_{23}N_4O$ requires 395.1872).

[(*E*)-2-(6-Benzooxazol-2-yl-1-hexyl-1*H*-benzoimidazol-2-yl)ethenyl]methylphenylamine (32)

To a solution of compound **86** (62 mg, 0.2 mmol) and **10** (32 mg, 0.2 mmol) in ethanol (10 mL), was added $ZrOCl_2 \cdot 8H_2O$ (32 mg, 0.1 mmol) to synthesize **32** using the procedure described for preparation of **17**. Flash column chromatography (hexane/ethyl acetate, 5:1)

afforded the product (58 mg, 64%) as a yellow solid; mp 88–89 °C; R_f = 0.40 (hexane/ethyl acetate, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, J = 13.1 Hz, 1H), 8.11-8.09 (m, 2H), 7.76 (m, 1H), 7.67 (dd, J_1 = 0.9 Hz, J_2 = 8.0 Hz, 1H), 7.56 (m, 1H), 7.38-7.30 (m, 4H), 7.23-7.21 (m, 2H), 7.10 (m, 1H), 5.37 (d, J = 13.1 Hz, 1H), 4.15 (t, J = 7.4 Hz, 2H), 3.37 (s, 3H), 1.85 (m, 2H), 1.35 (m, 6H), 0.89 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.5, 156.3, 150.8, 146.6, 143.4, 142.5, 135.8, 129.4 (× 2), 124.4, 124.3, 123.7, 121.9, 119.7 (× 3), 119.4, 119.0, 117.6, 110.3, 108.0, 86.2, 43.3, 36.5, 31.5, 29.8, 26.6, 22.6, 14.0; IR (film) ν max 3357, 3060, 2950, 2929, 2853, 1628, 1594, 1559, 1491, 1452, 1409, 1326, 1294, 1268, 1243, 1128, 1001, 823, 746, 695 cm^{-1}; HRMS (ESI+) *m/z* 451.2472 ($M+H^+$, $C_{29}H_{31}N_4O$ requires 451.2498).

Biological assays

To construct the PIV5 strain used for antiviral assays, recombinant PIV5 encoding GFP between the viral genes HN and L[32] was modified by replacement of GFP in the PIV5 genome with renilla luciferase. Recombinant PIV5 expressing renilla luciferase (rPIV5-RL) was confirmed by RT-PCR sequencing and functional assays. To examine whether luciferase activity of infected cells reflects the rate of viral infection, a serial dilution (2 ×) of rPIV5-RL virus was used to infect HeLa cells grown on a 96-well plate. At 1 day post infection (dpi), the cells were lysed and assayed for renilla luciferase activity (commercial detection kit from Promega Inc., Madison, WI). As shown in Figure 3, at 0.06 to 8 MOI (multiplicity of infectivity, corresponding to the number of infectious viruses per cell), the luciferase activity directly correlated with the amount of virus applied, indicating that rPIV5-RL can be used to quantitatively assess viral replication. To test the effects of compounds on replication of rPIV5-RL, HeLa cells in 96-well plates were infected with 1 MOI of rPIV5-RL. The cells were incubated with compounds, collected at 1 day post infection, and assayed for renilla luciferase activity.

To examine the toxicity of compounds to mammalian cells, cellular viability was quantified after 24 h (HeLa cells) or 40 h (NHBE cells) with a firefly luciferase-based CellTiter-Glo luminescent cell viability assay (Promega Inc., Madison, WI). Normal human bronchial epithelial (NHBE) cells were cultured in an air-liquid interface system as previously described.[35] Briefly, NHBE cells (passage-2, 2×10^4 cells/cm^2, Clonetics, San Diego, CA) were cultured in Transwell-clear culture inserts (24.5 mm, 0.45 μm pore size; Costar, Cambridge, MA) thin-coated with rat tail collagen, type I (Collaborative Res., Bedford, MA). Cells were cultured submerged for the first 5 to 7 days. After this period, the apical medium was removed to create the air-liquid interface, and cells were fed medium on their basal surface only. For an additional 28 days, the apical surface of the cells was exposed to a humidified 95% air/5% CO_2 environment. NHBE cells were cultured for a total of 35 days.

Cultures of *Mycobacterium fortuitum*, a generous gift of Dr. Fred Quinn, were maintained at 37 °C in 7H9 broth supplemented with OADC and 0.05% glycerol. The assay in infected HeLa cells was based on a related survival assay for *M. tuberculosis*.[36] HeLa cells were seeded in 24 well plates at 5×10^5 cells per well in EMEM containing 10% FBS (HeLa medium) overnight in a CO_2 (5%) incubator at 37 °C. *M. fortuitum* was washed with uptake buffer (1 part Basal Uptake Buffer (4.5 mg/mL glucose, 5 mg/mL BSA, 0.1 mg/mL $CaCl_2$, 0.1 mg/mL $MgCl_2$, 1 mg/mL gelatin in PBS): 4 parts Wash Buffer (5% FBS in DMEM)). After the wells had been washed to remove antibiotics, *M. fortuitum* in uptake buffer was added to the wells containing HeLa cells (MOI = 5). After infection for 24 h, the medium was removed, the wells were washed with warm DMEM, and this DMEM was replaced with HeLa medium containing compounds **1** (0.5 μM), **29** (0.5 μM), **30** (0.5 μM), **31** (0.5 μM), TritonX-100 (1%), or DMSO (0.1%, vehicle control). After an additional 24 h, the medium was removed and the cells were lysed, diluted, and plated for determination of colony forming units (CFU) on 7H11 agar plates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AKT	protein kinase B
AGC	cAMP-dependent, cGMP-dependent and protein kinase C
FOXO	forkhead box-O
NHBE	normal human bronchial epithelial
PIV5	parainfluenza virus 5
PI3K	phosphatidylinositol-3-kinase
PH	pleckstrin homology
RL	renilla luciferase
MOI	multiplicity of infection
CFU	colony forming units

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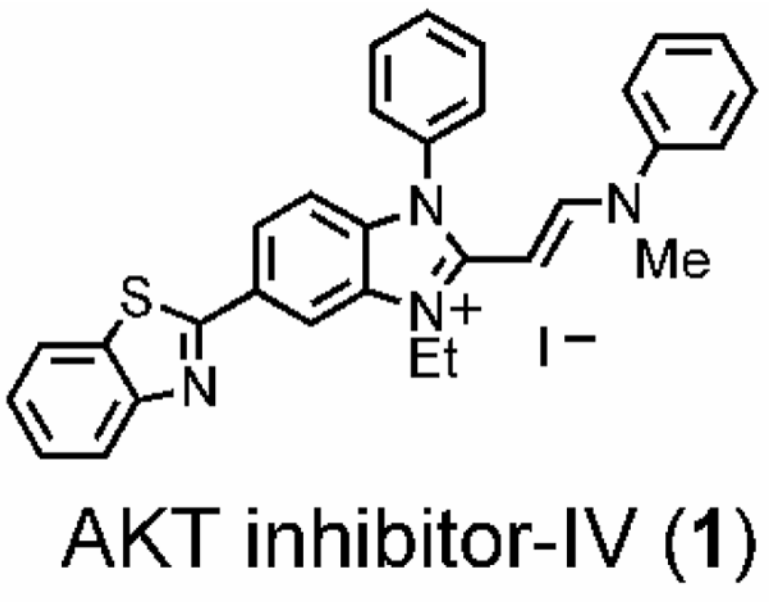


Figure 1.
Structure of AKT inhibitor IV (**1**).

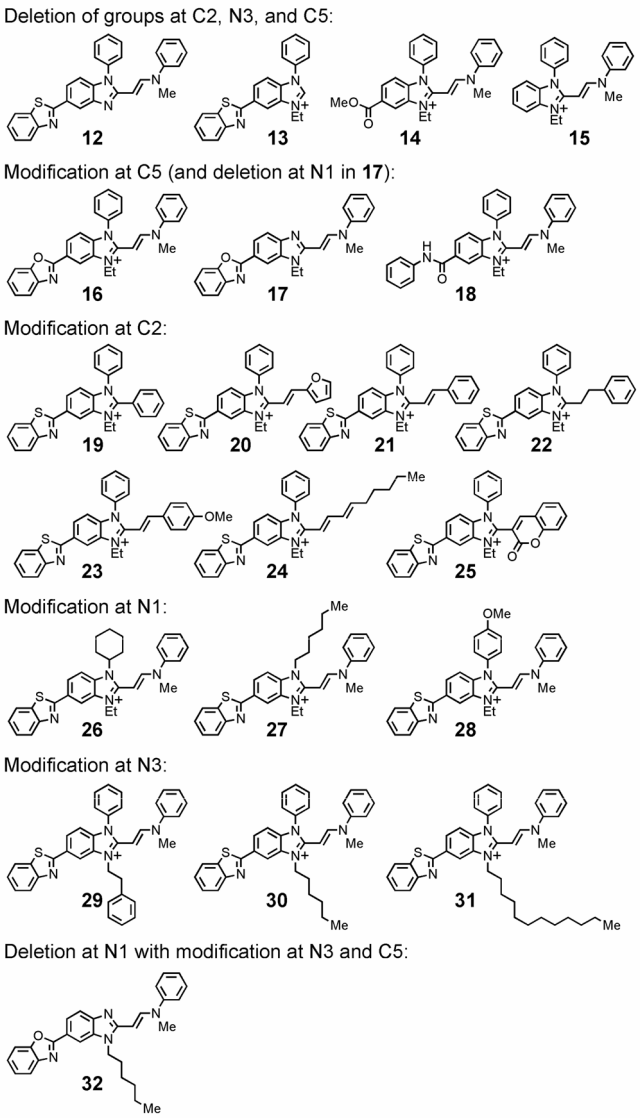


Figure 2.

Structures of analogues **12** to **32**. For **13** to **16** and **18** to **31**, compounds were isolated as ammonium iodides.

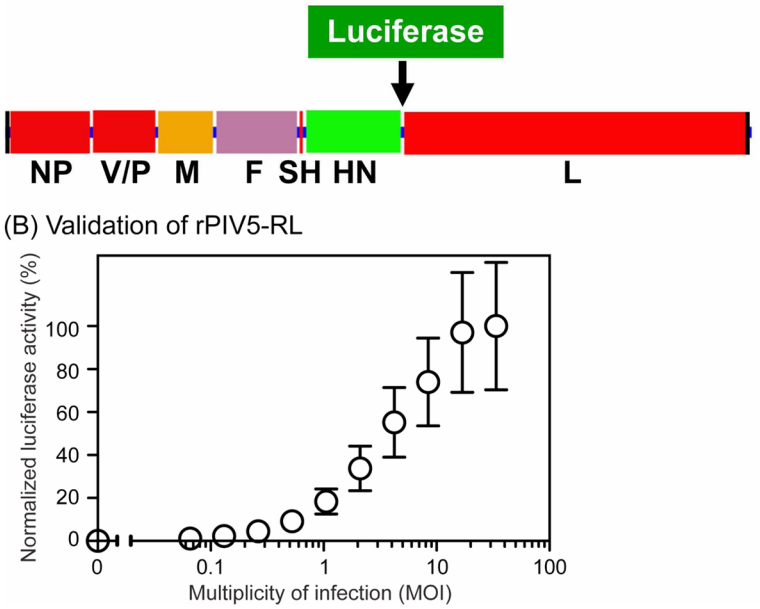


Figure 3.

Panel A: Design of recombinant PIV5 expressing renilla luciferase. The gene encoding luciferase was inserted into the PIV5 genome between the HN and L genes. Panel B: Validation of rPIV5-RL by luciferase assay of infected HeLa cells. The activity of the highest dose of infection, 34 infectious viruses per cell, was set as 100%. Error bars represent standard errors of the mean.

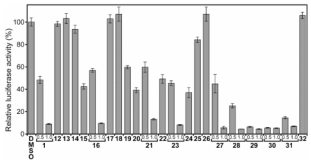


Figure 4.

Initial evaluation of compounds **1** and **12-32** against rPIV5-RL at one day post infection. All compounds were tested at 1 μM. Compounds comparable in activity to **1** were further examined at 0.5 μM (concentrations (μM) shown in brackets). Error bars represent standard errors of the mean.

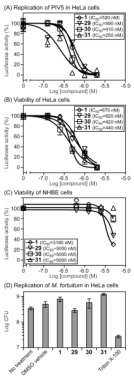
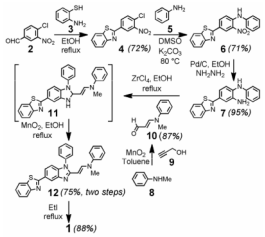
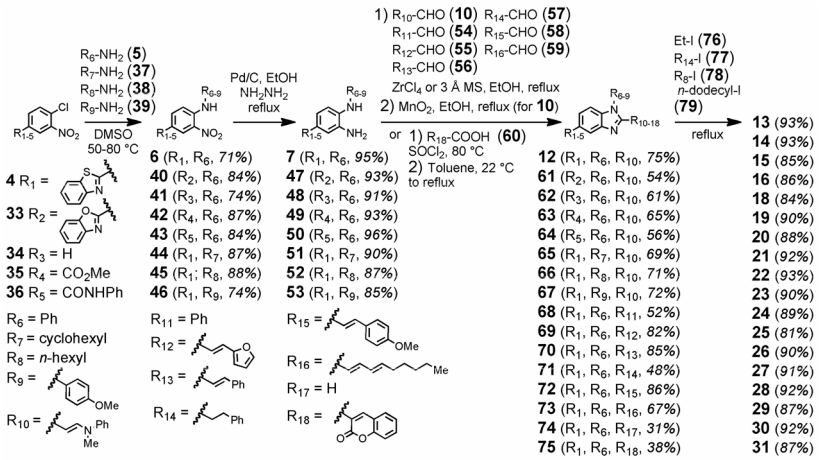


Figure 5.

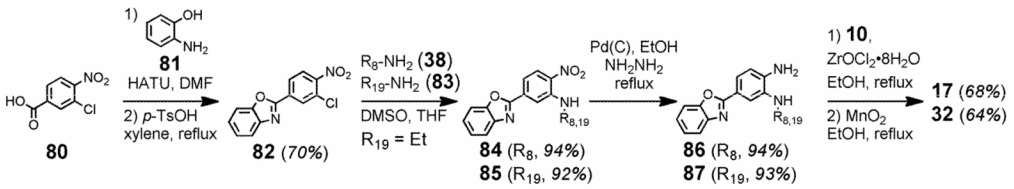
Panels A-C: Dose-dependent effects of compounds **1** and **29**-**31** on renilla luciferase expressed by rPIV5-RL in HeLa cells (Panel A), on viability of HeLa cells after 24 h (Panel B), and on viability of NHBE cells after 40 h (Panel C). Viability of mammalian cells was measured by quantification of cellular ATP with firefly luciferase. Panel D: Examination of effects of compounds (0.5 μM) compared with Triton X-100 (1%) on replication of intracellular *M. fortuitum* bacteria in HeLa cells. Data are in triplicate or larger numbers of measurements. Standard errors of the mean are ≤ 20%.



Scheme 1.
Synthesis of AKT inhibitor-IV (**1**).



Scheme 2.
Synthesis of **13**-**16** and **18**-**31**.



Scheme 3.
Synthesis of **17** and **32**.