# Synthesis and Structure–Activity Relationship of Novel Indole Acrylamide Derivatives as HCV Replication Inhibitors

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A series of indole acrylamide derivatives were synthesized and evaluated for their inhibitory effects on hepatitis C virus (HCV) replication. Previously, we have identified (*E*)-*N*-(4-*tert*-butylphenyl)-3-(5-cyano-1*H*-indol-3-yl)-2-methylacrylamide (**6c**) as one of the promising leads for anti-HCV chemotherapy. Based on the structural features of indole acrylamide, we have explored extended structure–activity relationship study using analogs with substituted indoles, various amides, and N-substitution at the indole ring. Among the newly synthesized series, 5-cyanoindole acrylamide analog with *N*-acetyl substitution (**13c**) ($EC_{50}$ = 0.98 μM, $CC_{50}$ = 40.74 μM, and SI = 41.6) exhibited the most potent antiviral activity with reasonable cytotoxicity in a cell-based J6/JFH1 reporter assay using Huh7.5 cells. In addition, improved water solubility of **13c** compared to **6c** further merits consideration of **13c** as a valuable candidate for anti-HCV therapeutics development.

**Keywords:** Hepatitis C virus, HCV replication inhibitors, Indole derivatives, Structure–activity relationship (SAR) study

## Introduction

Hepatitis C virus (HCV) is a clinically important human pathogen affecting over 180 million people worldwide.[1] Infection with HCV has a high risk of developing chronic liver cirrhosis and hepatocellular carcinoma.[2] Approximately, 40–45% of all liver transplantation occur due to HCV infection, and it is the leading cause for chronic liver diseases in the United States.[3] In spite of recent emergence of highly active, direct-acting antivirals (DAAs) targeting specific HCV proteins, the current standard therapy still relies on a combination of pegylated interferon-α and ribavirin because of the DAA's unaffordable price tags.[4] In addition, this interferon-based therapy can cause undesirable side effects such as flu-like symptoms, anemia, and depression in patients. Therefore, the new class of anti-HCV therapeutics with a novel mechanism of action is urgently needed to develop a more cost-effective and safer antiviral in the future.[5]

A number of small molecules targeting nonstructural viral proteins have been identified and have progressed to various stages of clinical development. They include inhibitors of NS3 protease[6]/helicase[7], NS4B,[8,9] NS5A,[10,11] and NS5B polymerase.[12] Especially, the HCV NS5B polymerase has been widely studied as an attractive target to develop HCV inhibitors because this polymerase, which is an RNA-dependent RNA polymerase, plays a critical role for viral replication in the overall life cycle of the virus.

We have recently reported the discovery of a novel series of indole 2-methylacrylamide derivatives as novel HCV NS5B polymerase inhibitors.[5] In order to develop more potent HCV replication inhibitors with improved water solubility, we further synthesized a series of indoles with N-substitution and evaluated their biological activities. Here, we report structure–activity relationship (SAR) of a novel series of indole acrylamide derivatives. We hope that this work will lead to highly potent HCV inhibitors for further development of a new class of HCV replication inhibitors.

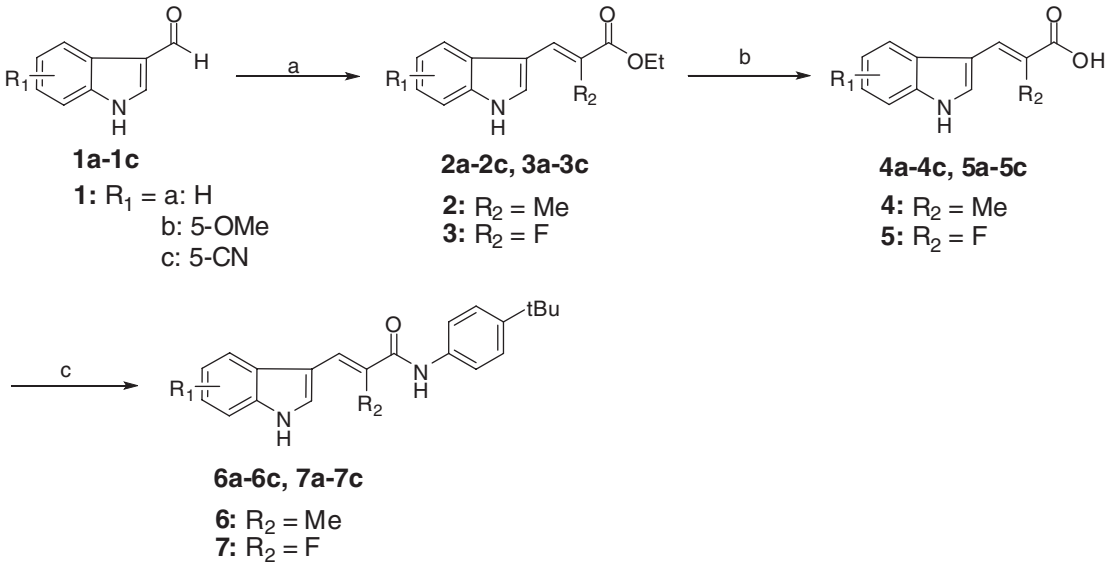
## Results and Discussion

As continuing efforts from our ongoing program for the identification of more effective HCV inhibitors, we have chosen three compounds **6a–6c** as the starting point for new compound generation. As shown in Scheme 1, commercially available reagents, such as unsubstituted or substituted indole-3-carboxaldehyde **1a–1c** were used for Wittig reaction with (carbethoxyethylidene) triphenylphosphorane or triethyl 2-fluoro-2-phosphonoacetate to provide compounds **2a–2c** and **3a–3c**. The ethyl esters **2a–2c** and **3a–3c** were then subjected to hydrolysis to yield carboxylic acid compounds **4a–4c** and **5a–5c**. Carboxylic acids **4a–4c** and **5a–5c** were used for coupling by using standard coupling reagent and 4-*tert*-butylaniline to provide a series of indole 2-mehtylacrylamide derivatives **6a–6c** and **7a–7c**.

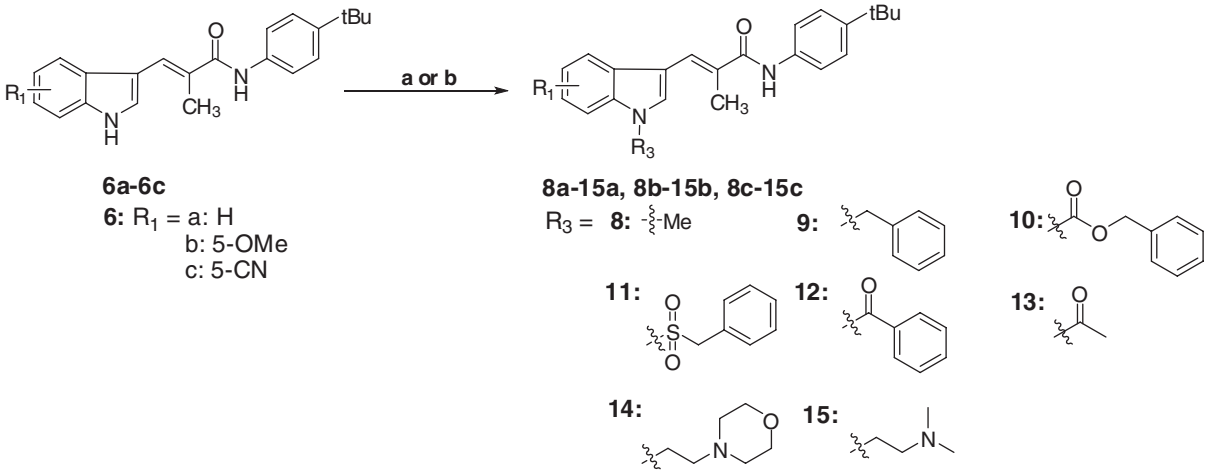
As shown in Scheme 2, we set out to explore the effect of N-substitution at indole 2-methylacrylamide. By using same method from Scheme 1, a series of indole 2-methylacrylamide derivatives **6a–6c** were prepared and further modified using

† These two authors contributed equally to this work.




**Scheme 1.** *Reagents and conditions*: (a) (Carbethoxyethylidene) triphenylphosphorane, $CH_2Cl_2$ for **2a–2c**; Triethyl 2-fluoro-2-phosphonoacetate, DBU, LiCl, THF for **3a–3c**. (b) NaOH, MeOH, THF, $H_2O$, and (c) 4-*tert*-butylaniline, HBTU, DIPEA, DMF, $CH_2Cl_2$.




**Scheme 2.** *Reagents and conditions*: (a) Alkyl halide, acyl halide or sulfonyl halide, NaH, DMF for **10a–15a**, **10b–15b**, **10c–15c**; (b) TBAB, 20% NaOH, alkyl halide, $CH_2Cl_2$ for **8a–9a**, **8b–9b**, **8c–9c**.

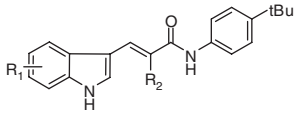
alkylation, acylation, or sulfonylation to give the desired compounds **8a–15a, 8b–15b,** and **8c–15c**.

All the newly synthesized compounds with N-substitution of indole ring were evaluated for their antiviral activity in a cell-based J6/JFH1 reporter assay using Huh7.5 cells. The *in-vitro* $EC_{50}$, $CC_{50}$, and selective index values for each analogue are listed in Table 1, and clemizole was used as a positive control. MTT(3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) based cytotoxicity ($CC_{50}$) was measured in parallel, to confirm whether the observed antiviral effect ($EC_{50}$) of each compound was due to its specific inhibitory action against HCV replication and not due to mere reduction of cell numbers by its cytotoxic effect. On the basis of the previous SAR study on indole 2-methylacrylamides,[5] we have chosen three compounds, **6a–6c**, as the most potent compounds among the series. These compounds share a common structure except for the substitution group in indole part, such as H, 5-OMe, and 5-CN. First, we explored the effect of fluoro-substitution instead of methyl group in the acryl amide portion, which maintains the remaining parts. Compounds **7a–7c** exhibited moderate to poor inhibitory activities (**7a**, $EC_{50}$ = 4.7 μM, $CC_{50}$ = 10.4 μM, and SI = 2.2; **7b**, $EC_{50}$ = 6.3 μM, $CC_{50}$ = 11.7 μM, and SI = 1.9; **7c**, $EC_{50}$ = 8.8 μM, $CC_{50}$ = 24.0 μM, and SI = 2.7). This result suggested the importance of the methyl acryl portion in comparison with the corresponding fluoro acryl portion. Accordingly, we synthesized N-substituted indole 2-methylacrylate derivatives for further study, as shown in Table2.

Of note, compound **6c**, the most potent inhibitor in the previous study, had poor water solubility, which may cause difficulties in further study. To develop a better analog with improved water solubility as well as high potency, we

**Table 1.** Structure–activity relationships of indole 2-methylacrylamides (**6a–6c**) or 2-fluoroacrlyamides (**7a–7c**).




| No. | Structure $R_1$ | Structure $R_2$ | Anti-HCV ($EC_{50}$, μM) | Cytotoxicity ($CC_{50}$, μM) | Selectivity index ($CC_{50}/EC_{50}$) |
|---|---|---|---|---|---|
| **6a** | H | Me | 2.2 | 17.3 | 7.9 |
| **6b** | 5-OMe | Me | 3.0 | 16.4 | 5.5 |
| **6c** | 5-CN | Me | 1.1 | 61.8 | 56.1 |
| **7a** | H | F | 4.7 | 10.4 | 2.2 |
| **7b** | 5-OMe | F | 6.3 | 11.7 | 1.9 |
| **7c** | 5-CN | F | 8.8 | 24.0 | 2.7 |
| **Clemizole** | N/A | N/A | 8.2 | 34.6 | 4.4 |

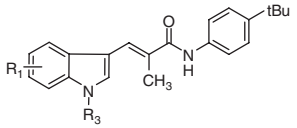
envisioned that the *N*-alkylated compounds **6a–6c** might show desired profiles. As shown in Table 2, a series of N-substituted indole ring derivatives **8a–15a, 8b–15b,** and **8c–15c**, which were derived from the leading compound **6a–6c**, were evaluated for their biological activity. Among the N-substituted indoles based on the **6a** derivative, the sulfonyl analog **11a** ($EC_{50}$ = 1.56 μM, $CC_{50}$ = 54.1 μM, and SI = 34.7) exhibited enhanced potency and selectivity index compared to the original compound **6a**. The corresponding benzyl (**9a**), acetyl (**13a**), and morpholine-4-ethyl (**14a**) substituted compounds showed moderate activity profiles, whereas methyl (**8a**), CBZ (carboxybenzyl) (**10a**), and benzoyl (**12a**) substituted compounds lost the activity. The *N*,*N*-dimethylaminoethyl analog **15a** showed moderate antiviral activity, but it was found to be toxic with the SI value of 0.4. In the case of 5-methyoxy indoles **8b–15b**, which were derived from **6b**, it was found that N-substitution at the indole ring was not tolerable on HCV inhibition, even though benzyl sulfonyl compound **11b** exhibited better selectivity ($EC_{50}$ = 5.3 μM, $CC_{50}$ = 66 μM, and SI = 12.5) than the original **6b**. As shown in Table 2, 5-cyano indoles **8c–15c** were also evaluated as potential HCV agents. Of note, indole **13a** and 5-OMe indole **13b,** which are substituted with the acetyl group, showed moderate potency, but the corresponding 5-CN indole **13c** exhibited enhanced potency (**13a**, $EC_{50}$ = 4.0 μM, $CC_{50}$ = 25.5 μM, and SI = 6.40; **13b**, $EC_{50}$ = 8.9 μM, $CC_{50}$ ≥100 μM, and SI ≥11.2; **13c**, $EC_{50}$ = 0.98 μM, $CC_{50}$ = 40.74 μM, and SI = 41.6). Interestingly, the benzoyl compound **12c** had improved potency, while compounds **12a**, and **12b,** which were substituted with benzoyl, were less potent (**12a**, $EC_{50}$ = 33.3 μM, $CC_{50}$ ≥100 μM, and SI ≥3.0; **12b**, $EC_{50}$ ≥100 μM, $CC_{50}$ ≥100 μM, and SI ≥1.0; **12c**, $EC_{50}$ = 1.16 μM, $CC_{50}$ = 69.0 μM, and SI = 59.5). In Table 3, especially, Compound **6c** had poor solubility in water, even though its antiviral activity was the best. Solubility in water is a general issue with synthetic compounds for further study, and therefore the kinetic solubilities of compounds **6c**, **12c**, and **13c** were measured by using an established nephelometry method. As in Table 3, an aromatic carbonyl-containing compound **12c** was not improved compared to the original compound **6c**. Interestingly, an alkyl carbonyl-containing compound **13c** exhibited much better water solubility at the three different pH conditions than original compound **6c.**

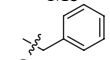
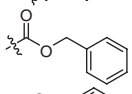
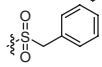
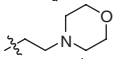
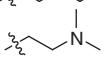
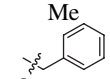
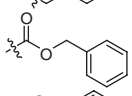
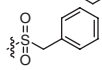
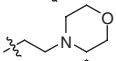
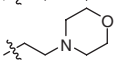
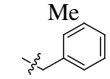
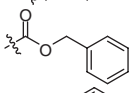
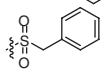
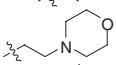
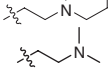
From the SAR study, we found that compounds **12c** and **13c** exhibited potent anti-HCV activities. Therefore, we further evaluated them for their *in-vitro* water solubility under various pH conditions. The kinetic solubility of compounds **6c**, **12c**, and **13c** were measured using an established nephlometry method. Of note, the solubility of benzoyl-substituted compound **12c** was not improved over the original compound **6c** (Table 3). Interestingly, the acetyl-substituted compound **13c** exhibited much better water solubility at the three different pH conditions than the original compound **6c.**

In order to confirm the antiviral effect of compound **13c** on HCV replication in a reporter-free system, we transfected *in-vitro*-transcribed genotype 2a J6/JFH1 RNAs into Huh7.5 cells and treated these cells with increasing amounts of compound **13c** for 72 h. Then, the viral as well as the host GAPDH RNA levels were measured by real-time polymerase chain traction (RT-PCR) analysis. As shown in Figure 1(a), a nearly threefold higher $EC_{50}$ value (3.2 μM) was observed in this system compared with the one (0.98 μM) measured previously using the renilla luciferase-linked reporter virus. In addition, when J6/JFH1 RNA-transfected cells were treated with compound **13c** at a concentration of 10 μM for different time periods, the length of time required for reducing HCV RNA levels by half ($T_{1/2}$) turned out to be 26.8 h (Figure 1(a)). We also wanted to test the inhibitory activity of compound **13c** against the more clinically relevant genotype 1b HCV to see whether compound **13c** had any cross-genotype antiviral activity. For this purpose, we used a Bart79I subgenomic replicon system, in which genotype 1b HCV RNAs encoding only the nonstructural components of stably replicating viral genes under selection pressure of G418 due to expression of a neomycin phosphotransferase gene were inserted in front of HCV genes. In this system, compound **13c** showed a 1.3-fold higher

**Table 2.** Structure–activity relationships of *N*-substituted indole 2-methylacrylamides (**8a–15a, 8b–15b, and 8c–15c**).




| No. | Structure | | Anti-HCV ($EC_{50}$, μM) | Cytotoxicity ($CC_{50}$, μM) | Selectivity index ($CC_{50}/EC_{50}$) |
|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | | | |
| **8a** | H | Me | 21.7 | 19.3 | 0.9 |
| **9a** | H |  | 6.8 | >100 | >14.7 |
| **10a** | H |  | >100 | 63.7 | >0.6 |
| **11a** | H |  | 1.56 | 54.1 | 34.7 |
| **12a** | H |  | 33.3 | >100 | >3.0 |
| **13a** | H |  | 4.0 | 25.5 | 6.4 |
| **14a** | H |  | 7.9 | 19.7 | 2.5 |
| **15a** | H |  | 18.5 | 6.9 | 0.4 |
| **8b** | 5-OMe | Me | 8.9 | 26.5 | 3.0 |
| **9b** | 5-OMe |  | >100 | >100 | >1.0 |
| **10b** | 5-OMe |  | 14.2 | >100 | 7.0 |
| **11b** | 5-OMe |  | 5.3 | 66 | 12.5 |
| **12b** | 5-OMe |  | >100 | >100 | >1.0 |
| **13b** | 5-OMe |  | 8.9 | >100 | >11.2 |
| **14b** | 5-OMe |  | 15.6 | 30.9 | 2.0 |
| **15b** | 5-OMe |  | >100 | >100 | >1.0 |
| **8c** | 5-CN | Me | 9.5 | 27.2 | 2.9 |
| **9c** | 5-CN |  | >100 | >100 | >1.0 |
| **10c** | 5-CN |  | 33.0 | 21.8 | 0.7 |
| **11c** | 5-CN |  | 10.4 | 17.0 | 1.6 |
| **12c** | 5-CN |  | 1.16 | 69.0 | 59.5 |
| **13c** | 5-CN |  | 0.98 | 40.74 | 41.6 |
| **14c** | 5-CN |  | 25.0 | >100 | >4.0 |
| **15c** | 5-CN |  | 1.8 | 2.1 | 1.2 |
| **Clemizole** | N/A | N/A | 8.2 | 34.6 | 4.4 |

potency against HCV replication activity ($EC_{50}$ = 2.4 μM) than that in the genotype 2a infectious clone system ($EC_{50}$ = 3.2 μM); accordingly, compound **13c** needed a 1.8-fold shorter time for reducing HCV RNA levels by half ($T_{1/2}$ = 14.6 h) than in the genotype 2a infectious clone system ($T_{1/2}$ = 26.8 h) (Figure 1(b)). These data demonstrated the cross-genotype potency of compound **13c** against both genotype 1b and genotype 2a HCV replicons in a reporter-free system.

After confirming suppression of HCV replication by compound **13c** at the RNA level, we wanted to see whether this compound **13c**-induced inhibition of HCV replication can also lead to the downregulation of viral protein levels. For this purpose, J6/JFH1 RNA-transfected Huh7.5 cells were treated with increasing concentrations of compound **13c** for 120 h. Then, levels of viral NS3 as well as host GAPDH proteins were measured by Western blot analyses. As shown in Figure 2(a), lower $EC_{50}$ as well as shorter $T_{1/2}$ values (0.5 μM and 16.1 h, respectively) were determined by this protein stability-based method when compared with those determined previously by the RNA stability-based method (3.2 μM and 26.8 h, respectively). When Huh7.5 cells stably maintaining genotype 1b subgenomic Bart79I RNAs were used to assess the dose- and time-dependent effects of compound **13c** on viral protein levels, a similar length of time was necessary to reduce HCV NS3 protein levels by half ($T_{1/2}$ = 14.2 h) in spite of a lower antiviral potency ($EC_{50}$ = 1.7 μM) (Figure 2(b)).

**Table 3.** *In-vitro* water solubility of **6c, 12c,** and **13c.**

| | Solubility (μM)[a] | | |
|---|---|---|---|
| No. | pH 2 | pH 7.4 | pH 9 |
| **6c** | 58.0 | 38.0 | 11.3 |
| **12c** | 65.0 | 35.0 | 57.0 |
| **13c** | 103.0 | 101.3 | 35.7 |

[a] Values are the mean of three experiments.

## Conclusion

In conclusion, we have designed a novel series of indole acrylamide derivatives as potential HCV replication inhibitors using a cell-based J6/JFH1 reporter assay using Huh7.5 cells. We have carried out SAR exploration based on structural modification using substituted indoles, various amides, and N-

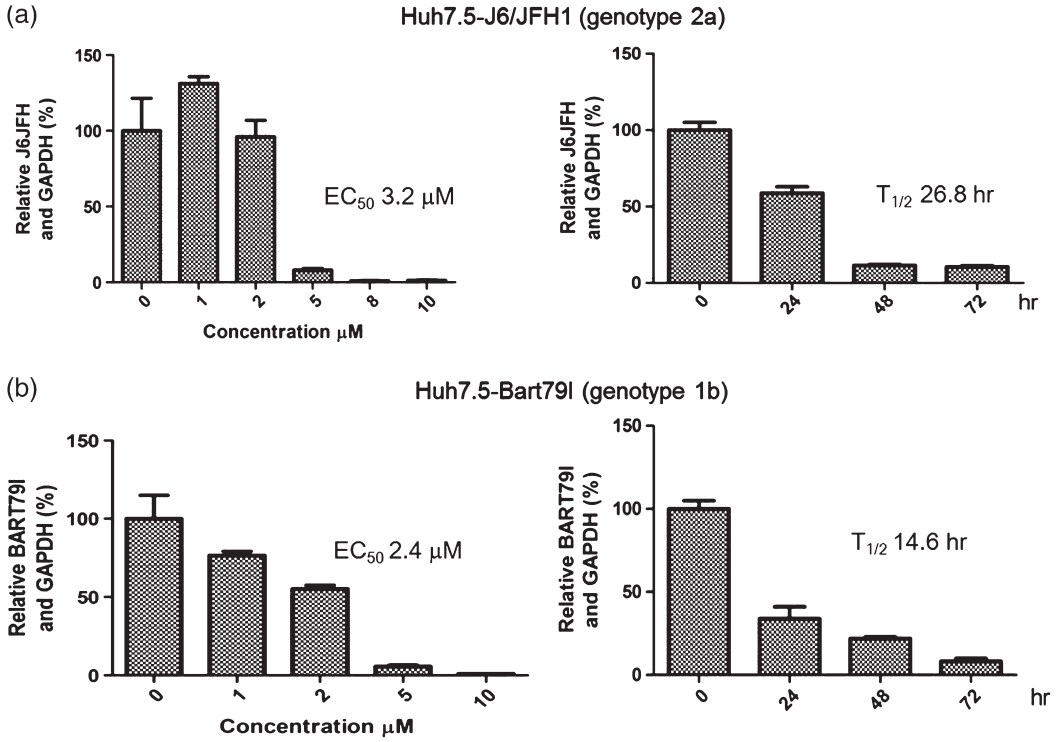



**Figure 1.** Effect of compound **13c** on HCV replication. (a) Dose–response graph determined by measuring relative HCV as well as GAPDH RNA levels via real-time qRT-PCR analysis of J6/JFH1 RNA-transfected Huh7.5 cells treated with increasing concentrations of compound **13c** for 72 h. The time–response graph was determined by measuring relative HCV as well as GAPDH RNA levels via real-time qRT-PCR analysis of J6/JFH1 RNA-transfected Huh7.5 cells treated with 10 μM of compound **13c** for increasing periods of time. (b) Dose–response graph determined by measuring relative HCV well as GAPDH RNA levels via real time qRT-PCR analysis of Bart79I RNA-transfected Huh7.5 cells treated with increasing concentrations of compound **13c** for 72 h. The time–response graph was determined by measuring relative HCV as well as GAPDH RNA levels via real time qRT-PCR analysis of Bart79I RNA replicon Huh7.5 cells treated with 10 μM of compound **13c** for increasing periods of time. Average values from triplicate experiments are depicted. $EC_{50}$ and $T_{1/2}$ values were determined based on each response curve. Error bars represent standard deviation values.

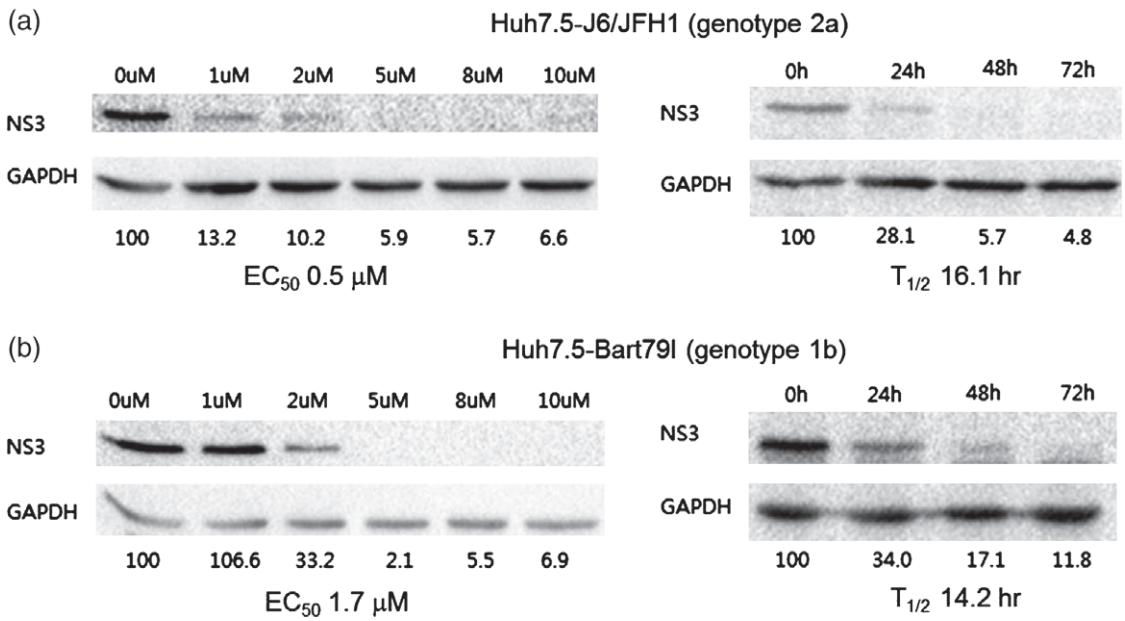



**Figure 2.** Effect of compound **13c** on levels of HCV proteins. (a) Dose–response determined by measuring relative levels of HCV NS3 as well as host GAPDH proteins via Western blot analyses of J6/JFH1 RNA-transfected Huh7.5 cells treated with increasing concentrations of compound **13c** for 120 h. The time–response curve was determined by measuring relative levels of HCV NS3 as well as host GAPDH proteins via Western blot analyses of J6/JFH1 RNA-transfected Huh7.5 cells treated with 10 μM of compound **13c** for increasing periods of time. (b) Dose–response curve determined by measuring relative levels of HCV NS3 as well as host GAPDH proteins via Western blot analyses of Bart79I replicon Huh7.5 cells treated with increasing concentrations of compound **13c** for 120 h. The time–response was determined by measuring relative levels of HCV NS3 as well as host GAPDH proteins via Western blot analyses of Bart79I replicon Huh7.5 cells treated with 10 μM of compound **13c** for increasing periods of time. Numbers below GAPDH blots represent relative quantitation of intensity of viral protein after normalization to levels of GAPDH proteins.

substitution of the indole ring. Among the synthesized compounds, *N*-acetyl-substituted indole acrylamide **13c** displayed the most potent antiviral activity with good selectivity and better water solubility compared to the previously identified **6c**. As a continuation of our study toward the identification of effective HCV inhibitors, we concluded that compound **13c** was the most potent inhibitor among the series. Compound **13c** may serve as a promising platform for further development of a new class of HCV NS5B RNA polymerase inhibitors in the future.

## Experimental

**General.** All the reagents used were commercial grade chemicals and used without further purification. All reactions were carried out under the atmosphere of dried argon in flame-dried glassware. All reaction products were purified by flash column chromatography (CC) with silica gel 60 (230–400 mesh Kieselgel 60). In addition, reactions were monitored by thin-layer chromatography (TLC) using glass-backed plates coated with 0.25 mm silica gel (E. Merck, silica gel 60 F254). The chromatograms were visualized using ultraviolet illumination, exposure to iodine vapors, and staining with PMA or Hanessian's solution. Proton nuclear magnetic resonance ($^1$H NMR) spectra were determined on a Varian (400 MHz) or a Bruker (500 MHz) spectrometer (Varian Medical Systems, Inc. Palo Alto, CA, USA). Chemical shifts are provided in parts per million (ppm) downfield with coupling constants in hertz (Hz) from tetramethylsilane (internal standard). Mass spectra were recorded with high-resolution mass spectrometry (HRMS) (electrospray ionization MS, ESI-MS) obtained on a Waters, Xevo, G2, QTof (Wilford, MA,USA) mass spectrometer or MS (electron ionization MS, EI-MS) obtained on a JMS-700, Jeol (Tokyo, Japan) mass spectrometer. Purities of the final products were checked by reversed phase high-pressure liquid chromatography (RP-HPLC), which was performed on a Waters Corp. HPLC system equipped with an ultraviolet (UV) detector set at 254 nm. The mobile phases were (A) $H_2O$ containing 0.05% trifluoroacetic acid and (B) CH3CN. HPLC had a YMC Hydrosphere C18 (HS-302) column (5-μM particle size, 12-nm pore size) that was 4.6 mm in diameter × 150 mm in size with a flow rate of 1.0 mL/min. Compound purity was assessed with (Method A) a gradient of 20% B to 100% B in 35 min or (Method B) a gradient of 25% B to 100% B in 35 min. Melting points were determined on a Fisher Scientific apparatus (Waltham, MA, USA; CAT No.: 12-144).

**Cell Culture.** Cells of the human hepatoma cell line Huh7.5 were cultured in monolayers as described previously,[13,14] with media consisting of DMEM (Sigma-Aldrich, St.Louis, MO, USA) supplemented with 1% L-glutamine (Hyclone, GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA), 1%

penicillin–streptomycin (Hyclone), 1% nonessential amino acid (Hyclone), and 10% fetal bovine serum (JR Scientific, CA, USA).

**Plasmids.** Rluc-J6/JFH1 (FL-J6/JFH-5′C19Rluc2AUbi) [15] is a monocistronic, full-length HCV genome that expresses renilla luciferase and was derived from the previously described infectious genotype 2a HCV genome J6/JFH1.[16] Bart79I is a high-efficiency bicistronic subgenomic replicon of HCV derived from the HCV genotype 1b Con1 sequence that harbors the neomycin phosphotransferase gene in the first cistron and the HCV nonstructural proteins in the second cistron under the translational control of an EMCV IRES.[14] This plasmid also has an adaptive mutation (S2204I) in NS5A, which increases the replication efficiency. FL-J6/JFH-5′C19Rluc2AUbi and Bart79I were gifts from Dr. Charles Rice of Rockefeller University.

***In-vitro* Transcription for Production of HCV RNA Genomes.** *In-vitro* transcription for production of HCV RNA genomes was performed as previously described.[17] Briefly, Wild-type Bart79I, J6/JFH1, or RLuc-J6/JFH1 plasmids were linearized by ScaI for Bart79I or XbaI for J6/JFH1 (NEB) digestion. The T7 promoter-driven *in-vitro* transcription of the digested plasmid was performed to produce the wild type HCV RNA genomes by using a MEGAscript kit (Ambion).

**Generation of Stable HCV Replicon Cell Lines.** The establishment of Huh7.5 cells stably maintaining a Bart79I subgenomic replicon in the presence of G418 selection has been described elsewhere.[9] Briefly, *in-vitro* transcribed Bart79I RNAs were transfected into Huh7.5 cells by using a lipofectamine 2000 transfection reagent. The transfected cells were supplemented with G418 at a final concentration of 750 μg/mL. This selection medium was replaced every 3 days for 3 weeks. The establishment of Huh7.5 cells stably maintaining a Bart79I-YFP subgenomic replicon in the presence of G418 selection was performed similarly. The establishment of Huh7.5 cells stably maintaining a J6/JFH1 infectious clone was performed by transfecting *in-vitro* transcribed J6/JFH1 RNAs into Huh7.5 cells by using a lipofectamine 2000 transfection reagent. The establishment of Huh7.5 cells stably maintaining an Rluc-J6/JFH1 clone was also performed similarly.

**Cell Viability and Anti-HCV Replication Analysis Using a Luciferase Assay.** *In-vitro* transcribed RLuc-J6/JFH1 RNAs were transfected into Huh7.5 cells by using a lipofectamine 2000 transfection reagent (Invitrogen) as described by the manufacturer. Transfected cells were plated onto a white 96-well plate (Costar 3610) and supplemented with DMSO or 1 nM, 10 nM, 100 nM, 1 μM, 10 μM, and 100 μM of the compounds. At 3 days after incubation, cells were incubated for 3 h at 37 °C in the presence of EZ-CYTOX (10% tetrazolium salt; Dogen) reagent to assess the cytotoxicity. Renilla luciferase activities were measured by using a luciferase reagent (1 mM coelenterazine in methanol-HCL; Goldbio). A time–response curve was also determined by measuring renilla luciferase activities as well as cell viabilities at 24, 48, 72 h after treating HCV RNA-transfected cells with 10 μM of the compound.

**Quantitative RT-PCR (qRT-PCR) Analysis.** Onto a 6-well plate (Costar 3610), $3 \times 10^5$ cells (Huh7.5-J6/JFH1 or Bart79I) were plated and supplemented with DMSO or compound **13c** at the indicated concentrations. At 3 days after incubation, total cellular RNA was extracted using the RNeasy® mini kit (Qiagen) in accordance with the manufacturer's instructions. The yield of extracted RNA was assessed spectrophotometrically. The expression of HCV subgenomic RNA and cellular RNA was measured by quantitative RT-PCR (qRT-PCR) analysis as previously described. Each sample was normalized using the endogenous reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The cDNA quantification was performed by the CFX384 RT PCR detection system (Bio-Rad, Hercules, CA, USA). The primers used for the qRT-PCR were as follows: FW-J6/JFH1-CTCCGCCATGAATCACTC, RV-J6/JFH1-ACGACACTCATACTAACGC, FW-Bart79I-AGAGCCATAGTGGTCT, RV-Bart79I-CCAAATCTCCAGGCATTGAGC, FW-GAPDH-TGGTCTCCTCTGACTTCA, and RV-GAPDH-CGTTGTCATACCAGGAAATG. A time–response curve was also determined by measuring renilla luciferase activities as well as cell viabilities at 24, 48, 72 h after treating HCV RNA-transfected cells with 10 μM of compound **13c**.

**Western Blot Analysis.** Onto a 6-well plate (Costar 3610), $1.5 \times 10^5$ cells (Huh7.5-J6/JFH1 or Bart79I) were plated and supplemented with DMSO or compound **13c** at the indicated concentrations. At 5 days after incubation, whole-cell extracts were prepared in RIPA buffer (150 mM NaCl, 1% Triton X-100, 1% deoxycholic acid sodium salt, 0.1% sodium dodecyl sulfate, 50 mM Tris-HCl , pH 7.5, 2 mM EDTA; genDEPOT) containing a cocktail of protease inhibitors (Complete, Roche Diagnostic at a final concentration of 1 tablet per 50 mL RIPA buffer) and quantitated by the Bradford assay (Bio-Rad). Equal amounts of protein were electrophoresed on an SDS polyacrylamide gel, subsequently transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore, Bedford, MA, USA), and probed with a mouse anti-NS3 monoclonal antibodies (1878 for NS3, Virostat). A time–response curve was also determined by performing similar Western blot analyses at 24, 48, 72 h after treating cells with 10 μM of compound **13c**.

**General Procedure for Wittig Reaction.** A solution of indole aldehyde (1.0 equiv) and (carbethoxyethylidene) triphenylphosphorane (1.3 equiv) in $CH_2Cl_2$ was refluxed for 18 h and the excess solvent was evaporated under reduced pressure. The reaction mixture was partitioned between $CH_2Cl_2$ and water. The organic layer was dried over magnesium sulfate ($MgSO_4$) and purified by CC on silica gel using 0–40% EtOAc in hexanes as eluent to obtain the desired compounds **2a–2c** as yellow solid. The mixture of indole aldehyde (1.0 equiv) and triethyl 2-fluoro-2-phosphonoacetate was dissolved in THF. DBU and LiCl were added to the mixture, and the resulting mixture was stirred overnight at room temperature. After the reaction was complete, the reaction mixture

was quenched by 10% citric acid and extracted with $CH_2Cl_2$. The organic layer was dried over $MgSO_4$ and purified by column chromatography on silica gel using 0–40% EtOAc in hexanes as eluent to yield compounds **3a–3c** as a green solid.

**(*E*)-Ethyl 3-(1*H*-indol-3-yl)-2-methylacrylate (2a):** white solid, yield 65%. m.p. 138–139 °C; $^1$H-NMR ($CDCl_3$, 500 MHz) $\delta$ 8.50 (brs, 1H), 8.05 (s, 1H), 7.83 (d, $J$ =7.8 Hz, 1H), 7.51 (d, $J$ = 2.4 Hz, 1H), 7.42 (d, $J$ = 8.0 Hz, 1H), 7.22–7.30 (m, 2H), 4.31 (q, $J$ = 7.1 Hz, 2H), 1.38 (t, $J$ = 7.1 Hz, 3H); $^{13}$C-NMR ($CDCl_3$, 100Hz) $\delta$ 168.11, 134.40, 128.93, 126.60, 124.54, 122.19, 122.07, 119.69, 117.91, 112.22, 110.22, 59.61, 14.03, 13.43; MS (EI) *m/z* 229 ($M^+$), HRMS (ESI) *m/z* calcd. for $C_{14}H_{16}NO_2$ [$(M + H)^+$] 230.1181, found: 230.1178; purity 100 % (as determined by RP-HPLC, $t_R$ = 16.5 min).

**(*E*)-Ethyl 3-(5-methoxy-1*H*-indol-3-yl)-2-methylacrylate (2b):** white solid, yield 99%. $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 8.38 (brs, 1H), 7.99 (s, 1H), 7.48 (d, $J$ = 2.8 Hz, 1H), 7.30 (d, $J$ = 8.8 Hz, 1H), 7.23 (d, $J$ = 2.4 Hz, 1H), 6.93 (dd, $J$ = 2.4, 8.4 Hz, 1H), 4.30 (q, $J$ = 7.2 Hz, 2H), 3.90 (s, 3H), 2.19 (s,3H), 1.38 (t, $J$ = 7.2 Hz, 3H); MS (ESI) *m/z* 260 $(M + H)^+$, 258 $(M - H)^-$.

**(*E*)-Ethyl 3-(5-cyano-1*H*-indol-3-yl)-2-methylacrylate (2c):** white solid, yield 99%. $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$ 8.15 (s, 1H), 7.98 (s, 1H), 7.81 (s, 1H), 7.58 (d, $J$ = 8.0 Hz, 1H), 7.49 (d, $J$ = 8.4 Hz, 1H), 4.28 (q, $J$ = 6.0 Hz, 2H), 2.18 (s, 3H), 1.37 (t, $J$ = 8.0 Hz, 3H); MS (ESI) *m/z* 255 $(M + H)^+$, 253 $(M - H)$.

**(*E*)-2-Fluoro-3-(1*H*-indol-3-yl)acrylic acid (3a):** green solid, yield 99%. $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$ 8.42 (s, 1H), 7.77 (s, 1H), 7.74 (d, $J$ = 8.0 Hz, 1H), 7.66 (d, $J$ = 0.8 Hz, 1H), 7.37–7.41 (m, 3H), 7.10–7.17 (m, 5H); HRMS (ESI) *m/z* calcd. for $C_{11}H_9NO_2F$ [$(M + H)^+$] 206.0617, found: 206.0617.

**(*E*)-2-Fluoro-3-(5-methoxy-1*H*-indol-3-yl)acrylic acid (3b):** green solid, yield 85%. $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$ 8.37 (s, 1H), 7.16–7.31 (m, 4H), 6.82–6.85 (m, 2H), 3.85 (s, 3H); HRMS (ESI) *m/z* calcd. for $C_{12}H_{11}NO_3F$ [$(M + H)^+$] 236.0723, found: 236.0730.

**(*E*)-3-(5-Cyano-1*H*-indol-3-yl)-2-fluoroacrylic acid (3c):** green solid, yield 72%. $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$ 8.54 (s, 1H), 8.16 (s, 1H), 7.56 (d, $J$ = 8.8 Hz, 1H), 7.47 (dd, $J$ = 1.4, 8.6 Hz, 1H), 7.25–7.31 (m, 1H); HRMS (ESI) *m/z* calcd. for $C_{12}H_8N_2O_2F$ [$(M + H)^+$] 231.0570, found: 231.0570.

**General Synthetic Procedure for Hydrolysis.** A solution of the acrylate (1.0 equiv.) in THF/$H_2O$/MeOH was treated with sodium hydroxide (10.0 equiv.) in water at 5 °C and then heated in an oil bath and refluxed for 2–4 h. The reaction mixture was acidified with 2 N HCl to pH ~2. The mixture was partitioned between EtOAc and water. The organic layer was dried over $MgSO_4$, filtered, and concentrated *in-vacuo* to give crude products **4a–4c** and **5a–5c**, which were used without further purification.

**(*E*)-3-(1*H*-Indol-3-yl)-2-methylacrylic acid (4a):** white solid, yield 61%. m.p. 237–238 °C; $^1$H-NMR (acetone-$d_6$, 500 MHz) $\delta$ 10.84 (brs, 1H), 8.10 (s, 1H), 7.79 (d, $J$ = 8.0 Hz, 1H), 7.50 (d, $J$ = 8.0 Hz, 1H), 7.16–7.24 (m, 3H), 2.17 (d, $J$ = 1.0 Hz, 3H); $^{13}$C-NMR ($CD_3OD$, 100 Hz) $\delta$ 172.91, 137.47, 132.35, 129.20, 127.99, 123.60, 121.33, 119,10, 113.40, 112.62, 15.19; MS (EI) *m/z* 201 ($M^+$); HRMS (EI) *m/z* calcd. for $C_{12}H_{11}NO_2$ 201.0790, found: 201.0791; purity >99.9 % (as determined by RP-HPLC, $t_R$ = 7.9 min).

**(*E*)-3-(5-Methoxy-1*H*-indol-3-yl)-2-methylacrylic acid (4b):** white solid, yield 78%. $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 8.43 (brs, 1H), 8.12 (s, 1H), 7.54 (d, $J$ = 2.8 Hz, 1H), 7.31 (d, $J$ = 8.8 Hz, 1H), 7.24 (d, $J$ = 2.4 Hz, 1H), 6.94 (dd, $J$ = 2.4, 8.8 Hz, 1H), 3.90 (s, 3H), 2.21 (s, 3H); MS (ESI) *m/z* 232 $(M + H)^+$.

**(*E*)-3-(5-Cyano-1*H*-indol-3-yl)-2-methylacrylic acid (4c):** brown solid, yield 99%. $^1$H-NMR (DMSO-$d_6$, 400 MHz) $\delta$ 12.21 (brs, 1H), 8.27 (s, 1H), 7.9 (d, $J$ = 16.0 Hz, 2H), 7.60 (d, $J$ = 8.8 Hz, 1H), 7.52 (d, $J$ = 8.4 Hz, 1H), 2.08 (s, 3H); MS (ESI) *m/z* 227 $(M + H)^+$.

**(*E*)-2-Fluoro-3-(1*H*-indol-3-yl)acrylic acid (5a):** green solid, yield 99%. $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$ 8.42 (s, 1H), 7.77 (s, 1H), 7.74 (d, $J$ = 8.0 Hz, 1H), 7.66 (d, $J$ = 0.8 Hz, 1H), 7.37–7.41 (m, 3H), 7.10–7.17 (m, 5H); HRMS (ESI) *m/z* calcd. for $C_{11}H_9NO_2F$ [$(M + H)^+$] 206.0617, found: 206.0617.

**(*E*)-2-Fluoro-3-(5-methoxy-1*H*-indol-3-yl)acrylic acid (5b):** green solid, yield 85%. $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$ 8.37 (s, 1H), 7.16–7.31 (m, 4H), 6.82–6.85 (m, 2H), 3.85 (s, 3H);HRMS (ESI) *m/z* calcd. for $C_{12}H_{11}NO_3F$ [$(M + H)^+$] 236.0723, found: 236.0730.

**(*E*)-3-(5-Cyano-1*H*-indol-3-yl)-2-fluoroacrylic acid (5c):** green solid, yield 72%. $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$ 8.54 (s, 1H), 8.16 (s, 1H), 7.56 (d, $J$ = 8.8 Hz, 1H), 7.47 (dd, $J$ = 1.4, 8.6 Hz, 1H), 7.25–7.31 (m, 1H); HRMS (ESI) *m/z* calcd. for $C_{12}H_8N_2O_2F$ [$(M + H)^+$] 231.0570, found: 231.0570.

**General Procedure for Preparation of Substituted Indole 2-methylacrylamide and 2-fluoroacrylamide Analogs.** The mixture of substituted (*E*)-3-(1*H*-indol-3-yl)-2-methylacrylic acid (1 equiv.) or (*E*)-2-fluoro-3-(1*H*-indol-3-yl)acrylic acid (1 equiv) was dissolved in a mixture of DMF and CH2Cl2 (v/v, 1:1). To the resulting mixture was added DIPEA (1.5 equiv) and HBTU (1.5 equiv) and stirred for 30 min at room temperature. After stirring, the amine (1.0 equiv) was added to the solution at room temperature and stirred overnight at room temperature. The mixture was partitioned with EtOAc and water. The organic layer was dried over $MgSO_4$ and purified by column chromatography on silica gel using 0–60% EtOAc in hexanes as eluent to provide compounds **6a–6c** and **7a–7c**.

**(*E*)-*N*-(4-*tert*-Butylphenyl)-3-(1*H*-indol-3-yl)-2-methylacrylamide (6a):** yellow solid, yield 77%. m.p. 178–179 °C; $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$ 7.80 (d, $J$ = 8.4 Hz, 1H), 7.76 (s, 1H), 7.60 (s, 1H), 7.54 (d, $J$ = 7.2 Hz, 2H), 7.37–7.43 (m, 3H), 7.13–7.19 (m, 3H), 2.26 (s, 3H), 1.33 (s, 9H); $^{13}$C-NMR ($CD_3OD$, 100Hz) $\delta$ 146.82, 136.13, 136.00, 127.73, 126.32, 125.73, 125.08, 122.00, 120.81, 119.61, 117.99, 111.91, 111.08, 33.78, 30.41, 14.43; MS (EI) *m/z* 332 ($M^+$); HRMS (EI) *m/z* calcd. for $C_{22}H_{24}N_2O$ 332.1889, found: 332.1888; purity >99.9% (as determined by RP-HPLC, $t_R$ = 21.2 min).

**(*E*)-*N*-(4-*tert*-Butylphenyl)-3-(5-methoxy-1*H*-indol-3-yl)-2-methylacrylamide (6b):** yellow solid, yield 61%. m. p. 160–161 °C; $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$ 7.74 (s, 1H), 7.53–7.57 (m, 3H), 7.39 (d, $J$ = 8.4 Hz, 2H), 7.31 (d, $J$ = 8.4 Hz, 2H), 6.84 (dd, $J$ = 2.4, 8.4 Hz, 1H), 3.86 (s, 3H), 2.25 (s, 3H), 1.33 (s, 9H); $^{13}$C-NMR ($CD_3OD$, 100 Hz) $\delta$ 171.95, 156.10, 148.28, 137.60, 132.50, 129.79, 127.96, 127.74, 126.54, 122.34, 113.83, 113.27, 101.20, 56.21, 35.26, 31.88, 15.84; HRMS (ESI) *m/z* calcd. for $C_{23}H_{27}N_2O_2$ [$(M+H)^+$] 363.2073, found: 363.2068; purity >99.9 % (as determined by RP-HPLC, $t_R$ = 20.4 min).

**(*E*)-*N*-(4-*tert*-Butylphenyl)-3-(5-cyano-1*H*-indol-3-yl)-2-methylacrylamide (6c):** yellow solid, yield 25%. m. p. 268–269 °C; $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$ 8.18 (s, 1H), 7.68 (s, 1H), 7.60(s, 1H), 7.45–7.49 (m, 3H), 7.39 (dd, $J$ = 1.6, 8.4 Hz, 1H), 7.29 (d, $J$ = 8.4 Hz, 2H), 2.16 (s, 3H), 1.24 (s, 9H); $^{13}$C-NMR ($CD_3OD$, 100 Hz) $\delta$ 171.48, 148.41, 139.26, 137.47, 129.89, 129.33, 129.05, 126.57, 126.22, 125.83, 125.56, 122.24, 121.61, 114.12, 113.86, 103.81, 35.27, 31.87, 15.88; HRMS (ESI) *m/z* calcd. for $C_{23}H_{24}N_3O$ [$(M+H)^+$] 358.1919, found: 358.1922; purity >99.9 % (as determined by RP-HPLC, $t_R$ = 20.1 min).

**(*E*)-*N*-(4-*tert*-Butylphenyl)-2-fluoro-3-(1*H*-indol-3-yl) acrylamide (7a):** yellow solid, yield 16%. m.p. 214–215 °C; $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$ 8.56 (s, 1H), 7.68 (d, $J$ = 7.2 Hz, 1H), 7.57–7.60 (m, 2H), 7.39–7.41 (m, 3H), 7.24 (s, 1H), 7.11–7.19 (m, 3H), 1.33 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{21}H_{22}N_2OF$ [$(M+H)^+$] 337.1716, found: 337.1720.

**(*E*)-*N*-(4-*tert*-Butylphenyl)-2-fluoro-3-(5-methoxy-1*H*-indol-3-yl)acrylamide (7b):** yellow solid, yield 48%. m. p. 198–199 °C; $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$ 8.5 (s, 1H), 7.87 (s, 1H), 7.57 (d, $J$ = 8.4 Hz, 2H), 7.39 (d, $J$ = 8.4 Hz, 2H), 7.29 (d, $J$ = 9.2 Hz, 1H), 7.21 (s, 1H), 7.17 (d, $J$ = 2.4 Hz, 1H), 7.13–7.15 (m, 2H), 6.82 (dd, $J$ = 2.4, 8.8 Hz, 1H), 6.67 (d, $J$ = 6.8 Hz, 1H), 3.85 (s, 3H), 1.33 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{21}H_{22}N_2OF$ [$(M+H)^+$] 337.1716, found: 337.1720.

**(*E*)-*N*-(4-*tert*-Butylphenyl)-3-(5-cyano-1*H*-indol-3-yl)-2-fluoroacrylamide (7c):** yellow solid, yield 16%. m. p. 271–272 °C; $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$ 8.68 (s, 1H), 8.17 (s, 1H), 7.39–7.60 (m, 7H), 7.21 (d, $J$ = 26.4, 2H), 1.33 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{21}H_{22}N_2OF$ [$(M+H)^+$] 337.1716, found: 337.1720.

**General Procedure for N-Substitution of Indole Ring.** Sodium hydride (3.0 equiv) was added slowly to the amide **6a–6c** (1.0 equiv.) in DMF at 0 °C. The resulting mixture was stirred for 1 h in an ice bath and transferred to room temperature. To the solution was added alkyl halide or acyl halide at room temperature, and stirred for 1 h at room temperature. The reaction was quenched by ice-water and partitioned with EtOAc and water. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using 0–30% EtOAc in hexanes as eluent to give compounds **10a–13a**, **10b–13b**, and **10c–13c**. The amides **6a–6c** (1.0 equiv) were dissolved in DMF, and sodium hydride (3.0 equiv) was added to the solution at room temperature. The resulting mixture was stirred for 1 h at room temperature and transferred to an oil bath. Alkyl halide was added to the solution, and the resulting mixture was refluxed for 1.5 h. The reaction was quenched by ice-water and partitioned with EtOAc and water. The organic layer dried over $MgSO_4$, filtered, and the excess solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using 0–30% EtOac in hexanes as eluent to yield products **14a–15a**, **14b–15b**, and **14c–15 c**. A solution of the amide **6a–6c** (1.0 equiv) and tetrabutylammonium bromide (TBAB, 0.5 equiv) in $CH_2Cl_2$ was added 20% NaOH and methyl iodide at room temperature. The resulting mixture was stirred overnight at room temperature and quenched by water. The mixture was extracted with $CH_2Cl_2$ and water. The organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography on silica gel using 0–30% EtOAc in hexanes as eluent to give compounds **8a–9a**, **8b–9b**, and **8c–9c**.

**(*E*)-*N*-(4-*tert*-Butylphenyl)-2-methyl-3-(1-methyl-1*H*-indol-3-yl)acrylamide (8a):** yellow oil, yield 37%. $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 7.78 (d, $J$ = 8.0 Hz, 1H), 7.71 (s, 1H), 7.52–7.56 (m, 3H), 7.30–7.39 (m, 5H), 7.23 (t, $J$ = 7.0 Hz, 2H), 3.88 (s, 3H), 2.80 (s, 3H), 1.33 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{23}H_{26}N_2O$ [$(M+H)^+$] 347.2123, found: 347.2129; purity >99.6% (as determined by RP-HPLC, $t_R$ = 23.2 min).

**(*E*)-3-(1-Benzyl-1*H*-indol-3-yl)-*N*-(4-*tert*-butylphenyl)-2-methylacrylamide (9a):** yellow solid, yield 34%.m.p. 165–166 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 7.20–7.35 (m, 7H), 7.15 (t, $J$ = 7.4 Hz, 1H), 7.10 (s, 1H), 7.00–7.08 (m, 6H), 5.07 (s, 2H), 1.86 (s, 3H), 1.26 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{29}H_{30}N_2O$ [$(M-H)^-$] 421.2258, found: 421.2262; purity >99.9% (as determined by RP-HPLC, $t_R$ = 28.4 min).

**(*E*)-benzyl 3-[3-(4-*tert*-Butylphenylamino)-2-methyl-3-oxoprop-1-enyl]-1*H*-indole-1-carboxylate (10a):** yellow solid, yield 32%. m.p. 197–198 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 8.17 (s, 1H), 7.79 (s, 1H), 7.67 (d, $J$ = 7.6 Hz, 1H), 7.50–7.55 (m, 6H), 7.37–7.46 (m, 6H), 7.26–7.37 (m, 1H), 5.51 (s, 2H), 2.28 (s, 3H), 1.32 (s, 9H);HRMS (ESI) *m/z* calcd. for $C_{30}H_{30}N_2O_3$ [$(M-H)^-$] 465.2178, found: 465.2171; purity >99.9% (as determined by RP-HPLC, $t_R$ = 27.9 min).

**(*E*)-3-[1-(Benzylsulfonyl)-1*H*-indol-3-yl]-*N*-(4-*tert*-butylphenyl)-2-methylacrylamide (11a):** yellow solid, yield 29%. m.p. 151–152 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 7.87 (dd, $J$ = 1.8, 7.0 Hz, 1H), 7.70 (dd, $J$ = 2.4, 6.8 Hz, 1H), 7.53 (d, $J$ = 8.4 Hz, 3H), 7.46 (s, 1H), 7.35–7.41 (m, 4H), 7.30 (d, $J$ = 7.2 Hz, 1H), 7.17 (t, $J$ = 7.6 Hz, 2H), 7.04 (s, 1H), 6.85 (d, $J$ = 7.6 Hz, 2H), 4.54 (s, 2H), 1.97 (s, 3H), 1.33 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{29}H_{30}N_2O_3S$ [$(M-H)$] 485.1899, found: 485.1902; purity >99.9% (as determined by RP-HPLC, $t_R$ = 25.5 min).

**(*E*)-3-(1-Benzoyl-1*H*-indol-3-yl)-*N*-(4-*tert*-butylphenyl)-2-methylacrylamide (12a):** yellow solid, yield 31%.m. p. 187–188 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 8.35 (d, $J$ = 8.0 Hz, 1H), 7.80 (s, 1H), 7.78 (d, $J$ = 1.6 Hz, 1H), 7.64–7.73

(m, 2H), 7.52–7.59 (m, 6H), 7.48 (s, 1H), 7.42–7.48 (m, 2H), 7.40 (s, 1H), 7.26 (s, 1H), 2.19 (s, 3H), 1.32 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{29}H_{28}N_2O_2$ [(M – H)$^-$] 435.2073, found: 435.2092; purity >99.9% (as determined by RP-HPLC, $t_R$ = 26.4 min).

**(*E*)-3-(1-Acetyl-1*H*-indol-3-yl)-*N*-(4-*tert*-butylphenyl)-2-methylacrylamide (13a):** yellow solid, yield 38%.m. p. 202–203 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 8.44 (d, $J$ = 8.4 Hz, 1H), 7.67 (d, $J$ = 7.2 Hz, 1H), 7.52–7.55(m, 5H), 7.34–7.45 (m, 4H), 2.71 (s, 3H), 2.30 (s, 3H), 1.33 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{24}H_{26}N_2O_2$ [(M – H)$^-$] 373.1916, found: 373.1922; purity >99.7% (as determined by RP-HPLC, $t_R$ = 22.9 min).

**(*E*)-*N*-(4-*tert*-Butylphenyl)-2-methyl-3-[1-(2-morpholinoethyl)-1*H*-indol-3-yl]acrylamide (14a):** yellow solid, yield 35%. m.p. 151–152 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 7.78 (d, $J$ = 8.0 Hz, 1H), 7.70 (s, 1H), 7.54–7.56 (m, 3H), 7.48 (s, 1H), 7.38 (d, $J$ = 6.8 Hz, 2H), 7.27 (t, $J$ = 7.6 Hz, 1H), 7.24 (t, $J$ = 7.4 Hz, 2H), 4.30 (t, $J$ = 6.8 Hz, 2H), 3.71 (t, $J$ = 4.0 Hz, 4H), 2.80 (t, $J$ = 7.7 Hz, 2H), 2.50 (t, $J$ = 4.4 Hz, 4H), 2.29 (s, 3H), 1.26 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{28}H_{35}N_3O_2$ [(M – H)$^-$] 444.2670, found: 444.2659; purity >99.7% (as determined by RP-HPLC, $t_R$ = 11.3 min).

**(*E*)-*N*-(4-*tert*-Butylphenyl)-3-{1-[2-(dimethylamino) ethyl]-1*H*-indol-3-yl}-2-methylacrylamide (15a):** yellow solid, yield 36%. m.p. 128–129 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 7.77 (d, $J$ = 8.0 Hz, 1H), 7.69 (s, 1H), 7.52–7.57 (m, 3H), 7.43 (s, 1H), 7.37 (d, $J$ = 18,4 Hz, 3H), 7.29 (t, $J$ = 7.4 Hz, 1H), 7.21 (t, $J$ = 7.6 Hz, 1H), 4.29 (t, $J$ = 7.0 Hz, 2H), 2.75 (t, $J$ = 7.2 Hz, 2H), 2.28 (s, 6H), 2.28 (s, 3H), 1.35 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{26}H_{33}N_3O$ [(M + H)$^+$] 404.2702, found: 404.2692; purity >99.6% (as determined by RP-HPLC, $t_R$ = 11.0 min).

**(*E*)-*N*-(4-*tert*-Butylphenyl)-3-(5-methoxy-1-methyl-1*H*-indol-3-yl)-2-methylacrylamide (8b):** yellow solid, yield 33%. m.p. 149–150 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$7.71 (s, 1H), 7.54 (t, $J$ = 8.0 Hz, 3H), 7.38 (d, $J$ = 8.8 Hz, 2H), 7.30 (s, 1H), 7.23 (s, 1H), 7.20 (d, $J$ = 2.4 Hz, 1H), 6.95 (dd, $J$ = 2.4, 9.2 Hz, 1H), 3.89 (s, 3H), 3.85 (s, 3H), 2.28 (s, 3H), 1.33 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{24}H_{28}N_2O_2$ [(M – H)$^-$] 375.2073, found: 375.2081; purity >99.8% (as determined by RP-HPLC, $t_R$ = 22.7 min).

**(*E*)-3-(1-Benzyl-5-methoxy-1*H*-indol-3-yl)-*N*-(4-*tert*-butylphenyl)-2-methylacrylamide (9b):** yellow solid, yield 31%.m.p. 119–120 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 7.27–7.35 (m, 5H), 7.24 (s, 1H), 7.09 (s, 1H), 7.03–7.07 (m, 4H), 7.01 (s, 1H), 6.93 (d, $J$ = 2.0 Hz, 1H), 6.81 (dd, $J$ = 2.4, 8.8 Hz, 1H), 5.06 (s, 2H), 3.84 (s, 3H), 1.76 (s, 3H), 1.24 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{30}H_{32}N_2O_2$ [(M + H)$^+$] 453.2515, found: 453.2551; purity >99.9% (as determined by RP-HPLC, $t_R$ = 27.7 min).

**(*E*)-Benzyl 3-[3-(4-*tert*-butylphenylamino)-2-methyl-3-oxoprop-1-enyl]-5-methoxy-1*H*-indole-1-carboxylate (10b):** yellow solid, yield 26%. m.p. 146–147 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 8.04 (s, 1H), 7.76 (s, 1H), 7.49–7.55 (m, 6H), 7.37–7.43 (m, 5H), 7.15 (d, $J$ = 44.4 Hz, 1H), 6.98 (dd, $J$ = 3.0, 9.0 Hz, 1H), 5.49 (s, 2H), 3.82 (s, 3H), 2.28 (s, 3H), 1.33 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{31}H_{32}N_2O_4$ [(M – H)$^-$] 495.2284, found: 495.2267; purity >99.9% (as determined by RP-HPLC, $t_R$ = 27.9 min).

**(*E*)-3-[1-(Benzylsulfonyl)-5-methoxy-1*H*-indol-3-yl]-*N*-(4-*tert*-butylphenyl)-2-methylacrylamide (11b):** yellow solid, yield 25%. m.p. 201–202 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 7.75 (d, $J$ = 9.2 Hz, 1H), 7.53 (d, $J$ = 8.8 Hz, 2H), 7.46 (d, $J$ = 10.4 Hz, 2H), 7.39 (d, $J$ = 8.8 Hz, 2H), 7.27–7.29 (m, 1H), 7.18 (t, $J$ = 7.6 Hz, 2H), 7.10 (d, $J$ = 2.4 Hz, 1H), 6.98–7.01 (m, 2H), 6.87 (d, $J$ = 8.0 Hz, 2H), 4.52 (s, 2H), 3.88 (s, 3H), 1.97 (s, 3H), 1.34 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{30}H_{32}N_2O_4S$ [(M – H)$^-$] 515.2005, found: 515.2003; purity >99.7% (as determined by RP-HPLC, $t_R$ = 25.5 min).

**(*E*)-3-(1-Benzoyl-5-methoxy-1*H*-indol-3-yl)-*N*-(4-*tert*-butylphenyl)-2-methylacrylamide (12b):** yellow solid, yield 32%.m.p. 180–181 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 8.25 (d, $J$ = 9.2 Hz, 1H), 7.78 (s, 1H), 7.76 (d, $J$ = 1.2 Hz, 1H), ; HRMS (ESI) *m/z* calcd. for $C_{30}H_{30}N_2O_3$ [(M – H)$^-$] 403.2022, found: 403.2018; purity >99.7% (as determined by RP-HPLC, $t_R$ = 26.3 min).

**(*E*)-3-(1-Acetyl-5-methoxy-1*H*-indol-3-yl)-*N*-(4-tert-butylphenyl)-2-methylacrylamide (13b):** yellow solid, yield 33%.m.p. 141–142 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 8.32 (d, $J$ = 10.0 Hz, 1H), 7.54 (t, $J$ = 8.4 Hz, 5H), 7.4 (dd, $J$ = 1.8, 6.6 Hz, 2H), 7.09 (d, $J$ = 2.4 Hz, 1H), 7.00–7.03 (m, 1H), 3.88 (s, 3H), 2.63 (s, 3H), 2.30 (s, 3H), 1.34 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{25}H_{28}N_2O_3$ [(M – H)$^-$] 465.2178, found: 465.2173; purity >99.8% (as determined by RP-HPLC, $t_R$ = 22.8 min).

**(*E*)-*N*-(4-*tert*-Butylphenyl)-3-[5-methoxy-1-(2-morpholinoethyl)-1*H*-indol-3-yl]-2-methylacrylamide (14b):**yellow solid, yield 28%. m.p. 185–186 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$7.70 (s, 1H), 7.53–7.58 (m, 3H), 7.43 (s, 1H), 7.37–7.39 (m, 2H), 7.25–7.27 (m, 1H), 7.19 (d, $J$ = 2.4 Hz, 1H), 6.93 (dd, $J$ = 2.4, 8.8 Hz, 1H), 4.25 (t, $J$ = 6.4 Hz, 2H), 3.88 (s, 3H), 3.70 (t, $J$ = 4.8 Hz, 4H), 2.77 (t, $J$ = 6.6 Hz, 2H), 2.49 (t, $J$ = 4.4 Hz, 4H), 2.28 (s, 3H), 1.33 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{29}H_{37}N_3O_3$ [(M – H)$^-$] 474.2757, found: 474.2753; purity >99.7% (as determined by RP-HPLC, $t_R$ = 11.1 min).

**(*E*)-*N*-(4-*tert*-Butylphenyl)-3-{1-[2-(dimethylamino) ethyl]-5-methoxy-1*H*-indol-3-yl}-2-methylacrylamide (15b):** yellow solid, yield 29%. m.p. 144–145 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 7.70 (s, 1H), 7.58 (s, 1H), 7.53–7.57 (m, 2H), 7.37–7.39 (m, 3H), 7.25–7.27 (m, 1H), 7.19 (d, $J$ = 2.4 Hz, 1H), 6.93 (dd, $J$ = 2.4, 8.8 Hz, 1H), 4.24 (t, $J$ = 7.0 Hz, 2H), 3.87 (s, 3H), 2.73 (t, $J$ = 6.8 Hz, 2H), 2.30 (s, 6H), 2.27 (s, 3H), 1.33 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{27}H_{35}N_3O_2$ [(M + H)$^+$] 434.2808, found: 434.2809; purity >99.8% (as determined by RP-HPLC, $t_R$ = 10.9 min).

**(*E*)-*N*-(4-*tert*-Butylphenyl)-3-(5-cyano-1-methyl-1*H*-indol-3-yl)-2-methylacrylamide (8c):** yellow solid, yield 38%. m.p. 188–189 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 8.08 (t, $J$ = 0.8 Hz, 1H), 7.69 (s, 1H), 7.52–7.57 (m, 3H), 7.50

(dd, $J$ = 1.4, 8.6 Hz, 1H), 7.36–7.39 (m, 4H), 3.90 (s, 3H), 2.26 (s, 3H), 1.33 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{24}H_{25}N_3O$ [(M–H)$^-$] 370.1919, found: 370.1908; purity >99.9% (as determined by RP-HPLC, $t_R$ = 22.0 min).

**(*E*)-3-(1-Benzyl-5-cyano-1*H*-indol-3-yl)-*N*-(4-*tert*-butylphenyl)-2-methylacrylamide (9c):** yellow solid, yield 39%.m.p. 175–176 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 7.51 (s, 1H), 7.37 (dd, $J$ = 1.6, 8.8 Hz, 1H), 7.31–7.33 (m, 4H), 7.29 (s, 3H), 7.18 (s, 1H), 7.00–7.06 (m, 4H), 6.79 (s, 1H), 5.06 (s, 2H), 1.90 (s, 3H), 1.25 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{30}H_{29}N_3O$ [(M – H)$^-$] 446.2232, found: 446.2214; purity >99.9% (as determined by RP-HPLC, $t_R$ = 26.7 min).

**(*E*)-Benzyl 3-[3-(4-*tert*-butylphenylamino)-2-methyl-3-oxoprop-1-enyl]-5-cyano-1*H*-indole-1-carboxylate (10c):** yellow solid, yield 36%. m.p. 180–181 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 8.30 (d, $J$ = 7.6 Hz, 1H), 8.00 (s, 1H), 7.87 (s, 1H), 7.48–7.56 (m, 4H), 7.39–7.41 (m, 3H), 7.30–7.39 (m, 1H), 7.22 (s, 1H), 7.17 (t, $J$ = 7.8 Hz, 2H), 6.87 (d, $J$ = 7.2 Hz, 2H), 4.58 (s, 2H), 2.03 (s, 3H), 1.33 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{31}H_{29}N_3O_3$ [(M – H)$^-$] 490.2131, found: 490.2130; purity >99.8% (as determined by RP-HPLC, $t_R$ = 27.1 min).

**(*E*)-3-[1-(Benzylsulfonyl)-5-cyano-1*H*-indol-3-yl]-*N*-(4-*tert*-butylphenyl)-2-methylacrylamide (11c):** yellow solid, yield 28%.m.p. 237–238 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 8.01 (s, 1H), 7.81 (d, $J$ = 8.4 Hz, 1H), 7.48–7.56 (m, 4H), 7.39–7.41 (m, 3H), 7.28–7.32 (m, 1H), 7.22 (s, 1H), 7.17 (t, $J$ = 7.8 Hz, 2H), 6.87 (d, $J$ = 7.2 Hz, 2H), 4.58 (s, 2H), 2.03 (s, 3H), 1.33 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{30}H_{29}N_3O_3S$ [(M – H)$^-$] 510.1851, found: 510.1859; purity >99.9% (as determined by RP-HPLC, $t_R$ = 25.0 min).

**(*E*)-3-(1-Benzoyl-5-cyano-1*H*-indol-3-yl)-*N*-(4-*tert*-butylphenyl)-2-methylacrylamide (12c):** yellow solid, yield 42%. m.p. 170–171 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 8.44 (dd, $J$ = 0.8, 8.4 Hz, 1H), 8.05 (d, $J$ = 1.2 Hz, 1H), 7.80 (s, 1H), 7.86 (d, $J$ = 12.0 Hz, 1H), 7.69 (dd, $J$ = 1.4, 9.0 Hz, 2H), 7.62 (s, 1H), 7.59 (d, $J$ = 5.6 Hz, 2H), 7.54 (s, 1H), 7.47–7.52 (m, 3H), 7.38–7.41 (m, 2H), 2.23 (s, 3H), 1.33 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{30}H_{27}N_3O_2$ [(M – H)$^-$] 460.2025, found: 460.2023; purity >99.7% (as determined by RP-HPLC, $t_R$ = 20.1 min).

**(*E*)-3-(1-Acetyl-5-cyano-1*H*-indol-3-yl)-*N*-(4-*tert*-butylphenyl)-2-methylacrylamide (13c):** yellow solid, yield 37%. m.p. 206–207°C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 8.58 (d, $J$ = 8.4 Hz, 1H), 8.00 (s, 1H), 7.68 (dd, $J$ = 1.2, 8.8 Hz, 1H), 7.65 (s, 1H), 7.53–7.55 (m, 1H), 7.48 (s, 1H), 7.41 (d, $J$ = 8.8 Hz, 2H), 2.69 (s, 3H), 2.29 (s, 3H), 1.34 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{25}H_{25}N_3O_2$ [(M – H)$^-$] 398.1869, found: 398.1867; purity >99.6% (as determined by RP-HPLC, $t_R$ = 20.0 min).

**(*E*)-*N*-(4-*tert*-Butylphenyl)-3-[5-cyano-1-(2-morpholinoethyl)-1*H*-indol-3-yl]-2-methylacrylamide (14c):** yellow solid, yield 31%. m.p. 180–181 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 8.10 (d, $J$ = 0.8 Hz, 1H), 7.54–7.60 (m, 4H), 7.51 (dd, $J$ = 1.6, 8.8 Hz, 1H), 7.38–7.42 (m, 3H), 4.30 (t, $J$ = 6.4 Hz, 2H), 3.69 (t, $J$ = 4.4 Hz, 4H), 2.79 (t, $J$ = 6.4 Hz, 2H), 2.50 (t, $J$ = 4.4 Hz, 4H), 2.28 (s, 3H), 1.33 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{29}H_{34}N_4O_2$ [(M – H)$^-$] 469.2604, found: 469.2586; purity >99.6% (as determined by RP-HPLC, $t_R$ = 11.1 min).

**(*E*)-*N*-(4-*tert*-Butylphenyl)-3-{5-cyano-1-[2-(dimethylamino)ethyl]-1*H*-indol-3-yl}-2-methylacrylamide (15c):** yellow solid, yield 38%. m.p. 179–180 °C; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ 8.07 (d, $J$ = 0.8 Hz, 1H), 7.54 (s, 1H), 7.54–7.57 (m, 3H), 7.53 (s, 1H), 7.47 (dd, $J$ = 1.2, 8.4 Hz, 1H), 7.36–7.42 (m, 3H), 4.27 (t, $J$ = 6.6 Hz, 2H), 2.73 (t, $J$ = 6.6 Hz, 2H), 2.33 (s, 6H), 2.23 (s, 3H), 1.32 (s, 9H); HRMS (ESI) *m/z* calcd. for $C_{27}H_{32}N_4O$ [(M – H)$^-$] 427.2498, found: 427.2490; purity >99.9% (as determined by RP-HPLC, $t_R$ = 10.8 min).

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