Discovery of Novel Arylethynyltriazole Ribonucleosides with Selective and Effective Antiviral and Antiproliferative Activity

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Novel ethynyltriazole ribonucleosides were synthesized using a simple and efficient two-step procedure involving Sonogashira coupling and subsequent ammonolysis. Compounds **2f** and **3o** inhibited hepatitis C virus (HCV) replication efficiently, whereas compound **3f** demonstrated potent apoptosis-induced antiproliferative activity against pancreatic cancer MiaPaCa-2 cells both in vitro and in vivo. Most interestingly, the notable selective antiviral and antiproliferative activities were achieved respectively for **2f** and **3f** by modulating the ribose sugar moiety into deprotected and protected forms while retaining a similar trifluoromethylphenylethynyltriazole as the nucleobase. Preliminary structure−activity relationship study revealed that not only the ribose moiety but also the CF_3 group at the *p*-position of the phenyl ring and the rigid triple bond functionality contributed critically to the observed antiviral activity of **2f** against HCV and antiproliferative activity of **3f** against pancreatic cancer. These two compounds constitute therefore promising leads in the search for new antiviral and anticancer candidates.

Introduction

Synthetic nucleoside analogues with modified nucleobase and/or sugar moieties are of considerable importance in the search for new structural leads endowed with antiviral and anticancer activity.[1] These nucleoside analogues can mimic natural nucleosides and serve as building units or inhibitors that interfere in nucleic acid synthesis or block nucleos(t)ide-dependent biological processes. Some well-known synthetic nucleoside drugs include the antiviral drugs ribavirin, acyclovir, and zidovudine, as well as the anticancer drugs gemcitabine and cladribine (Figure 1).

Introducing various functionalities to the natural nucleobase is the most frequently used method of obtaining novel nucleoside mimics with base modifications. Such nucleosides can be imparted with not only novel biologically interesting activities but also particular resistance to nucleos(t)ide metabolizing enzymes, thus leading to better in vivo stability and efficiency. A successful example is the clinical anticancer drug cladribine (Figure 1) with a chlorine atom attached to the 2-position of adenine nucleobase.[2] Recently, 6-arylpurine nucleosides (Figure 1), with aromatic systems appended on the 6-position of adenine, have been shown to elicit interesting anti-HCV[a] and anticancer activity.[3] Another example includes HEPT (Figure 1), an acyclonucleoside analogue with the pyrimidine nucleobase bearing a phenylthio group at the 6-position, which has been reported to show potent and selective anti-HIV activity.[4] The appended aryl at the 6-position is one of the key factors contributing to this anti-HIV activity because of interactions with the amino acid residues Tyr188 and Leu100 in the enzyme HIV reverse transcriptase, leading to its conformational change.[5]

Using unnatural heterocycles as nucleobases is another approach to develop novel nucleoside analogues. Ribavirin was the first synthetic nucleoside with an unnatural triazole heterocycle as the nucleobase and exhibits a broad spectrum of antiviral activity against a variety of RNA and DNA viruses.[6] Used in combination with pegylated interferon-α to treat patients infected with hepatitis C virus (HCV),[7] this therapy with ribavirin is unfortunately only effective in 40−60% of the patients (depending on the genotype) and is often associated with serious side effects.[7] An estimated 3% of the world population, about 170 millon people, are infected by HCV and at increased risk of developing liver cirrhosis and primary hepatocellular carcinoma. The development of new drugs with improved efficacity and tolerance therefore represents an urgent need to treat HCV infection.[8] Moreover, it is also of paramount importance to explore new drug candidates for the treatment of pancreatic cancer, one of the most lethal forms of human cancers, on which conventional cancer therapy has little impact because of both the aggressivity of this cancer and the rapid development of drug resistance.[9] The current first-line treatment is based on gemcitabine, which is moderately effective, and results in a mere 12% response and 3% overall survival rate.[9]

Although ribavirin was discovered over 30 years ago, the search for triazole nucleoside analogues with improved antiviral activity and selectivity has achieved little success. One reason

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[a] Abbreviations: HCV, hepatitis C virus; EC_{50}, 50% effective concentration; CC_{50}, 50% cytostatic concentration; MEM, minimum essential medium; DMEM, Dulbecco's modified Eagle media; FCS, fetal calf serum; FBS, fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; FACS, fluorescence activated cell sorting; ELISA, enzyme linked immunosorbent assay; ip, intraperitoneal; O/N, overnight; SE, standard error.

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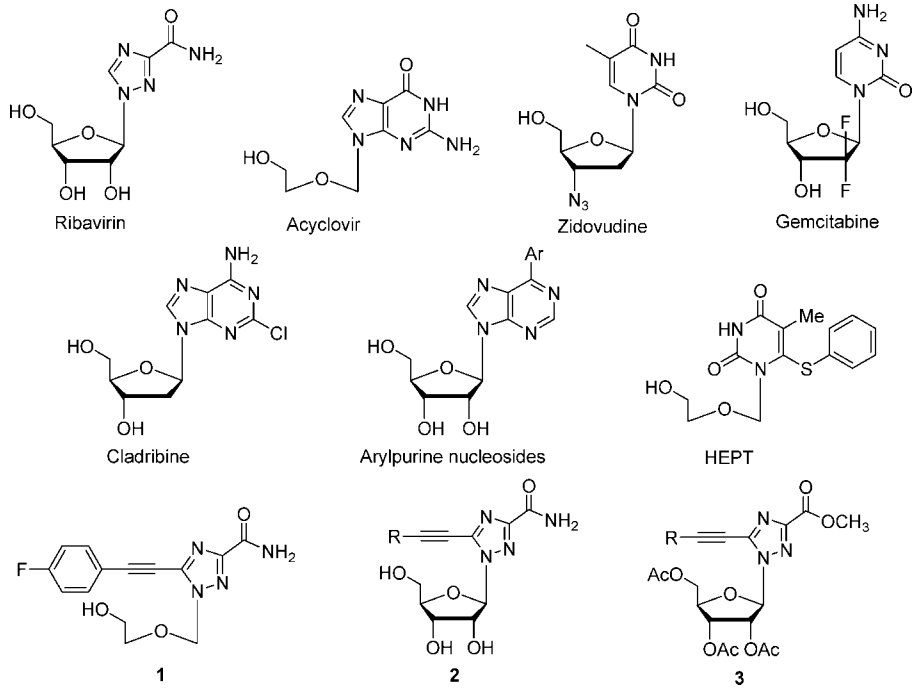


Figure 1. Ribavirin, acyclovir, zidovudine, gemcitabine, cladribine, 6-arylpurine ribonucleoside, HEPT, arylethynyltriazole acyclonucleoside **1**, and proposed ethynyltriazole ribonucleosides **2** and **3**.

for this may be the limited synthetic methods available to conveniently obtain structurally diverse triazole nucleosides. Over the past few years, our laboratories have been actively engaged in elaborating different synthetic strategies for the development of novel structural motifs of triazole nucleosides in an attempt to identify new antiviral and anticancer drug candidates.[10−16] We have mainly focused our efforts on appending π-conjugated systems onto the triazole nucleobase.[11−16] Triazole nucleosides may possess unique and intrinsic properties due to the triazole heterocycle which is a universal base[17] for base-pairing and has broad scope for H-bonding and special geometry between purine and pyrimidine, as well as a high polarity. We also expect the appended π-conjugated systems to offer advantageous binding properties with respect to the corresponding biological targets via the stronger interactions afforded by a larger aromatic binding surface and better shape-complementary conjugated system. Furthermore, we would expect nucleosides with these unnatural triazole nucleobases to be generally resistant to nucleos(t)ide metabolizing enzymes that may result in increased in vivo stability and efficiency.

Using Suzuki coupling, Huisgen cycloaddition, Sonogashira reaction, and *N*-arylation, we have previously synthesized aryltriazolyl,[11,12] bitriazolyl,[13,14] ethynyltriazolyl,[15] and *N*-aryltriazolyl nucleosides,[16] respectively. Some of these triazole nucleoside analogues displayed promising antiviral activity against TMV (tobacco mosaic virus)[13,14] and HCV.[15] Of particular interest is the arylethynyltriazolyl acyclonucleoside[15] (**1** in Figure 1) discovered in our laboratories, which showed interesting anti-HCV activity and a greatly reduced toxicity compared to the current clinical drug in use, ribavirin. In our continuing efforts to develop novel triazole nucleosides as potential drug candidates, we became interested in synthesizing ethynyltriazole ribonucleosides (**2** and **3** in Figure 1). Here, we report on the synthesis and characterization of these triazole nucleosides as well as the assessment of their biological activity. Two of the newly synthesized ethynyltriazole ribonucleosides in this work elicited effective antiviral activity against HCV, and another revealed potent antiproliferative activity on Mia-PaCa-2 cells, a drug-resistant pancreatic cancer cell line.

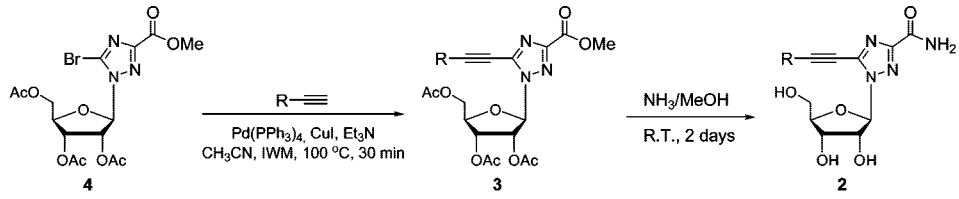
Results and Discussion

1. Chemistry. Similar to the synthesis of ethynyltriazole acyclonucleosides,[15] we employed the Sonogashira coupling reaction to prepare the corresponding ethynyltriazole ribonucleosides **2** and **3** (Scheme 1). However, contrary to our previous one-pot synthetic protocol for acyclonucleosides, here we used a two-step procedure with a view to obtaining both sugar-protected and -deprotected nucleosides **3** and **2** (Scheme 1). The synthesis was achieved via Sonogashira coupling reaction between the bromotriazole nucleoside **4**[11] and various alkynes to obtain first the protected nucleosides **3** (Scheme 1). These were then subjected to subsequent ammonolysis to yield the deprotected nucleosides **2** (Scheme 1).

On the basis of our previous experience synthesizing aryltriazole nucleosides,[11,15] we attempted to optimize the Sonogashira coupling reaction between the bromotriazole nucleoside **4** and phenylacetylene under microwave irradiation by varying the catalysts ($Pd(PPh_3)_4$, $Pd(PPh_3)_2Cl_2$, Pd_2dba_3, $Pd(OAc)_2$, $PdCl_2$, etc.), bases (K_2CO_3, Li_2CO_3, triethylamine, TBAF, etc.), solvents (CH_3CN, THF, toluene, etc.), reaction temperature, and microwave irradiation duration (data not shown). The best results for synthesizing **3** were achieved with the cocatalyst system of $Pd(PPh_3)_4$/CuI (1/2) in the presence of triethylamine in CH_3CN at 100 °C under microwave irradiation for 30 min.

It is worth noting that the presence of the electron-donating or electron-withdrawing groups on phenylacetylene did not significantly affect the Sonogashira coupling yields (Scheme 1, entries 1−6). Neither was any noteworthy steric effect observed (Scheme 1, entries 6−9). Furthermore, both heterocyclic arylacetylenes (Scheme 1, entries 10−12) and alkylacetylenes (Scheme 1, entries 14−16) gave good to excellent yields. The reaction was also tolerated for a large range of alkyne substrates having various functionalities including a halide, double bond, and hydroxyl group, etc. (Scheme 1, entries

Scheme 1. Synthesis of **3** and **2** via the Sonogashira Reaction and the Subsequent Ammonolysis



entry	R	Products	Yields (%)	Products	Yields (%)
1		**3a**	81	**2a**	84
2		**3b**	88	**2b**	78
3		**3c**	82	**2c**	82
4		**3d**	62	**2d**	76
5		**3e**	82	**2e**	77
6		**3f**	64	**2f**	73
7		**3g**	71	**2g**	78
8		**3h**	69	**2h**	75
9		**3i**	65	**2i**	82
10		**3j**	77	**2j**	85
11		**3k**	89	**2k**	76
12		**3l**	87	**2l**	91
13		**3m**	86	**2m**	86
14		**3n**	80	**2n**	71
15		**3o**	89	**2o**	85
16		**3p**	96	**2p**	88
17		**3q**	0	-	-
18		**3r**	0	-	-

13−16). However, no coupling product could be obtained when alkynes were directly linked to the electron-withdrawing functionalities (Scheme 1, entries 17−18), similar to that observed previously with ethynyltriazole acyclonucleosides.[15] This is mainly due to the significantly reduced nucleophilicity and increased electrophilicity of the corresponding alkyne induced by the strong electron-withdrawing group, making transmetalation difficult and at the same time leading to undesired Michael additions.[18,19]

Treatment of **3** in NH_3/MeOH at room temperature resulted in the deprotection of the sugar moiety and amination of the carboxylester group, yielding the corresponding deprotected nucleoside **2** in good to excellent yields (Scheme 1).

We next synthesized several structural analogues of **2f** and **3f** (Figure 2) with a view to undertaking the relevant structure−activity relationship studies. Compounds **5**, **6**, **9**, and **11** were prepared according to our previous synthetic procedure.[11,15] Compound **7** was obtained by selective acetylation of **6**[15] using acetic anhydride in the presence of triethylamine at room temperature, while **8** was synthesized by direct Sonogashira coupling between the corresponding bromotriazole acyclonucleoside[12] and the *p*-trifluoromethylphenylacetylene. Finally, **10** and **12** were achieved via Pd-catalyzed hydrogenation of **2f** and **3f**, respectively, with almost quantitative yields.

We further succeeded in obtaining crystal structures of **2a** (Figure 3). Two different conformational structures were observed in the same unit cell: one with the syn conformation and the other the anti. This finding suggests that the glycosidic bond in **2a** is relatively flexible allowing different rotational conformations. In both structures, the phenyl ring in **2a** extended

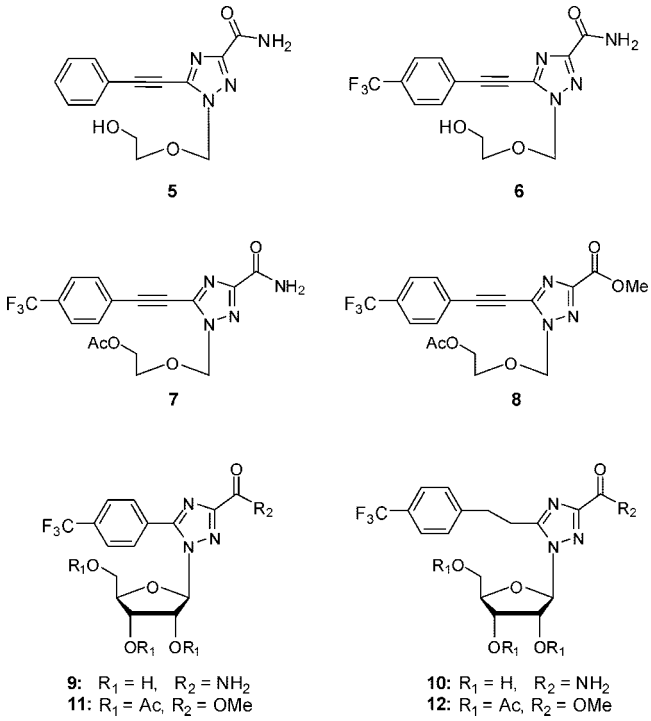


Figure 2. Structural analogues of **2f** and **3f**.

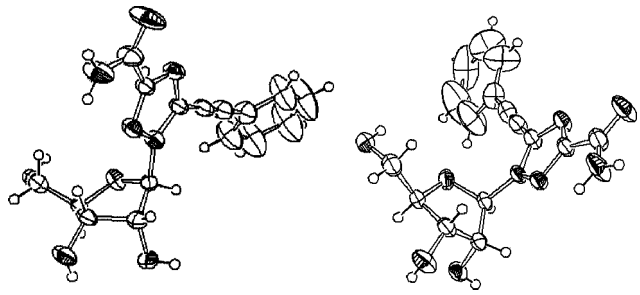


Figure 3. X-ray structure of **2a**.

out of the triazole ring plane via the triple bond linkage, similar to that observed for its acyclic nucleoside homologue **5**[15] but different from the corresponding bitriazolyl ribonucleosides.[13]

2. Biology. Antiviral Activity against Hepatitis C Virus. We first evaluated the anti-HCV activity of the above synthesized nucleosides using a HCV subgenomic RNA replicon assay in Huh-5-2 cells (Table 1).[20] Compounds with interesting antiviral activity with no or little effect on the proliferation of the host cells were further evaluated on HCV subgenomic RNA replicon assays using Huh-9-13 and Huh-6 cells.[20] Two compounds **2f** and **3o** resulted in reproducible anti-HCV activity in all three replicon assays and had limited or no adverse effects on the host cell. Neither was any noteworthy toxicity observed on various cell lines such as L1210, HepG2, Hela, MiaPaCa-2, and PC-3 cells, even with concentrations up to 200 μM (data not shown).

Some interesting observations were made during the structure−activity relationship studies with the acyclo- and ribonucleoside analogues of **2f** (Figure 4). We previously noted that the acyclonucleoside **5** (Figure 4), with a simple phenyl ring connected to the triple bond, had elusive anti-HCV activity (Table 1).[15] By introduction of one F atom at the *p*-position, the corresponding analogue **1** (Figure 4) resulted in reproducible anti-HCV activity (Table 1).[15] Replacing this F with CF_3 in **1** led to analogue **6** (Figure 4) and an improved anti-HCV activity but unfortunately also an increased toxicity (Table 1). Changing the acyclic sugar part in **6** to the cyclic ribose sugar moiety gave the ribonucleoside **2f** (Figure 4), which has an antiviral potential at a level similar to **6** though markedly reduced cytotoxicity (Table 1). These data demonstrate the importance of the cyclic ribose sugar component in compound **2f** in the view to both maintain anti-HCV activity and reduce the associated toxicity.

Furthermore, the effect of the CF_3 group at the *p*-position of the phenyl ring in **2f** was also studied using **2g** and **2h** containing this group at the *m*- and *o*-position, respectively (Figure 4). Neither **2g** nor **2h** displayed favorable anti-HCV activity. Therefore, the CF_3 group at the *p*-position of the phenyl ring seems to be critical for the observed anti-HCV activity.

We next studied the importance of the triple bond bridging the trifluoromethylphenyl ring and the triazole ring in **2f**. Removing the rigid ethynyl triple bond in **2f** resulted in an inactive analogue **9** (Figure 4),[11] whereas replacing it with a more flexible ethylene group (compound **10** in Figure 4) completely abolished the antiviral activity (data not shown). The triple bond linker is therefore an important requirement for the observed antiviral activity of **2f**, which is in line with results obtained earlier with acyclonucleoside **1**.[15]

The alkylnyltriazole ribonucleoside **3o** also inhibited HCV replication. In a previous study with triazole acyclonucleosides, only arylethynyltriazole nucleosides exhibited antiviral activity.[15] Alkylnyltriazole ribonucleoside **3o** may also constitue a new structural anti-HCV hit. It is worth noting that compound **2o**, the corresponding deprotected analogue of **3o**, was devoid of anti-HCV activity.

Antiproliferative Activity on Pancreatic Cancer Mia-PaCa-2 Cells. We next evaluated the antiproliferative activity on MiaPaCa-2 cells, a drug-resistant pancreatic cancer cell line. Identification of active compounds against cell proliferation of MiaPaCa-2 may lead to an anticancer candidate to treat pancreatic cancer. We first used a MTT assay to evaluate the antiproliferative activity of all the synthesized nucleosides. Our results reveal a significant reduction in MiaPaCa-2 cell proliferation and/or viability by 59% after 2 days post-treatment with compound **3f**. Under the same conditions, gemcitabine only moderately inhibited (29%) cell proliferation.

It is interesting to note that **2f**, the corresponding deprotected form of **3f**, which elicited effective anti-HCV activity, displayed no antiproliferative activity on MiaPaCa-2 cells. This is in agreement with the low toxicity of **2f** observed during anti-HCV assay in different Huh cells and in L1210, HepG2, Hela, and PC-3 cells. In addition, an acyclic nucleoside analogue of **2f**, **6** (Figure 5), which displayed important toxicity on Huh cells (Table 1), exhibited no antiproliferative activity on MiaPaCa-2 cells nor did the acetyl-protected acylconucleoside analogues **7** and **8** (Figure 5). Therefore, the acetyl-protected ribose component represents a crucial factor contributing to the antiproliferative activity of **3f**. Modulation of the protection/deprotection state of the ribose sugar moiety is able to generate distinct and selective antiviral/antiproliferative activity. It is not clear at this stage whether **3f** exerts its antiproliferative activity per se or through hydrolysis of its acetyl protecting groups in vivo. Also, the mechanism of action of **2f** remains the subject of further study. It can be hypothesized that **2f** may (i) akin to ribavirin be incorporated into RNA or (ii) be a non-nucleoside inhibitor of DNA/RNA polymerase.

The preliminary structure−activity relationship was further studied using various analogues of **3f** (Figure 5). Two isomers of **3f**, **3h** and **3g**, bearing the CF_3 group at the *o*- and *m*-position,

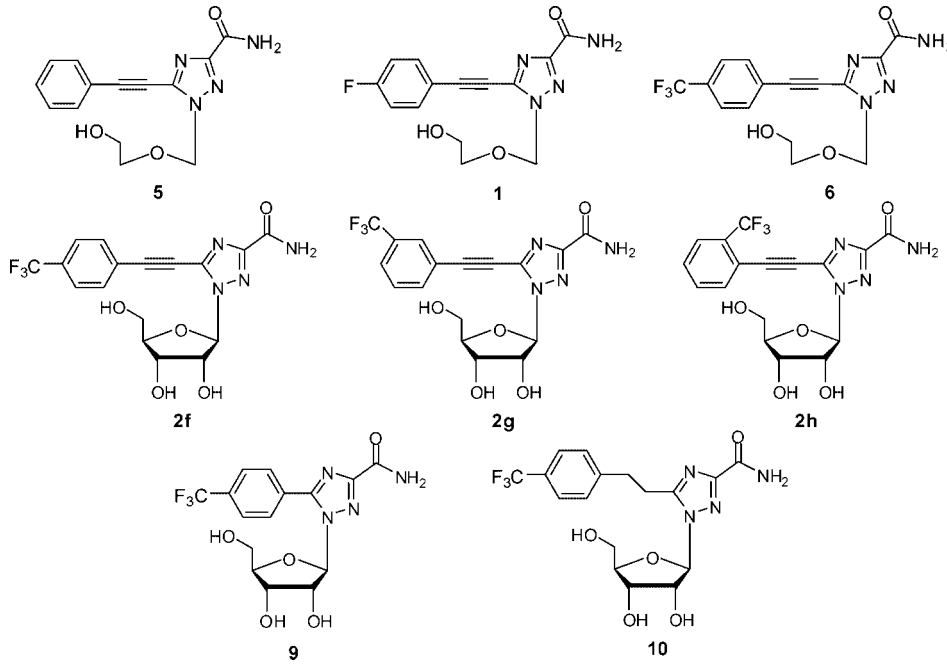


Figure 4. Structural analogues of **2f** for the structure−activity relationship analysis related to its anti-HCV activity.

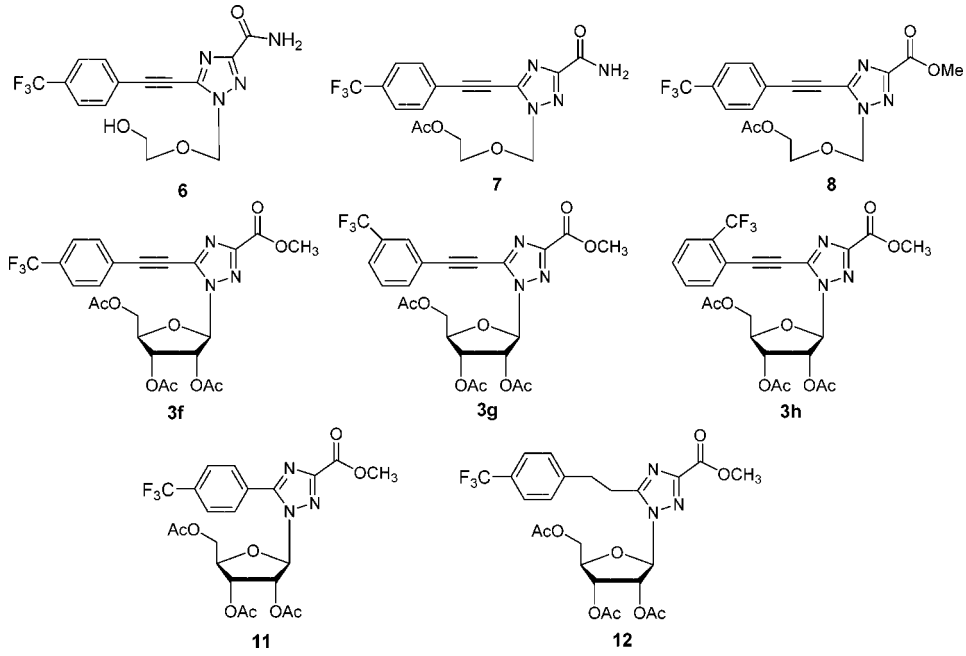


Figure 5. Structural analogues of **3f** for the structure−activity relationship analysis related to its antiproliferative activity on MiaPaCa-2 cells.

Table 1. Antiviral Activities of Ethynyltriazole Nucleoside Analogues on HCV Subgenomic Replicon Replication in Huh-5-2, Huh-9-13 and Huh-6 Cells[a]

	Huh-5-2		Huh-9-13		Huh-6	
entry	EC_{50}	CC_{50}	EC_{50}	CC_{50}	EC_{50}	CC_{50}
5	62.9 ± 14	>170	>164	>170	112 ± 1.7	>170
1	72.3 ± 9.9	>160	125 ± 6.6	>160	95.3 ± 3.3	132 ± 18
6	14.1 ± 3.7	56.5 ± 14	36.7 ± 23	79.0 ± 19	50.8 ± 21	87.5 ± 14
2f	17.7 ± 1.9	82.5 ± 9.7	19.4 ± 7.0	>120	43.7 ± 20	>120
3o	52.3 ± 3.1	>105	54.3 ± 18	>105	25.1 ± 4.8	>105
ribavirin	28.7 ± 8.2	86.0 ± 45	84.0 ± 15	229	33.0	>100

[a] EC_{50}: 50% effective concentration or the concentration (μM) required to inhibit replicon replication by 50%. CC_{50}: 50% cytostatic concentration or the concentration (μM) required to inhibit Huh cell proliferation by 50%. Values are expressed as the mean ± SE ($n \geq 3$).

respectively, showed no notable inhibition on MiaPaCa-2 cell proliferation. Moreover, removing the triple bond and replacing it with the flexible $-CH_2CH_2-$ connection in **3f** led to inactive analogues **11** and **12**, respectively (Figure 5). These data suggest

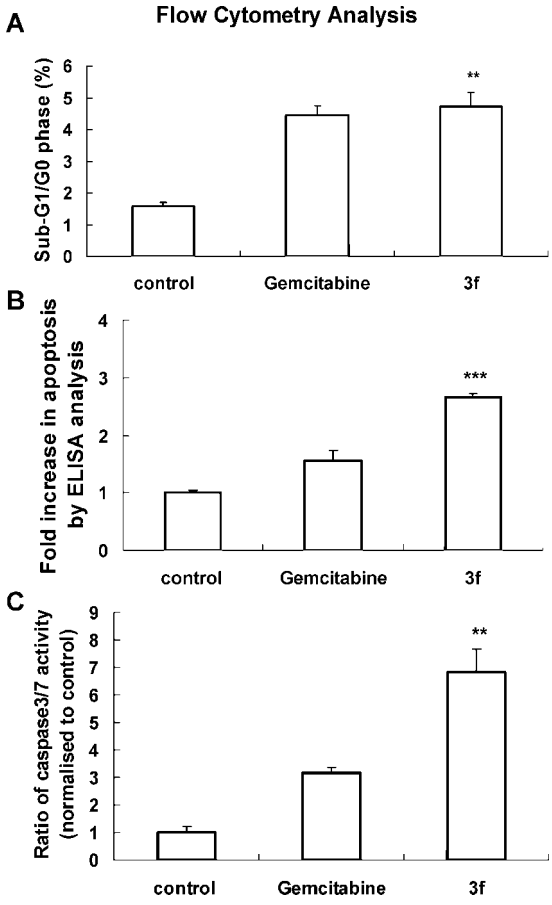


Figure 6. Apoptosis−induction in MiaPaCa-2 cells after 48 h of treatment with **3f** or gemcitabine as well as no treatment as control: (A) flow cytometry analysis to quantify the percentage of cells undergoing apoptosis (cells in sub-G1/G0); (B) ELISA test for detection of DNA/histone release; (C) caspase-3/7 activity measurements. All the experiments were done in triplicate. All of the results were expressed as the mean ± SE ($n = 3$): points, mean values of triplicate analysis; bars, standard errors; *, **, and ***, differing from control ($p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively) by Student's *t* test.

that both the CF_3- functionality at the *p*-position of the phenyl ring and the triple bond connection between the phenyl group and the triazole ring are critical for the antiproliferative activity of **3f**.

We further studied the apoptosis-inducing activity of **3f** in MiaPaCa-2 cells using fluorescence-activated cell sorting (FACS) flow cytometry,[21] ELISA test for DNA/histone release[22] and caspase-3/7 activity assay[21] (Figure 6). FACS flow analysis revealed a 3-fold increase in the fraction of cells undergoing apoptosis (sub-G1/G0 fraction) after treatment with **3f** compared to nontreatment control (Figure 6A). This suggests that for **3f**, the induction of apoptosis represents the main mode of antiproliferative activity. Importantly, DNA fragmentation and the release of DNA/histone nucleosomes into the cytoplasm represent early events in apoptosis. Results from ELISA test show a 3-fold increase in DNA/histone nucleosome release after treatment with **3f** compared to nontreatment control (Figure 6B), in good agreement with those obtained from FACS flow (Figure 6A) and further confirming the apoptosis-induced antiproliferative activity of **3f**. Caspase-3/7 activation, another hallmark of apoptosis, was also demonstrated with **3f** using caspase-3/7 colorimetric assay. Our results demonstrate a 6.8-fold increase in caspase-3/7 activation with **3f** treatment compared to nontreatment and a 2-fold increase compared to gemcitabine control

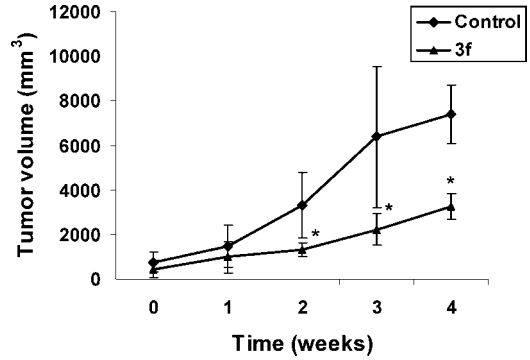


Figure 7. Effect of **3f** treatment on chemoresistant MiaPaCa-2 tumor growth in vivo. Nude mice bearing MiaPaCa-2 tumors of 100 mm^3 were randomly selected for treatment with **3f** and no treatment as control. **3f** was injected intraperitoneally (150 (mg/kg)/mice) every 3 days for 4 weeks and tumor volume measured once a week. All of the results were expressed as the mean ± SE ($n = 5$): points, mean values of analysis; bars, standard errors; *, differing from control ($p \leq 0.05$) by Student's *t* test.

(Figure 6C), suggesting a caspase-3/7-dependent mechanism of apoptosis-induction.

We next evaluated the antitumor effect of **3f** in nude mice bearing MiaPaCa-2-xenografed tumors (Figure 7).[23] Mice with mean tumor volume of 100 mm^3 were randomly selected for treatment with **3f** versus no treatment as a control. Compound **3f** was administrated intraperitoneally (ip) once every 3 days for 4 consecutive weeks. Figure 7 shows that treatment with **3f** significantly reduced MiaPaCa-2 tumor volume by 60% after just two weeks of treatment (*, $p \leq 0.05$). Under the experimental conditions used, no adverse effects were observed. Altogether, our results reveal that compound **3f** has potent antiproliferative activity and may constitute a promising novel anticancer lead.

Conclusion

We succeeded in developing novel ethynyltriazole ribonucleosides based on our earlier anti-HCV leads of triazole acyclonucleosides. Their synthesis was achieved using a simple and efficient Sonogashira coupling with or without subsequent ammonolysis. Among the new ethynyltriazole ribonucleosides synthesized here, two compounds **2f** and **3o** exhibited effective inhibition of HCV replication in vitro and another compound **3f** demonstrated potent antiproliferative activity in vitro and in vivo in chemoresistant pancreatic cancer MiaPaCa-2 cells.

Compared to the acyclic nucleoside homologue, the ribonucleoside **2f** developed in this work had considerably reduced toxicity, while retaining in vitro anti-HCV activity. Furthermore, replacing the acyclic sugar component with the ribose resulted in a new anti-HCV lead, alkylethynyltriazole ribonucleoside **3o**. This finding differs from our previous results obtained with acyclonucleosides, although **3o** may have potential carcinogenic toxicity.

Most interestingly, we have also identified the lead compound **3f**, which displayed potent and effective apoptosis-induced antitumor activity in vitro and in vivo in the chemoresistant pancreatic cancer MiaPaCa-2 model with no adverse effects. It is mentioned here that **2f** and **3f** have a similar triazole nucleobase and **3f** differs from **2f** by having the ribose sugar moiety protected. Surprisingly, simple modulation of the protection form of the ribose sugar component resulted in distinct antiviral (**2f**) and anticancer (**3f**) activities. This may provide new direction for searching of novel antiviral and anticancer nucleoside candidates.

The preliminary structure−activity relationship study of **2f** and **3f** revealed also that the CF_3 group at the *p*-position of the phenyl ring and the rigid triple bond connection between the phenyl group and the triazole ring contribute importantly to both the antiviral (**2f**) and antiproliferative (**3f**) activity against our HCV and pancreatic cancer MiaPaCa-2 cells. The mechanism of action and the pharmacological properties of these compounds remain the subject for further study.

The two arylethynyltriazole ribonucleosides **2f** and **3f** discovered in this work present simple and concise structural motifs, which can be conveniently synthesized using the Sonogashira reaction and modified for further structural optimization and structure−activity relationship study. Altogether, both compounds constitute promising structural leads in the search for novel antiviral and antiproliferative candidates against HCV and pancreatic cancer, respectively. A lead-optimization process will be initiated, and we are actively pursuing this angle in current work.

Experimental Section

General. All the terminal alkynes and catalysts were purchased from Acros or Lancaster. The microwave assisted reactions were performed on an Initiator Creator produced by Biotage. The ^{1}H NMR spectra were recorded at 300 or 600 MHz and the ^{13}C NMR spectra recorded at 75 or 150 MHz on Varian Mercury-VX300 and Varian Inova-600 spectrometers, respectively. The chemical shifts were recorded in parts per million (ppm) with TMS as the internal reference. ESI mass spectra were determined using Finnigan LCQ Advantage mass spectrometers. MALDI mass spectra and high resolution mass spectra were obtained by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) using an IonSpec 4.7 T Fourier transform mass spectrometer, with 2,5-dihydroxybenzoic acid (DHB) being the matrix. All compounds were purified by performing flash chromatography on silica gel (200−300 mesh). The purity of compound was further verified by HPLC analysis using an analytical column of Hypersil C18 (ELITE) (4.6 mm × 250 mm) with an isocratic elution of CH_3CN/H_2O 90/10 (method 1) and an analytical column of Nucleosil 120-5C8 (HICHROM) (4.6 mm × 250 mm) with an isocratic elution with CH_3CN/H_2O 90/10 (method 2).

General Procedure for Preparing 3 via a Microwave Assisted Sonogashira Reaction. 5-Bromo-1-[2,3,5-tri-*O*-acetyl-*β*-D-ribofuranosyl]-1,2,4-triazole-3-carboxylate **4** (92.8 mg, 0.2 mmol), the terminal alkynes (0.4 mmol), tetrakis(triphenylphosphine)-palladium(0) (11.6 mg, 0.01 mmol), CuI (3.8 mg, 0.02 mmol), and triethylamine (0.4 mL) were suspended in 3 mL of fresh distilled MeCN under argon. The vessel was sealed and irradiated at 100 °C for 30 min and then cooled to room temperature. The reaction mixture was concentrated under reduced pressure and the crude residue purified by flash chromatography on silica gel (petroleum ether/ethyl acetate, 2:1). The purified material was dried in vacuo to afford the corresponding product **3a**−**p**.

3a. A total of 78.6 mg (81%) of product was obtained, isolated as a colorless oil. HPLC: t = 6.58 min, purity >99% (method 1); t = 6.64 min, purity 96.2% (method 2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.61−7.63 (m, 2H, phenyl-H), 7.42−7.49 (m, 3H, phenyl-H), 6.28 (d, 1H, J = 3.6 Hz, H-1′), 5.86−5.89 (m, 1H, H-2′), 5.74−5.75 (m, 1H, H-3′), 4.47−4.52 (m, 2H, H-4′ + H-5′), 4.18−4.21 (m, 1H, H-5′), 3.99 (s, 3H, −OCH_3), 2.14 (s, 9H, −C(O)CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.9, 169.7, 169.4, 159.6, 155.1, 142.0, 132.5, 130.9, 128.9, 120.1, 99.1, 89.0, 81.5, 74.4, 74.1, 71.1, 63.1, 53.1, 20.9, 20.9, 20.8, 20.7. ESI-MS: *m*/*z* 486.0 $[M + H]^+$. HRMS: calcd for $C_{23}H_{23}N_3O_9Na^+$ 508.1327, found 508.1322. IR: 2228.8 cm^{-1} (−C≡C−).

3b. A total of 80.3 mg (78%) of product was obtained, isolated as a colorless oil. HPLC: t = 6.57 min, purity >99% (method 1); t = 6.51 min, purity 96.2% (method 2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56 (d, 2H, J = 8.1 Hz, phenyl-H), 6.93 (d, 2H, J = 8.1 Hz, phenyl-H), 6.28 (d, 1H, J = 3.9 Hz, H-1′), 5.85−5.88 (m, 1H, H-2′), 5.72−5.76 (m, 1H, H-3′), 4.44−4.54 (m, 2H, H-4′ + H-5′), 4.16−4.21 (m, 1H, H-5′), 3.99 (s, 3H, −OCH_3), 3.86 (s, 3H, −OCH_3), 2.15 (s, 9H, −C(O)CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.9, 169.6, 169.4, 161.7, 159.7, 155.1, 142.3, 134.3, 114.6, 112.0, 99.6, 89.0, 81.4, 74.4, 73.3, 71.2, 63.1, 55.7, 53.0, 20.9, 20.7. Maldi-MS: *m*/*z* 538.1 $[M + Na]^+$. HRMS: calcd for $C_{24}H_{25}N_3O_{10}Na^+$ 538.1432, found 538.1430. IR: 2221.6 cm^{-1} (−C≡C−).

3c. A total of 81.8 mg (82%) of product was obtained, isolated as a colorless oil. HPLC: t = 6.69 min, purity >99% (method 1); t = 6.39 min, purity >99% (method 2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51 (d, 2H, J = 5.1 Hz, phenyl-H), 7.24 (d, 2H, J = 5.1 Hz, phenyl-H), 6.28 (d, 1H, J = 3.6 Hz, H-1′), 5.85−5.88 (m, 1H, H-2′), 5.72−5.76 (m, 1H, H-3′), 4.46−4.53 (m, 2H, H-4′ + H-5′), 4.16−4.22 (m, 1H, H-5′), 3.99 (s, 3H, −OCH_3), 2.41 (s, 3H, −CH_3), 2.14 (s, 9H, −C(O)CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.8, 169.6, 169.4, 159.6, 155.0, 142.1, 141.5, 132.4, 129.7, 116.9, 99.5, 88.9, 81.4, 74.4, 73.6, 71.1, 63.0, 52.9, 21.9, 20.6. Maldi-MS: *m*/*z* 522.1 $[M + Na]^+$. HRMS: calcd for $C_{24}H_{25}N_3O_9Na^+$ 522.1483, found 522.1480. IR: 2224.0 cm^{-1} (−C≡C−).

3d. A total of 64.4 mg (62%) of product was obtained, isolated as a white solid. HPLC: t = 6.70 min, purity 97.6% (method 1); t = 6.40 min, purity 97.3% (method 2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.55 (d, 2H, J = 8.1 Hz, phenyl-H), 7.41 (d, 2H, J = 8.1 Hz, phenyl-H), 6.26 (d, 1H, J = 3.0 Hz, H-1′), 5.84−5.87 (m, 1H, H-2′), 5.71−5.75 (m, 1H, H-3′), 4.49−4.54 (m, 2H, H-4′ + H-5′), 4.16−4.21 (m, 1H, H-5′), 4.00 (s, 3H, OCH_3), 2.14 (s, 9H, C(O)CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 169.7, 168.4, 168.2, 158.3, 153.9, 140.4, 136.0, 132.5, 128.2, 117.3, 96.6, 87.8, 80.2, 73.7, 73.2, 69.8, 61.8, 51.9, 19.6, 19.5. Maldi-MS: *m*/*z* 542.1 $[M + Na]^+$. HRMS: calcd for $C_{23}H_{23}N_3O_9Cl^+$ 520.1117, found 520.1106. IR: 2228.6 cm^{-1} (−C≡C−).

3e. A total of 77.5 mg (82%) of product was obtained, isolated as a colorless oil. HPLC: t = 6.56 min, purity >99% (method 1); t = 6.55 min, purity 96.7% (method 2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60−7.64 (m, 2H, phenyl-H), 7.13 (dd, 2H, $^3J_{HF}$ = 9.0 Hz, $^3J_{HH}$ = 8.7 Hz, phenyl-H), 6.26 (d, 1H, J = 3.3 Hz, H-1′), 5.85−5.88 (m, 1H, H-2′), 5.73−5.76 (m, 1H, H-3′), 4.46−4.52 (m, 2H, H-4′ + H-5′), 4.18−4.21 (m, 1H, H-5′), 3.99 (s, 3H, −OCH_3), 2.15 (s, 3H, −C(O)CH_3), 2.14 (s, 3H, −C(O)CH_3), 2.13 (s, 3H, −C(O)CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8, 169.6, 169.4, 164.0 (d, $^1J_{CF}$ = 252.4 Hz), 159.5, 155.1, 141.7, 134.7 (d, $^3J_{CF}$ = 8.6 Hz), 116.4 (d, $^2J_{CF}$ = 22.4 Hz), 97.9, 89.0, 81.4, 74.4, 74.0, 71.1, 62.9, 53.0, 20.8, 20.7, 20.6. Maldi-MS: *m*/*z* 526.1 $[M + Na]^+$. HRMS: calcd for $C_{23}H_{22}N_3O_9FNa^+$ 526.1232, found 526.1242. IR: 2228.5 cm^{-1} (−C≡C−).

3f. A total of 70.9 mg (64%) of product was obtained, isolated as a colorless oil. HPLC: t = 7.26 min, purity >99% (method 1); t = 6.45 min, purity 98.9% (method 2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68−7.76 (m, 4H, phenyl-H), 6.27 (d, 1H, J = 3.0 Hz, H-1′) 5.85−5.88 (m, 1H, H-2′), 5.72−5.75 (m, 1H, H-3′), 4.46−4.54 (m, 2H, H-4′ + H-5′), 4.17−4.23 (m, 1H, H-5′), 4.00 (s, 3H, −OCH_3), 2.15 (s, 6H, −C(O)CH_3), 2.13 (s, 3H, −C(O)CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.8, 169.9, 169.5, 159.4, 155.3, 141.3, 132.8, 132.6 (q, $^2J_{CF}$ = 33.5 Hz), 123.8, 123.7 (q, $^1J_{CF}$ = 271.4 Hz), 97.0, 89.2, 81.5, 76.0, 74.5, 71.1, 63.0, 53.1, 20.8, 20.71, 20.66. Maldi-MS: *m*/*z* 576.1 $[M + Na]^+$. HRMS: calcd for $C_{24}H_{22}N_3O_9F_3Na^+$ 576.1200, found 576.1204. IR: 2233.6 cm^{-1} (−C≡C−).

3g. A total of 78.3 mg (71%) of product was obtained, isolated as a colorless oil. HPLC: t = 6.86 min, purity >99% (method 1); t = 6.83 min, purity 96.9% (method 2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 (s, 1H, phenyl-H), 7.73−7.81 (m, 2H, phenyl-H), 7.55−7.61 (m, 1H, phenyl-H), 6.28 (d, 1H, J = 3.9 Hz, H-1′), 5.84−5.87 (m, 1H, H-2′), 5.73 (t, 1H, J = 5.4 Hz, H-3′), 4.48−4.54 (m, 2H, H-4′ + H-5′), 4.19−4.23 (m, 1H, H-5′), 4.00 (s, 3H, −OCH_3), 2.15 (s, 9H, −C(O)CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.9, 169.7, 169.5, 159.4, 155.3, 141.4, 135.6, 131.6 (q, $^2J_{CF}$ = 33.3 Hz), 129.7, 129.2, 127.4, 123.6 (q, $^1J_{CF}$ = 271.7 Hz), 121.1, 97.0, 89.1, 81.5, 75.4, 74.5, 71.1, 63.0, 53.1, 20.8, 20.71, 20.66.

Maldi-MS: *m/z* 576.1 $[M + Na]^+$. HRMS: calcd for $C_{24}H_{22}N_3O_9F_3Na^+$ 576.1200, found 576.1195. IR: 2235.3 cm^{-1} (−C≡C−).

3h. A total of 76.3 mg (69%) of product was obtained, isolated as a colorless oil. HPLC: $t = 6.64$ min, purity >99% (method 1); $t = 6.87$ min, purity 97.8% (method 2). ^1H NMR (300 MHz, $CDCl_3$): δ 7.75−7.79 (m, 2H, phenyl-H), 7.59−7.66 (m, 2H, phenyl-H), 6.35 (d, 1H, $J = 5.1$ Hz, H-1′), 5.81−5.84 (m, 1H, H-2′), 5.67−5.71 (m, 1H, H-3′), 4.46−4.58 (m, 2H, H-4′ + H-5′), 4.17−4.23 (m, 1H, H-5′), 4.00 (s, 3H, $-OCH_3$), 2.18 (s, 3H, $-C(O)CH_3$), 2.15 (s, 3H, $-C(O)CH_3$), 2.10 (s, 3H, $-C(O)CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.9, 169.8, 169.4, 159.5, 155.4, 141.5, 135.0, 132.3, 131.8, 130.7, 126.5, 123.6 (q, $^1J_{CF} = 271.7$ Hz), 118.3, 94.4, 88.7, 81.5, 79.0, 74.2, 71.3, 63.0, 53.1, 20.9, 20.6, 20.4. Maldi-MS: *m/z* 576.1 $[M + Na]^+$. HRMS: calcd for $C_{24}H_{22}N_3O_9F_3Na^+$ 576.1200, found 576.1196. IR: 2233.8 cm^{-1} (−C≡C−).

3i. A total of 79.1 mg (65%) of product was obtained, isolated as a yellow solid. HPLC: $t = 8.14$ min, purity 96.5% (method 1); $t = 8.73$ min, purity 96.2% (method 2). ^1H NMR (300 MHz, $CDCl_3$): δ 8.60 (d, 1H, $J = 8.7$ Hz, pyrene-H), 8.07−8.31 (m, 8H, pyrene-H), 6.51 (d, 1H, $J = 3.6$ Hz, H-1′), 5.94−5.97 (m, 1H, H-2′), 5.77−5.81 (m, 1H, H-3′), 4.54−4.59 (m, 2H, H-4′ + H-5′), 4.23−4.27 (m, 1H, H-5′), 4.04 (s, 3H, $-OCH_3$), 2.16 (s, 9H, $-C(O)CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.9, 169.7, 169.5, 159.7, 155.3, 142.3, 133.1, 131.2, 131.0, 130.3, 129.7, 129.6, 127.3, 126.9, 126.6, 125.0, 124.7, 124.4, 124.1, 113.8, 98.9, 89.1, 81.6, 79.3, 74.6, 71.3, 63.1, 53.1, 20.9, 20.8, 20.7. Maldi-MS: *m/z* 632.2 $[M + Na]^+$. HRMS: calcd for $C_{33}H_{28}N_3O_9^+$ 610.1820, found 610.1824. IR: 2217.9 cm^{-1} (−C≡C−).

3j. A total of 75.6 mg (77%) of product was obtained, isolated as a colorless oil. HPLC: $t = 6.62$ min, purity >99% (method 1); $t = 6.49$ min, purity 96.6% (method 2). ^1H NMR (300 MHz, $CDCl_3$): δ 7.49−7.50 (m, 2H, thiphenyl-H), 7.08−7.11 (m, 1H, thiophenyl-H), 6.25 (d, 1H, $J = 3.6$ Hz, H-1′), 5.85−5.88 (m, 1H, H-2′), 5.71−5.74 (m, 1H, H-3′), 4.48−4.53 (m, 2H, H-4′ + H-5′), 4.16−4.21 (m, 1H, H-5′), 3.99 (s, 3H, $-OCH_3$), 2.15 (s, 9H, $-C(O)CH_3$). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.9, 169.7, 169.4, 159.5, 155.2, 141.8, 135.6, 130.9, 127.9, 119.7, 92.7, 89.0, 81.5, 77.8, 74.4, 71.1, 63.0, 53.1, 20.7. Maldi-MS: *m/z* 514.1 $[M + Na]^+$. HRMS: calcd for $C_{21}H_{22}N_3O_9S^+$ 492.1071, found 492.1062. IR: 2223.7 cm^{-1} (−C≡C−).

3k. A total of 77.6 mg (89%) of product was obtained, isolated as a colorless oil. HPLC: $t = 6.84$ min, purity 99.0% (method 1); $t = 6.29$ min, purity 98.5% (method 2). ^1H NMR (300 MHz, $CDCl_3$): δ 7.78 (d, 1H, $J = 1.5$ Hz, thiphenyl-H), 7.37−7.40 (m, 1H, thiophenyl-H), 7.28 (s, 1H, thiophenyl-H), 6.27 (d, 1H, $J = 3.6$ Hz, H-1′), 5.85−5.88 (m, 1H, H-2′), 5.72−5.75 (m, 1H, H-3′), 4.48−4.54 (m, 2H, H-4′ + H-5′), 4.11−4.22 (m, 1H, H-5′), 3.99 (s, 3H, $-OCH_3$), 2.14 (s, 9H, $-C(O)CH_3$). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.9, 169.7, 169.4, 159.5, 155.1, 141.9, 132.9, 129.9, 126.6, 119.2, 94.5, 89.0, 81.4, 74.4, 73.9, 71.1, 63.0, 53.0, 20.8, 20.7. Maldi-MS: *m/z* 514.1 $[M + Na]^+$. HRMS: calcd for $C_{21}H_{22}N_3O_9S^+$ 492.1071, found 492.1062. IR: 2225.1 cm^{-1} (−C≡C−).

3l. A total of 85.1 mg (87%) of product was obtained, isolated as a pink solid. HPLC: $t = 7.19$ min, purity >99% (method 1); $t = 6.35$ min, purity 97.2% (method 2). ^1H NMR (300 MHz, $CDCl_3$): δ 7.58 (s, 1H, imidazole-H), 7.56 (s, 1H, imidazole-H), 6.23 (d, 1H, $J = 3.0$ Hz, H-1′), 5.84−5.86 (m, 1H, H-2′), 5.71−5.74 (m, 1H, H-3′), 4.49−4.54 (m, 2H, H-4′ + H-5′), 4.17−4.22 (m, 1H, H-5′), 4.00 (s, 3H, $-OCH_3$), 3.78 (s, 3H, -NCH_3), 2.15 (s, 9H, $-C(O)CH_3$). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.9, 169.7, 169.5, 159.5, 155.2, 141.5, 140.3, 138.2, 113.8, 89.1, 87.4, 81.7, 81.4, 74.5, 71.0, 62.9, 53.2, 32.7, 20.9, 20.7. Maldi-MS: *m/z* 490.2 $[M + H]^+$. HRMS: calcd for $C_{21}H_{24}N_5O_9^+$ 490.1569, found 490.1548. IR: 2226.1 cm^{-1} (−C≡C−).

3m. A total of 86.5 mg (86%) of product was obtained, isolated as a colorless oil. HPLC: $t = 7.10$ min, purity 98.9% (method 1); $t = 6.44$ min, purity 96.7% (method 2). ^1H NMR (300 MHz, $CDCl_3$): δ 6.45−6.47 (m, 1H, alkene-H), 6.18 (d, 1H, $J = 3.0$ Hz, H-1′), 5.80−5.83 (m, 1H, H-2′), 5.70−5.74 (m, 1H, H-3′), 4.41−4.52 (m, 2H, H-4′ + H-5′), 4.15−4.20 (m, 1H, H-5′), 3.97 (s, 3H, $-OCH_3$), 2.18−2.22 (m, 4H, $-CH_2-$), 2.13 (s, 9H, $-C(O)CH_3$), 1.63−1.70 (m, 4H, $-CH_2-$). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.9, 169.6, 169.4, 160.4, 159.7, 154.9, 142.3, 119.0, 101.2, 88.8, 81.3, 74.3, 71.8, 71.1, 63.0, 52.9, 28.3, 26.2, 22.1, 21.3, 20.7. Maldi-MS: *m/z* 512.2 $[M + Na]^+$. HRMS: calcd for $C_{23}H_{27}N_3O_9Na^+$ 512.1639, Found 512.1637. IR: 2214.5 cm^{-1} (−C≡C−).

3n. A total of 77.6 mg (80%) of product was obtained, isolated as a colorless oil. HPLC: $t = 6.52$ min, purity 98.6% (method 1); $t = 6.45$ min, purity 98.2% (method 2). ^1H NMR (300 MHz, $CDCl_3$): δ 6.19 (d, 1H, $J = 3.6$ Hz, H-1′), 5.79−5.82 (m, 1H, H-2′), 5.68−5.72 (m, 1H, H-3′), 4.46−4.53 (m, 2H, H-4′ + H-5′), 4.14−4.20 (m, 1H, H-5′), 3.97 (s, 3H, $-OCH_3$), 3.71 (t, 2H, $J = 6.2$ Hz, $-CH_2-$), 2.74 (t, 2H, $J = 6.9$ Hz, $-CH_2-$), 2.09−2.14 (m, 11H, $-C(O)CH_3$ and $-CH_2-$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8, 169.7, 169.5, 159.6, 154.8, 141.7, 100.0, 88.8, 81.3, 74.3, 71.1, 67.3, 62.9, 53.0, 43.5, 30.4, 20.8, 20.7, 20.6, 17.0. Maldi-MS: *m/z* 508.1 $[M + Na]^+$. HRMS: calcd for $C_{20}H_{24}N_3O_9ClNa^+$ 508.1093, Found 508.1087. IR: 2217.8 cm^{-1} (−C≡C−).

3o. A total of 87.7 mg (89%) of product was obtained, isolated as a colorless oil. HPLC: $t = 6.51$ min, purity >99% (method 1); $t = 6.38$ min, purity 98.6% (method 2). ^1H NMR (300 MHz, $CDCl_3$): δ 6.20 (d, 1H, $J = 2.4$ Hz, H-1′), 5.74−5.77 (m, 1H, H-2′), 5.66−5.70 (m, 1H, H-3′), 4.43−4.54 (m, 2H, H-4′ + H-5′), 4.19−4.24 (m, 1H, H-5′), 3.97 (s, 3H, $-OCH_3$), 3.00 (br s, 1H, −OH), 2.14 (s, 9H, $-C(O)CH_3$), 2.06−2.08 (m, 4H, cyclopentanyl-H), 1.77−1.93 (m, 4H, cyclopentanyl-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.9, 169.8, 159.4, 154.6, 141.4, 104.6, 89.1, 81.0, 74.4, 70.8, 68.1, 62.9, 52.9, 42.2, 23.7, 20.7, 20.6. Maldi-MS: *m/z* 516.2 $[M + Na]^+$. HRMS: calcd for $C_{22}H_{27}N_3O_{10}Na^+$ 516.1589, found 516.1586. IR: 2240.8 cm^{-1} (−C≡C−).

3p. A total of 97.3 mg (96%) of product was obtained, isolated as a colorless oil. HPLC: $t = 6.56$ min, purity 98.9% (method 1); $t = 6.47$ min, purity 97.8% (method 2). ^1H NMR (300 MHz, $CDCl_3$): δ 6.21 (d, 1H, $J = 2.7$ Hz, H-1′), 5.72−5.75 (m, 1H, H-2′), 5.65−5.69 (m, 1H, H-3′), 4.45−4.53 (m, 2H, H-4′ + H-5′), 4.18−4.24 (m, 1H, H-5′), 3.98 (s, 3H, $-OCH_3$), 3.42 (br s, 1H, −OH), 2.13 (s, 9H, $-C(O)CH_3$), 2.05−2.06 (m, 2H, cyclohexanyl-H), 1.61−1.74 (m, 4H, cyclohexanyl-H), 1.56−1.59 (m, 4H, cyclohexanyl-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.9, 169.7, 169.6, 159.4, 154.6, 141.3, 104.5, 89.0, 81.0, 74.4, 70.8, 69.1, 68.8, 62.9, 52.9, 39.2, 25.1, 23.1, 20.7, 20.6. Maldi-MS: *m/z* 530.2 $[M + Na]^+$. HRMS: calcd for $C_{23}H_{29}N_3O_{10}Na^+$ 530.1745, found 530.1740. IR: 2234.7 cm^{-1} (−C≡C−).

General Procedure for Preparing 2. 3 was dissolved in 10 mL of saturated NH_3/MeOH and stirred at room temperature for 2 days. Then the solvent was removed, the residue was dissolved in MeOH and crystallized by precipitation in CH_2Cl_2. Then the crystalline state precipitation was washed with CH_2Cl_2 and dried in vacuo to afford the corresponding product **2a**−**p**.

2a. An amount of 64.4 mg (0.133 mmol) of **3a** was used for the reaction. A total of 38.4 mg (84%) of product was obtained, isolated as a white solid. HPLC: $t = 8.04$ min, purity 96.0% (method 1); $t = 6.12$ min, purity 98.7% (method 2). ^1H NMR (300 MHz, DMSO-d_6): δ 8.05 (br, 1H, $-C(O)NH_2$), 7.77 (br, 1H, $-C(O)NH_2$), 7.71−7.73 (m, 2H, phenyl-H), 7.51−7.62 (m, 3H, phenyl-H), 6.01 (d, 1H, $J = 5.2$ Hz, H-1′), 5.65 (d, 1H, $J = 6.0$ Hz, −OH), 5.29 (d, 1H, $J = 5.1$ Hz, −OH), 4.82 (t, 1H, $J = 5.6$ Hz, −OH), 4.51−4.54 (m, 1H, H-2′), 4.24−4.27 (m, 1H, H-3′), 3.98−4.00 (m, 1H, H-4′), 3.42−3.63 (m, 2H, H-5′). ^{13}C NMR (75 MHz, DMSO-d_6): δ 157.7, 140.7, 132.7, 131.6, 129.8, 119.9, 97.9, 91.3, 87.0, 75.4, 74.8, 71.3, 62.7. Maldi-MS: *m/z* 367.1 $[M + Na]^+$. HRMS: calcd for $C_{16}H_{16}N_4O_5Na^+$ 367.1013, found 367.1012. IR: 2226.7 cm^{-1} (−C≡C−).

A single crystal of product **2a** suitable for X-ray crystallographic analysis was obtained via slow evaporation of H_2O solution. Crystallographic data of **2a**: colorless, triclinic space group *P*1, $Z = 2$, $a = 7.5538$ (11) Å, $b = 11.114$ (17) Å, $c = 11.5618$ (17) Å, $\alpha = 66.563°$ (2), $\beta = 89.504°$ (3), $\gamma = 78.045°$ (3), $V = 868.3$ (2)

Å^3, R ($F^2 > 2\sigma F^2$) = 0.0505 and wR = 0.0894 ($w = 1/[\sigma^2(F_o^2) + (0.0426P)^2 + 0.0000P]$, $P = (F_o^2 + 2F_c^2)/3$.

2b. An amount of 54.1 mg (0.105 mmol) of **3b** was used for the reaction. A total of 30.6 mg (78%) of product was obtained, isolated as a white solid. HPLC: t = 7.95 min, purity >99% (method 1); t = 6.17 min, purity 97.3% (method 2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.03 (br, 1H, −C(O)NH$_2$), 7.76 (br, 1H, −C(O)NH$_2$), 7.66 (d, 2H, J = 8.7 Hz, phenyl-H), 7.08 (d, 2H, J = 8.1 Hz, phenyl-H), 5.99 (d, 1H, J = 3.6 Hz, H-1′), 5.64 (d, 1H, J = 5.7 Hz, −OH), 5.29 (d, 1H, J = 6.0 Hz, −OH), 4.78−4.85 (m, 1H, −OH), 4.51−4.52 (m, 1H, H-2′), 4.23−4.24 (m, 1H, H-3′), 3.97−3.99 (m, 1H, H-4′), 3.84 (s, 3H, −OCH$_3$), 3.42−3.58 (m, 2H, H-5′). ^{13}C NMR (75 MHz, DMSO-d_6): δ 161.8, 160.4, 157.6, 141.0, 134.6, 115.5, 111.6, 98.4, 91.1, 86.9, 74.8, 74.4, 71.2, 62.7, 56.2. Maldi-MS: m/z 397.1 [M + Na]$^+$. HRMS: calcd for $C_{17}H_{18}N_4O_6Na^+$ 397.1119, found 397.1119. IR: 2222.1 cm^{-1} (−C≡C−).

2c. An amount of 65.9 mg (0.105 mmol) of **3c** was used for the reaction. A total of 38.8 mg (82%) of product was obtained, isolated as a white solid. HPLC: t = 7.92 min, purity >99% (method 1); t = 6.17 min, purity >99% (method 2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.04 (br, 1H, −C(O)NH$_2$), 7.76 (br, 1H, −C(O)NH$_2$), 7.60 (d, 2H, J = 7.5 Hz, phenyl-H), 7.34 (d, 2H, J = 8.1 Hz, phenyl-H), 5.99 (d, 1H, J = 3.6 Hz, H-1′), 5.64 (d, 1H, J = 5.7 Hz, −OH), 5.29 (d, 1H, J = 5.4 Hz, −OH), 4.81 (t, 1H, J = 5.1 Hz, −OH), 4.50−4.52 (m, 1H, H-2′), 4.22−4.24 (m, 1H, H-3′), 3.97−3.98 (m, 1H, H-4′), 3.43−3.58 (m, 2H, H-5′), 2.39 (s, 3H, −CH$_3$). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.4, 157.7, 141.8, 140.8, 132.7, 130.4, 116.9, 98.2, 91.2, 86.9, 74.9, 74.8, 71.3, 62.7, 21.9. Maldi-MS: m/z 381.1 [M + Na]$^+$. HRMS: calcd for $C_{17}H_{18}N_4O_5Na^+$ 381.1169, found 381.1169. IR: 2223.9 cm^{-1} (−C≡C−).

2d. An amount of 48.9 mg (0.094 mmol) of **3d** was used for the reaction. A total of 27.1 mg (76%) of product was obtained, isolated as a white solid. HPLC: t = 7.94 min, purity 97.1% (method 1); t = 6.18 min, purity 98.1% (method 2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.03 (br s, 1H, −C(O)NH$_2$), 7.74−7.76 (m, 3H, C(O)NH$_2$ + phenyl-H), 7.61 (d, 2H, J = 8.7 Hz, phenyl-H), 6.00 (d, 1H, J = 4.5 Hz, H-1′), 5.63 (d, 1H, J = 5.7 Hz, −OH), 5.28 (d, 1H, J = 5.7 Hz, −OH), 4.80 (t, 1H, J = 6.0 Hz, −OH), 4.50−4.52 (m, 1H, H-2′), 4.22−4.24 (m, 1H, H-3′), 3.97−3.99 (m, 1H, H-4′), 3.43−3.58 (m, 2H, H-5′). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.3, 157.8, 140.5, 136.5, 134.5, 130.0, 118.8, 96.6, 91.3, 87.0, 76.2, 74.8, 71.2, 62.7. Maldi-MS: m/z 401.1 [M + Na]$^+$. HRMS: calcd for $C_{16}H_{16}N_4O_5Cl^+$ 379.0804, found 379.0804. IR: 2230.7 cm^{-1} (−C≡C−).

2e. An amount of 75.5 mg (0.15 mmol) of **3e** was used for the reaction. A total of 41.8 mg (77%) of product was obtained, isolated as a white solid. HPLC: t = 7.77 min, purity >99% (method 1); t = 6.07 min, purity 98.3% (method 2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.03 (br s, 1H, −C(O)NH$_2$), 7.77−7.82 (m, 3H, −C(O)NH$_2$ + phenyl-H), 7.39 (dd, 2H, $^3J_{HF}$ = 8.7 Hz, $^3J_{HH}$ = 9.0 Hz, phenyl-H), 6.00 (d, 1H, J = 3.9 Hz, H-1′), 5.63 (d, 1H, J = 5.7 Hz, −OH), 5.28 (d, 1H, J = 5.7 Hz, −OH), 4.80 (t, 1H, J = 5.0 Hz, −OH), 4.50−4.52 (m, 1H, H-2′), 4.23−4.24 (m, 1H, H-3′), 3.97−3.99 (m, 1H, H-4′), 3.44−3.60 (m, 2H, H-5′). ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.2, 160.3, 157.7, 140.6, 135.5 (d, $^3J_{CF}$ = 9.6 Hz), 117.2 (d, $^2J_{CF}$ = 22.4 Hz), 116.4, 96.9, 91.3, 87.0, 75.2, 74.8, 71.2, 62.7. Maldi-MS: m/z 385.1 [M + Na]$^+$. HRMS: calcd for $C_{16}H_{15}N_4O_5FNa^+$ 385.0919, found 385.0917. IR: 2229.4 cm^{-1} (−C≡C−).

2f. An amount of 87.7 mg (0.159 mmol) of **3f** was used for the reaction. A total of 47.7 mg (73%) of product was obtained, isolated as a yellow solid. HPLC: t = 8.26 min, purity >99% (method 1); t = 6.11 min, purity >99% (method 2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.07 (br s, 1H, −C(O)NH$_2$), 7.89−7.98 (m, 4H, phenyl-H), 7.80 (br s, 1H, −C(O)NH$_2$), 6.05 (d, 1H, J = 3.6 Hz, H-1′), 5.66 (d, 1H, J = 5.1 Hz, −OH), 5.31 (d, 1H, J = 5.7 Hz, −OH), 4.83 (t, 1H, J = 5.6 Hz, −OH), 4.51−4.56 (m, 1H, H-2′), 4.24−4.29 (m, 1H, H-3′), 3.99−4.01 (m, 1H, H-4′), 3.45−3.62 (m, 2H, H-5′). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.3, 157.8, 140.2, 133.6, 131.2 (q, $^2J_{CF}$ = 31.5 Hz), 126.7, 124.4 (q, $^1J_{CF}$ = 271.2 Hz), 124.2, 96.0, 91.4, 87.0, 77.3, 74.9, 71.2, 62.6. Maldi-MS: m/z 435.1 [M + Na]$^+$. HRMS: calcd for $C_{17}H_{15}N_4O_5F_3Na^+$ 435.0887, found 435.0885. IR: 2232.8 cm^{-1} (−C≡C−).

2g. An amount of 55.7 mg (0.101 mmol) of **3g** was used for the reaction. A total of 31.1 mg (78%) of product was obtained, isolated as a white solid. HPLC: t = 7.08 min, purity >99% (method 1); t = 6.12 min, purity >99% (method 2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.13 (s, 1H, phenyl-H), 8.03−8.05 (m, 2H, −C(O)NH$_2$ + phenyl-H), 7.96 (d, 1H, J = 8.1 Hz, phenyl-H), 7.76−7.81 (m, 2H, phenyl-H), 6.08 (d, 1H, J = 3.6 Hz, H-1′), 5.65 (d, 1H, J = 5.7 Hz, −OH), 5.30 (d, 1H, J = 5.7 Hz, −OH), 4.82 (t, 1H, J = 5.9 Hz, −OH), 4.50−4.55 (m, 1H, H-2′), 4.24−4.29 (m, 1H, H-3′), 4.00−4.01 (m, 1H, H-4′), 3.46−3.62 (m, 2H, H-5′). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.3, 157.8, 140.2, 136.8, 131.1, 130.6 (d, $^2J_{CF}$ = 31.8 Hz), 129.3, 128.1, 124.2 (d, $^1J_{CF}$ = 270.6 Hz), 121.2, 95.9, 91.3, 86.9, 76.5, 74.9, 71.2, 62.7. ESI-MS: m/z 435.1 [M + Na]$^+$. HRMS: calcd for $C_{17}H_{15}N_4O_5F_3Na^+$ 435.0887, found 435.0882. IR: 2234.2 cm^{-1} (−C≡C−).

2h. An amount of 59.0 mg (0.107 mmol) of **3h** was used for the reaction. A total of 30.8 mg (75%) of product was obtained, isolated as a white solid. HPLC: t = 7.04 min, purity >99% (method 1); t = 6.14 min, purity >99% (method 2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.06 (br s, 1H, −C(O)NH$_2$), 7.94−8.01 (m, 2H, phenyl-H), 7.82−7.85 (m, 2H, phenyl-H), 7.78 (br s, 1H, −C(O)NH$_2$), 5.99 (d, 1H, J = 4.2 Hz, H-1′), 5.62 (d, 1H, J = 6.0 Hz, −OH), 5.25 (d, 1H, J = 6.0 Hz, −OH), 4.80 (t, 1H, J = 5.6 Hz, −OH), 4.49−4.52 (m, 1H, H-2′), 4.24−4.28 (m, 1H, H-3′), 3.96−4.00 (m, 1H, H-4′), 3.41−3.58 (m, 2H, H-5′). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.3, 157.9, 140.1, 135.7, 133.7, 132.0, 130.9 (q, $^2J_{CF}$ = 30.2 Hz), 127.2, 123.9 (q, $^1J_{CF}$ = 272.4 Hz), 117.6, 93.2, 91.3, 86.9, 79.9, 74.8, 71.1, 62.6. ESI-MS: m/z 435.1 [M + Na]$^+$. HRMS: calcd for $C_{17}H_{15}N_4O_5F_3Na^+$ 435.0887, found 435.0878. IR: 2232.9 cm^{-1} (−C≡C−).

2i. An amount of 65.9 mg (0.108 mmol) of **3i** was used for the reaction. A total of 41.6 mg (82%) of product was obtained, isolated as a yellow solid. HPLC: t = 8.23 min, purity 98.8% (method 1); t = 7.02 min, purity 97.3% (method 2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.14−8.55 (m, 11H, pyrene-H and br, 1H, −C(O)NH$_2$), 7.83 (br s, 1H, −C(O)NH$_2$), 6.25 (d, 1H, J = 3.6 Hz, H-1′), 5.75 (d, 1H, J = 5.7 Hz, −OH), 5.36 (d, 1H, J = 6.0 Hz, −OH), 4.87 (t, 1H, J = 5.7 Hz, −OH), 4.62−4.67 (m, 1H, H-2′), 4.29−4.34 (m, 1H, H-3′), 4.07−4.11 (m, 1H, H-4′), 3.51−3.67 (m, 2H, H-5′). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.5, 157.8, 141.0, 133.1, 132.6, 131.3, 130.93, 130.86, 130.5, 130.2, 127.84, 127.76, 127.4, 127.3, 125.7, 124.6, 124.1, 123.7, 113.7, 97.2, 91.4, 87.1, 80.7, 74.8, 71.3, 62.7. Maldi-MS: 491.1 [M + Na]$^+$. HRMS: calcd for $C_{26}H_{20}N_4O_5Na^+$ 491.1326, found 491.1314. IR: 2218.7 cm^{-1} (−C≡C−).

2j. An amount of 51.1 mg (0.104 mmol) of **3j** was used for the reaction. A total of 31.0 mg (85%) of product was obtained, isolated as a yellow solid. HPLC: t = 7.11 min, purity >99% (method 1); t = 6.05 min, purity >99% (method 2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.04 (br s, 1H, −C(O)NH$_2$), 7.91−7.92 (m, 1H, thiophene-H), 7.77 (br s, 1H, −C(O)NH$_2$), 7.72−7.73 (m, 1H, thiophene-H), 7.24−7.26 (m, 1H, thiophene-H), 5.94 (d, 1H, J = 3.9 Hz, H-1′), 5.65 (d, 1H, J = 5.4 Hz, −OH), 5.30 (d, 1H, J = 5.4 Hz, −OH), 4.75−4.85 (m, 1H, −OH), 4.51−4.53 (m, 1H, H-2′), 4.23−4.25 (m, 1H, H-3′), 3.97−3.99 (m, 1H, H-4′), 3.44−3.60 (m, 2H, H-5′). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.4, 157.7, 140.6, 136.7, 132.9, 129.0, 119.0, 91.6, 91.2, 86.9, 78.8, 74.7, 71.2, 62.6. ESI-MS: m/z 373.1 [M + Na]$^+$. HRMS: calcd for $C_{14}H_{14}N_4O_5SNa^+$ 373.0577, found 373.0585. IR: 2216.9 cm^{-1}(−C≡C−).

2k. An amount of 68.7 mg (0.14 mmol) of **3k** was used for the reaction. A total of 37.2 mg (76%) of product was obtained, isolated as a yellow solid. HPLC: t = 7.08 min, purity >99% (method 1); t = 6.05 min, purity >99% (method 2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.24 (d, 1H, J = 1.5 Hz, thiophenyl-H), 8.03 (br s, 1H, −C(O)NH$_2$), 7.76−7.78 (m, 2H, thiophenyl-H and −C(O)NH$_2$), 7.42 (d, 1H, J = 4.5 Hz, thiophenyl-H), 5.98 (d, 1H, J = 4.5 Hz, H-1′), 5.65 (d, 1H, J = 5.7 Hz, −OH), 5.30 (d, 1H, J = 5.7 Hz, −OH), 4.84 (t, 1H, J = 5.6 Hz, −OH), 4.49−4.54 (m, 1H, H-2′), 4.22−4.27 (m, 1H, H-3′), 3.96−3.99 (m, 1H, H-4′), 3.46−3.61 (m,

2H, H-5′). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.4, 157.7, 140.8, 134.6, 130.3, 128.7, 118.8, 93.6, 91.1, 86.9, 74.9, 74.8, 71.2, 62.7. Maldi-MS: *m*/*z* 373.1 $[M + Na]^+$. HRMS: calcd for $C_{14}H_{15}N_4O_5S^+$ 351.0758, found 351.0760. IR: 2223.6 cm^{-1}(−C≡C−).

2l. An amount of 60.0 mg (0.123 mmol) of **3l** was used for the reaction. A total of 38.8 mg (91%) of product was obtained, isolated as a yellow solid. HPLC: t = 9.29 min, purity 98.5% (method 1); t = 6.18 min, purity 96.0% (method 2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.04 (br s, 1H, −C(O)NH$_2$), 7.94 (s, 1H, H-imidazole), 7.77 (br s, 1H, −C(O)NH$_2$), 7.62 (s, 1H, H-imidazole), 5.96 (d, 1H, J = 4.5 Hz, H-1′), 5.64 (d, 1H, J = 5.4 Hz, −OH), 5.29 (d, 1H, J = 5.1 Hz, −OH), 4.81 (t, 1H, J = 4.5 Hz, −OH), 4.51−4.53 (m, 1H, H-2′), 4.21−4.26 (m, 1H, H-3′), 3.97−3.98 (m, 1H, H-4′), 3.76 (s, 3H, CH$_3$), 3.45−3.62 (m, 2H, H-5′). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.4, 157.7, 141.9, 140.6, 137.9, 113.4, 91.3, 87.1, 86.9, 82.5, 74.7, 71.2, 62.6, 32.7. Maldi-MS: *m*/*z* 349.1 $[M + H]^+$. HRMS: calcd for $C_{14}H_{17}N_6O_5^+$ 349.1255, found 349.1247. IR: 2223.9 cm^{-1}(−C≡C−).

2m. An amount of 76.6 mg (0.157 mmol) of **3m** was used for the reaction. A total of 45.2 mg (86%) of product was obtained, isolated as a yellow solid. HPLC: t = 7.38 min, purity >99% (method 1); t = 6.23 min, purity >99% (method 2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.01 (br s, 1H, −C(O)NH$_2$), 7.73 (br s, 1H, −C(O)NH$_2$), 6.51 (s, 1H, alkene-H), 5.85 (d, 1H, J = 4.2 Hz, H-1′), 5.61 (d, 1H, J = 5.1 Hz, −OH), 5.28 (d, 1H, J = 5.1 Hz, −OH), 4.80 (t, 1H, J = 5.1 Hz, −OH), 4.46−4.48 (m, 1H, H-2′), 4.19−4.21 (m, 1H, H-3′), 3.93−3.95 (m, 1H, H-4′), 3.40−3.60 (m, 2H, H-5′), 2.19−2.20 (m, 4H, −CH$_2$−), 1.58−1.65 (m, 4H, −CH$_2$−). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.4, 157.6, 141.6, 141.1, 118.8, 99.9, 91.0, 86.9, 74.6, 73.1, 71.2, 62.7, 28.4, 26.1, 22.1, 21.3. Maldi-MS: *m*/*z* 371.1 $[M + Na]^+$. HRMS: calcd for $C_{16}H_{20}N_4O_5Na^+$ 371.1326, found 371.1324. IR: 2214.1 cm^{-1} (−C≡C−).

2n. An amount of 80.1 mg (0.165 mmol) of **3n** was used for the reaction. A total of 40.3 mg (71%) of product was obtained, isolated as a white solid. HPLC: t = 7.29 min, purity >99% (method 1); t = 5.99 min, purity >99% (method 2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.96 (br s, 1H, −C(O)NH$_2$), 7.71 (br s, 1H, −C(O)NH$_2$), 5.87 (d, 1H, J = 4.5 Hz, H-1′), 5.59 (d, 1H, J = 6.0 Hz, −OH), 5.25 (d, 1H, J = 5.1 Hz, −OH), 4.79 (t, 1H, J = 5.6 Hz, −OH), 4.44−4.49 (m, 1H, H-2′), 4.18−4.23 (m, 1H, H-3′), 3.92−3.97 (m, 1H, H-4′), 3.77 (t, 2H, J = 6.3 Hz, −CH$_2$−), 3.43−3.59 (m, 2H, H-5′), 2.76 (t, 2H, J = 6.9 Hz, −CH$_2$−), 2.01−2.10 (m, 2H, −CH$_2$−). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.4, 157.3, 140.8, 99.9, 90.8, 86.8, 74.6, 71.2, 67.8, 62.7, 44.6, 30.8, 16.9. Maldi-MS: *m*/*z* 367.1 $[M + Na]^+$. HRMS: calcd for $C_{13}H_{17}N_4O_5ClNa^+$ 367.0780, found 367.0784. IR: 2246.1 cm^{-1} (−C≡C−).

2o. An amount of 77.0 mg (0.156 mmol) of **3o** was used for the reaction. A total of 46.7 mg (85%) of product was obtained, isolated as a white solid. HPLC: t = 7.48 min, purity >99% (method 1); t = 5.94 min, purity 97.7% (method 2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.98 (br s, 1H, −C(O)NH$_2$), 7.72 (br s, 1H, −C(O)NH$_2$), 5.88 (d, 1H, J = 3.9 Hz, H-1′), 5.77 (s, 1H, −OH), 5.60 (d, 1H, J = 6.0 Hz, −OH), 5.26 (d, 1H, J = 5.1 Hz, −OH), 4.79 (t, 1H, J = 5.4 Hz, −OH), 4.44−4.49 (m, 1H, H-2′), 4.20−4.25 (m, 1H, H-3′), 3.94−3.95 (m, 1H, H-4′), 3.39−3.60 (m, 2H, H-5′), 1.94−1.96 (m, 4H, cyclopentane-H), 1.73−1.75 (m, 4H, cyclopentane-H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.4, 157.4, 140.8, 104.5, 91.0, 86.7, 74.7, 73.4, 71.2, 68.3, 62.7, 42.3, 23.7. Maldi-MS: *m*/*z* 375.1 $[M + Na]^+$. HRMS: calcd for $C_{15}H_{20}N_4O_6Na^+$ 375.1275, found 375.1274. IR: 2241.5 cm^{-1} (−C≡C−).

2p. An amount of 69.0 mg (0.136 mmol) of **3p** was used for the reaction. A total of 43.8 mg (88%) of product was obtained, isolated as a white solid. HPLC: t = 7.48 min, purity 98.5% (method 1); t = 5.97 min, purity 98.6% (method 2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.01 (br s, 1H, −C(O)NH$_2$), 7.74 (br s, 1H, −C(O)NH$_2$), 5.91 (s, 1H, −OH), 5.89 (d, 1H, J = 4.2 Hz, H-1′), 5.62 (d, 1H, J = 5.1 Hz, −OH), 5.27 (d, 1H, J = 5.1 Hz, −OH), 4.80 (t, 1H, J = 5.4 Hz, −OH), 4.43−4.48 (m, 1H, H-2′), 4.20−4.25 (m, 1H, H-3′), 3.91−3.94 (m, 1H, H-4′), 3.40−3.58 (m, 2H, H-5′), 1.44−1.88 (m, 10H, cyclohexane-H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.4, 157.5, 140.7, 104.5, 91.0, 86.7, 74.7, 71.2, 69.4, 67.9, 62.7, 25.3, 23.2. Maldi-MS: *m*/*z* 389.1 $[M + Na]^+$. HRMS: calcd for $C_{16}H_{22}N_4O_6Na^+$ 389.1432, found 389.1438. IR: 2231.8 cm^{-1} (−C≡C−).

7. An amount of 35.9 mg (0.1 mmol) of **6** was dissolved in 3 mL acetic anhydride and stirred at 75 °C until TLC indicated the complete consumption of **6**. Then the solvent was removed and the residue purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 40:1). The purified material was dried in vacuo to afford 37.8 mg (94%) of product **7** as a white solid. HPLC: t = 6.91 min, purity >99% (method 1); t = 6.35 min, purity 97.8% (method 2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.69−7.77 (m, 4H, phenyl-H), 7.07 (br s, 1H, −C(O)NH$_2$), 6.01 (br s, 1H, −C(O)NH$_2$), 5.75 (s, 2H, −CH$_2$−), 4.23 (t, 2H, J = 4.4 Hz, −CH$_2$−), 3.91 (t, 2H, J = 4.4 Hz, −CH$_2$−), 2.06 (s, 3H, −C(O)CH$_3$). ^{13}C NMR (150 MHz, $CDCl_3$): δ 171.1, 160.5, 156.5, 140.7, 132.8, 132.4 (q, $^2J_{CF}$ = 31.8 Hz), 126.0 (d, $^3J_{CF}$ = 3.2 Hz), 123.7, 123.7 (q, $^1J_{CF}$ = 271.7 Hz), 96.7, 78.6, 76.2, 68.6, 62.9, 21.1. Maldi-MS: 419.1 $[M + Na]^+$. HRMS: calcd for $C_{17}H_{16}F_3N_4O_4^+$ 397.1118, found 397.1123. IR: 2237.8 cm^{-1} (−C≡C−).

8. 5-Bromo-1-[(2-acetyl)methyl]-1,2,4-triazole-3-carboxylate (63.5 mg, 0.2 mmol), 4-trifluoromethylphenylacetylene 39 μL (0.24 mmol), tetrakis(triphenylphosphine)palladium(0) (11.2 mg, 0.01 mmol), CuI (1.8 mg, 0.01 mmol), and triethylamine (0.3 mL) were suspended in 3 mL of fresh distilled dioxane under argon. The vessel was sealed and irradiated at 100 °C for 30 min and then cooled to room temperature. The reaction mixture was concentrated under reduced pressure, and the crude residue purified by flash chromatography on silica gel (petroleum ether/ethyl acetate, 2:1). The purified material was dried in vacuo to afford the corresponding product **8**. A total of 48.6 mg (60%) of product was obtained, isolated as a white oil. HPLC: t = 6.76 min, purity >99% (method 1); t = 6.43 min, purity >99% (method 2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64−7.75 (m, 4H, phenyl-H), 5.75 (s, 2H, −CH$_2$−), 4.23 (t, 2H, J = 4.8 Hz, −CH$_2$−), 4.04 (s, 3H, −OCH$_3$), 3.89 (t, 2H, J = 4.7 Hz, −CH$_2$−), 2.04 (s, 3H, −C(O)CH$_3$). ^{13}C NMR (150 MHz, $CDCl_3$): δ 171.0, 159.7, 154.8, 141.4, 132.8, 126.0, 123.9, 96.7, 78.7, 76.3, 68.5, 62.8, 53.3, 21.0. Maldi-MS: 434.1 $[M + Na]^+$. HRMS: calcd for $C_{18}H_{17}F_3N_3O_5^+$ 412.1115, found 412.1123. IR: 2234.0 cm^{-1} (−C≡C−).

10. An amount of 37.2 mg (0.09 mmol) of **2f** was dissolved in 5 mL of MeOH and 4.0 mg of 10% Pd/C added. H_2 was flushed in, and the reaction mixture was stirred at room temperature overnight. The insoluble residue was filtered by Celite and the solvent removed by reduced pressure. The residue was dried in vacuo to afford 37.0 mg (98.5%) of product **10** as a white solid. HPLC: t = 7.44 min, purity 98.8% (method 1); t = 6.03 min, purity 98.1% (method 2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.74 (br s, 1H, −C(O)NH$_2$), 7.66 (d, 2H, J = 8.1 Hz, phenyl-H), 7.61 (br s, 1H, −C(O)NH$_2$), 7.53 (d, 2H, J = 8.1 Hz, phenyl-H), 5.83 (d, 1H, J = 4.2 Hz, H-1′), 5.46 (d, 1H, J = 5.7 Hz, −OH), 5.22 (d, 1H, J = 5.1 Hz, −OH), 4.78 (t, 1H, J = 5.6 Hz, −OH), 4.45−4.48 (m, 1H, H-2′), 4.19−4.21 (m, 1H, H-3′), 3.93−3.94 (m, 1H, H-4′), 3.42−3.58 (m, 2H, H-5′), 3.15−3.22 (m, 4H, −CH$_2$−). ^{13}C NMR (75 MHz, DMSO-d_6): δ 161.2, 157.8, 156.3, 145.8, 129.9, 127.7 (q, $^2J_{CF}$ = 32.3 Hz), 125.8, 125.1 (d, $^1J_{CF}$ = 270.5 Hz), 90.1, 86.5, 74.7, 71.2, 62.7, 33.2, 26.9. Maldi-MS: 439.1 $[M + Na]^+$. HRMS: calcd for $C_{17}H_{19}F_3N_4O_5Na^+$ 439.1200, found 439.1200.

12. An amount of 39.0 mg (0.071 mmol) of **3f** was dissolved in 5 mL of CH_2Cl_2 and 4.0 mg of 10% Pd/C added. H_2 was flushed in, and the reactants were stirred at room temperature overnight. The insoluble residue was filtered by Celite and the solvent removed by reduced pressure. The residue was dried in vacuo to afford 39.0 mg (99.3%) of product **12** as colorless oil. HPLC: t = 6.76 min, purity >99% (method 1); t = 6.21 min, purity >99% (method 2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56 (d, 2H, J = 8.1 Hz, phenyl-H), 7.32 (d, 2H, J = 8.1 Hz, phenyl-H), 5.81 (br, 2H, H-1′ and H-2′), 5.68 (br, 1H, H-3′), 4.40−4.47 (m, 2H, H-5′), 4.11−4.16 (m, 1H, H-4′), 3.98 (s, 3H, −OCH$_3$), 3.21−3.23 (m, 2H, −CH$_2$−), 3.11−3.17 (m, 2H, −CH$_2$−), 2.13 (s, 3H, −C(O)CH$_3$), 2.11 (s, 3H, −C(O)CH$_3$), 2.07 (s, 3H, −C(O)CH$_3$). ^{13}C NMR (150 MHz,

$CDCl_3$): δ 170.7, 169.6, 160.2, 157.9, 154.2, 144.0, 129.2 (q, $^2J_{CF}$ = 32.6 Hz), 128.9, 125.8, 124.3 (q, $^1J_{CF}$ = 270.2 Hz), 88.2, 81.3, 74.5, 71.1, 62.9, 52.9, 33.5, 27.7, 20.72, 20.65, 20.57. Maldi-MS: 580.1 $[M + Na]^+$. HRMS: calcd for $C_{24}H_{26}N_3O_9F_3Na^+$ 580.1513, found 580.1498.

Anti-HCV Assay in Huh-5-2 Cells. Huh-5-2 cells were seeded at a density of 5000 per well in a tissue culture-treated white 96-well view plate (Packard, Canberra, Canada) in complete Dulbecco's modified Eagle medium (DMEM) supplemented with 2% fetal calf serum (FCS) and 250 μg/mL G418. After incubation for 24 h at 37 °C (5% CO_2) the medium was replaced by 3-fold serial dilutions in complete DMEM (without G418) of the test compounds in a total volume of 100 μL. After 4 days of incubation at 37 °C, cell culture medium was removed and luciferase activity determined using the Steady-Glo luciferase assay system (Promega, Leiden, The Netherlands) and was measured using a Luminoskan ascent (Thermo, Vantaa, Finland).

Anti-HCV Assay in Huh-9-13 Cells. Huh-9-13 cells were seeded at a density of 5000 cells per well in 96-well cell culture plates in complete DMEM supplemented with 2% fetal calf serum (FCS) and 1000 μg/mL G418. After 24 h of incubation at 37 °C, cell culture medium was replaced by 3-fold serial dilutions of the test compounds in complete DMEM without G418 in a total volume of 100 μL. After 4 days of incubation at 37 °C, cell culture fluid was removed and monolayers were washed once with phosphate-buffered saline. Cells were lysed in 350 μL of RLT buffer (Qiagen, Venlo, The Netherlands) according to the manufacturer's instruction. Lysates were used to determine the amount of HCV replicon RNA by means of quantitative real-time PCR. The values of EC_{50} were calculated as the concentration of compound causing a 50% reduction in HCV RNA levels compared to untreated control.

Anti-HCV Assay in Huh-6 Cells. Huh-6 cells were seeded at a density of 15 000 cells per well in 96-well cell culture plates as described for Huh-9-13. After a 3-day incubation period at 37 °C, cells were lysed in cells-to-cDNA lysis buffer (Ambion, Cambridgeshire, U.K.), and the amount of HCV replicon RNA was analyzed by quantitative real-time PCR. The values of EC_{50} were calculated as the concentration of compound causing a 50% reduction in HCV RNA levels compared to that of the untreated control.

Anticancer Assay in MiaPaCa-2 Cells. In Vitro Cell Growth Inhibition. Pancreatic cancer chemoresistant MiaPaCa-2 cells were cultured in DMEM medium (Gibco) supplemented with 10% fetal bovine serum (FBS). Cells were seeded at a densitiy of 15 000 cells per well in a 96-well view plate (Packard) in 250 μL of medium containing the same components as described above. Cells were allowed to attach overnight (O/N), and then culture medium was removed and replaced with fresh media alone as control or containing different concentrations of compounds. Plates were further incubated at 37 °C and 5% CO_2 for 48 h. The number of viable cells remaining after the appropriate treatment was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay.

Apoptosis Assay by Flow Cytometry (FACS). Cells were seeded in 10 cm dishes at a density of 10^6 cells/dish and allowed to adhere and proliferate O/N. Culture medium was then removed, and fresh medium containing the proper concentration of compound was added. No treatment was done as negative controls. After 48 h of treatment, the cells were trypsinized and the collected cell pellet was washed with PBS and fixed in cold 70% ethanol overnight at 4 °C. After a wash with phosphate−citrate buffer, cells were treated with 200 μL of RNase (500 μg/mL), labeled with 1 mL of propidium iodide (50 μg/mL), and immediately analyzed by fluorescence activated cell sorting (FACS Calibur, Becton Dickinson, Le Pont-De-Claix, France). Cell death analysis was done on 10^6 cells, evaluating the sub-G1/G0 ratio. Each sample was performed in triplicate.

Apoptosis Assay by ELISA. Cells were seeded for 24 h in 96-well plates (15 000 cells/well), and cells were treated by test compounds or not as negative control. After 48 h, apoptosis was assessed by an enzyme linked immunoassay (ELISA) that quantifies cytoplasmic nucleosomes produced during apoptosis (Cell Death Detection ELISA plus, Roche). The 96-well plates were centrifuged (200*g*) for 10 min, the supernatant was discarded, and lysis buffer was added. After lysis, the samples were centrifuged and an amount 20 μL of the supernatant was transferred to a streptavidin-coated microtiter plate. Biotin-labeled antihistone antibodies and peroxidase conjugated anti-DNA antibodies were added to each well, and the plate was incubated at room temperature for 2 h. After three washes with buffer, the peroxidase substrate was added to each well to quantitate the captured nucleosomes. After 20 min of incubation, the plates were read at 405/490 nm in a microplate reader. The enrichment in histone−DNA fragments is expressed as a fold increase in absorbance compared with control.

Apoptosis Assay by Caspase-3/7 Cleavage Assay. Caspase-3/7 activity was measured using the Apo-ONE homogeneous caspase-3/7 assay fluorometric kit (Promega). MiaPaCa-2 cells were initially seeded at 15 000 cells/well on 96-well plates. Twenty-four hours later, cells were treated with the test compound for 48 h and caspase-3 activity was measured by the cleavage of the fluorometric substrate Z-DEVD-R110 according to the instructions of the manufacturer (Promega). Next 100 μL of Apo-ONE homogeneous caspase-3/7 reagent was added to each well of a black 96-well plate containing 100 μL of blank, control, or cells in culture. Each experiment was performed in triplicate. The plate was covered with a plate sealer and incubated at room temperature for 30 min before the fluorescence of each well was measured.

Assessment of in Vivo Tumor Growth. Institutional guidelines for the proper and human use of animals in research were followed. Approximately 1×10^7 MiaPaCa-2 cells were inoculated subcutaneously with 0.1 mL of Matrigel (BD Biosciences Discovery Labware) to 6-week-old male xenografed nude mice. When MiaPaCa-2 tumors reached 100 mm^3, mice were randomly selected for treatment with test compound, and no treated mice were used as control. Each experimental group consisted of five mice. After randomization, 150 mg/kg test compound was injected every 3 days by ip injection for 4 weeks. Tumor volume measurements were performed once weekly and calculated by the formula length $\times$ width $\times$ depth $\times$ 0.5236.

Statistical Analysis. All of the results were expressed as the mean $\pm$ standard error (SE). Statistical analysis was performed by a one way ANOVA followed by Fisher's protected least significant difference (PLSD) test (Statview 512, Brain Power Inc., Calabases, CA). $p \leq 0.05$ was considered significant (*); $p \leq 0.01$ (**); $p \leq 0.001$ (***).

Cytotoxicity Assay. MiaPaCa-2, PC-3, and Hela cells were cultured in DMEM medium (Gibco) supplemented with 10% fetal bovine serum (FBS). HepG2 cells were cultured in MEM medium supplemented with 10% FBS. Cells were seeded at a density of 15 000 cells per well in a 96-well view plate (Packard) in 250 μL of medium containing the same components as described above. L1210 cells were cultured in RPMI 1640 medium supplemented with 5% FBS and were seeded at a density of 10 000 cells per well in a 96-well view plate (Packard) in 50 μL of medium. Cells were allowed to attach O/N, and then culture medium was removed and replaced with fresh media alone as control or containing serial dilutions of the test compounds from 10 nM to 200 μM. Plates were further incubated at 37 °C and 5% CO_2 for 48 h. The number of viable cells remaining after the appropriate treatment was determined by MTT colorimetric assay. The cell viability of L1210 cells were determined by CellTiter 96 AQ_{ueous} One solution cell proliferation assay (Promega).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra and HPLC analysis results for all new compounds described in the work. This material is available free of charge via the Internet at http://pubs.acs.org.

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