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# Novel Triazole Ribonucleoside Down-Regulates Heat Shock Protein 27 and Induces Potent Anticancer Activity on Drug-Resistant Pancreatic Cancer

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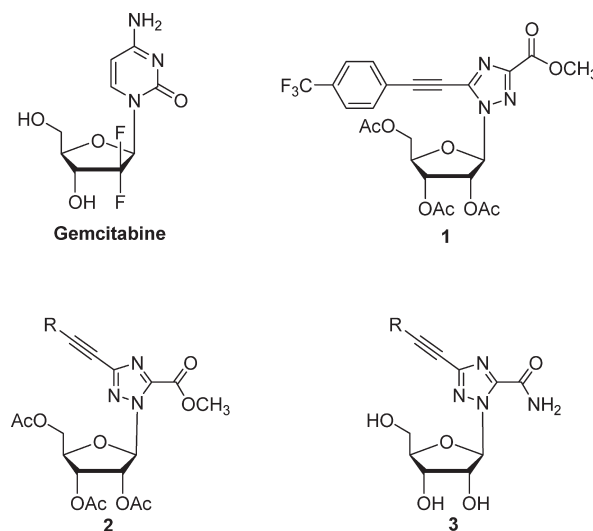
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A series of novel 3-arylethynyltriazolyl ribonucleosides were synthesized and assessed for their anti-cancer activity on the drug-resistant pancreatic cancer cell line MiaPaCa-2. Among them, one compound exhibited potent apoptosis-inducing properties and anticancer activity against the pancreatic cancer model MiaPaCa-2 both in vitro and in vivo with no adverse effects. This compound did not inhibit DNA synthesis and therefore does not resemble the clinical drug gemcitabine. It did, however, significantly down-regulate the expression of heat shock protein 27 (Hsp27), a small molecular chaperone playing an important role in drug resistance and highly expressed in drug-resistant cancer forms, and thus represents the first small molecular anticancer lead with such a mode of action.

## Introduction

Pancreatic cancer is one of the most lethal and devastating forms of human cancer, ranking as the fifth most common cause of death by cancer in the Western world and with a 5-year survival rate as low as 3%.<sup>1</sup> Conventional cancer treatments have little impact on this extremely aggressive form of cancer, and drug resistance develops very quickly. The current first-line treatment is based on gemcitabine (Figure 1), a nucleoside drug that exerts its anticancer activity mainly via inhibiting DNA synthesis alongside other possible modes of action.<sup>2</sup> Unfortunately, gemcitabine is only moderately effective, yielding a mere 12% response rate and a median survival period of 5 months.<sup>1,2</sup> There is therefore an urgent need to explore new drug candidates for combating pancreatic cancer, particularly drug-resistant pancreatic cancer.

The resistance of pancreatic cancer to apoptosis is an important factor contributing to its drug resistance. The investigation of several proteins such as p8,<sup>3</sup> Bcl2, Bcl2-like proteins,<sup>4</sup> and their respective signaling pathways has provided evidence to this effect. Of particular interest is the heat shock protein 27 (Hsp27<sup>a</sup>), which plays an important role in the resistance shown by patients with pancreatic cancer to gemcitabine.<sup>5</sup> Hsp27 is an ATP-independent small molecular chaperone functioning to protect against drug toxicity, which displays enhanced synthesis in response to stress and anti-



**Figure 1.** Gemcitabine, 5-arylethynyltriazole ribonucleoside **1**, and 3-ethynyltriazole ribonucleosides **2** and **3** described in this study.

apoptotic properties.<sup>6</sup> Increased levels of Hsp27 expression render tumors more resistant to many chemotherapeutic agents. Inhibiting Hsp27 function can increase cell sensitivity to drugs, thus overcoming the problem of drug resistance. Antisense oligonucleotides and siRNA molecules, developed to down-regulate Hsp27, have potent caspase-dependent apoptotic anticancer activity on prostate cancer<sup>7,8</sup> and are currently in clinical trials for use in the treatment of several human cancers, such as lung, breast, prostate, bladder, and ovarian cancers.<sup>9</sup> One recent study has shown that down-regulation of Hsp27 via interferon- $\gamma$  could also produce effective anticancer activity in drug-resistant pancreatic cancer cells, further demonstrating the general utility and importance of down-regulating Hsp27 in treating drug-resistant cancers.<sup>10</sup>

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<sup>a</sup>Abbreviations: Hsp27, heat shock protein 27; DMEM, Dulbecco's modified Eagle medium; 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; LDH, lactate dehydrogenase; 7-AAD, 7-aminoactinomycin D; qRT-PCR, quantitative real-time polymerase chain reaction; DAPI, 4,6-diamidino-2-phenylindole; ip, intraperitoneal; O/N, overnight; SE, standard error.

**Table 1.** Synthesis of 3-Arylethynyltriazole Nucleosides **2** and **3** via the Sonogashira Reaction and the Subsequent Ammonolysis

| entry | R | Products  | Yields (%) | Products  | Yields (%) |
|-------|---|-----------|------------|-----------|------------|
| 1     |   | <b>2a</b> | 77         | <b>3a</b> | 81         |
| 2     |   | <b>2b</b> | 75         | <b>3b</b> | 72         |
| 3     |   | <b>2c</b> | 63         | <b>3c</b> | 78         |
| 4     |   | <b>2d</b> | 69         | <b>3d</b> | 83         |
| 5     |   | <b>2e</b> | 73         | <b>3e</b> | 71         |
| 6     |   | <b>2f</b> | 49         | <b>3f</b> | 77         |
| 7     |   | <b>2g</b> | 73         | <b>3g</b> | 80         |
| 8     |   | <b>2h</b> | 64         | <b>3h</b> | 77         |
| 9     |   | <b>2i</b> | 64         | <b>3i</b> | 77         |
| 10    |   | <b>2j</b> | 58         | <b>3j</b> | 74         |
| 11    |   | <b>2k</b> | 64         | <b>3k</b> | 76         |
| 12    |   | <b>2l</b> | 55         | <b>3l</b> | 73         |
| 13    |   | <b>2m</b> | 73         | <b>3m</b> | 81         |
| 14    |   | <b>2n</b> | 52         | <b>3n</b> | 76         |
| 15    |   | <b>2o</b> | 61         | <b>3o</b> | 86         |
| 16    |   | <b>2p</b> | 62         | <b>3p</b> | 74         |
| 17    |   | <b>2q</b> | 75         | <b>3q</b> | 74         |
| 18    |   | <b>2r</b> | 65         | <b>3r</b> | 79         |
| 19    |   | <b>2s</b> | 79         | <b>3s</b> | 63         |
| 20    |   | <b>2t</b> | 79         | <b>3t</b> | 60         |

However, as yet, no small molecular weight compound able to down-regulate Hsp27 has been identified and the related drug

discovery process is difficult due to the lack of structural information on Hsp27.

We recently discovered a triazole ribonucleoside **1** (Figure 1), which showed potent anticancer activity against the drug-resistant pancreatic cancer cell line MiaPaCa-2 both in vitro and in vivo while having no adverse effect.<sup>11</sup> **1** belongs to the family of arylethynyltriazolyl ribonucleosides, the chemistry and biological activity of which have never been described before.<sup>12</sup> This family of triazole nucleosides may thus provide a new structural lead in the search for drug candidates with novel mode of action to treat drug-resistant pancreatic cancer. In addition, arylethynyltriazolyl nucleosides also exhibit antiviral activity against HCV.<sup>11,13</sup> Consequently, we feel that arylethynyltriazolyl nucleosides may represent an important structural motif in the search of bioactive compounds.

In our continuing efforts to identify novel triazole nucleosides as potential anticancer drug candidates and to provide molecular diversity of triazole nucleosides for further structure–activity relationship analysis, we are particularly interested in 3-arylethynyltriazolyl ribonucleosides **2** and **3** (Figure 1), the structural isomers of the corresponding 5-arylethynyltriazolyl ribonucleoside **1** previously developed in our laboratory.<sup>11</sup> We wished to investigate their potential anticancer activity and more particularly their ability to down-regulate Hsp27, with the aim of developing small molecular weight compounds having novel mechanisms of action. This represents part of our ongoing program of developing structurally novel and diverse triazole nucleosides by appending  $\pi$ -conjugated systems onto the triazole nucleobase.<sup>11–18</sup> These compounds are expected to offer advantageous binding properties to their corresponding biological targets via more efficient and stronger interactions provided by their larger aromatic systems.<sup>14</sup> In addition, they may lead to better in vivo stability and efficiency due to the unnatural triazole heterocyclic system. Here we report on the chemical synthesis of the 3-arylethynyltriazolyl ribonucleosides **2** and **3** (Figure 1), the biological assessment of their anticancer activity against drug-resistant pancreatic cancer MiaPaCa-2 cell line, and the results of our preliminary investigation on their mechanism of action. One of the newly synthesized 3-arylethynyltriazolyl ribonucleosides showed effective anticancer activity on the MiaPaCa-2 cell line both in vitro and in vivo, probably via a mechanism involving the down-regulation of Hsp27.

## Results and Discussion

**1. Chemistry.** The synthesis of 3-ethynyltriazole ribonucleosides was carried out via a microwave-assisted Sonogashira coupling reaction, using a strategy similar to that used to synthesize 5-ethynyltriazole ribonucleosides (Table 1).<sup>11</sup> The Sonogashira coupling reaction between the 3-bromotriazole nucleoside and various alkynes led to the nucleosides **2**, which were then subjected to ammonolysis to give the deprotected nucleosides **3** in good yields (Table 1). Similar to that observed previously with 5-ethynyltriazole ribonucleosides, there was no important difference in the yields of the Sonogashira reaction between using alkynes with electron-donating and those with electron-withdrawing groups appended on phenylacetylene. In addition, very good yields were obtained with both heterocyclic arylacetylenes (Table 1, entries 13–15) and alkylacetylenes (Table 1, entries 16–20) bearing various functionalities including a chloride, a double bond, and a hydroxyl group etc (Table 1, entries 16–20).

We further synthesized several structural analogues of **3e** (Figure 2 and Scheme 1) for the relevant structure–activity

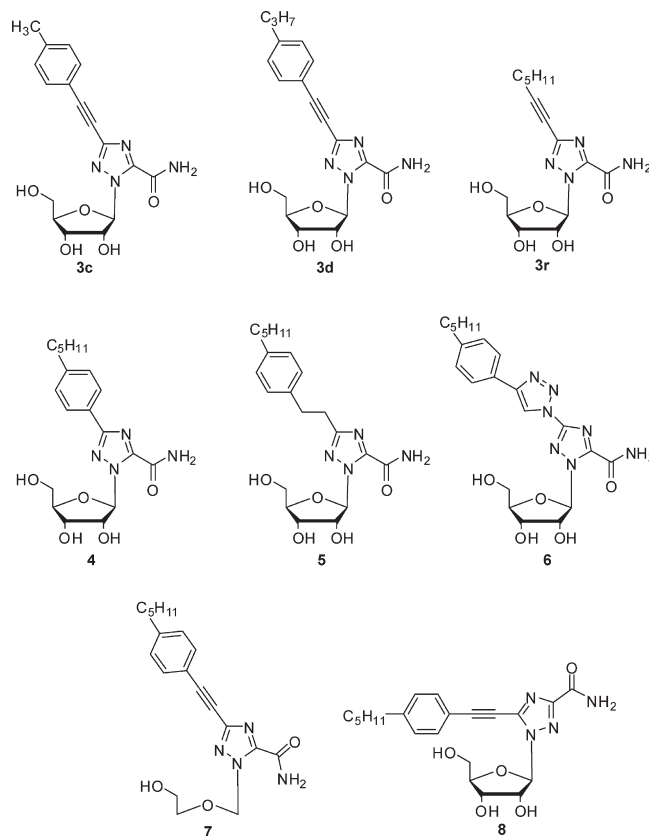


Figure 2. Structural analogues for SAR analysis of **3e**.

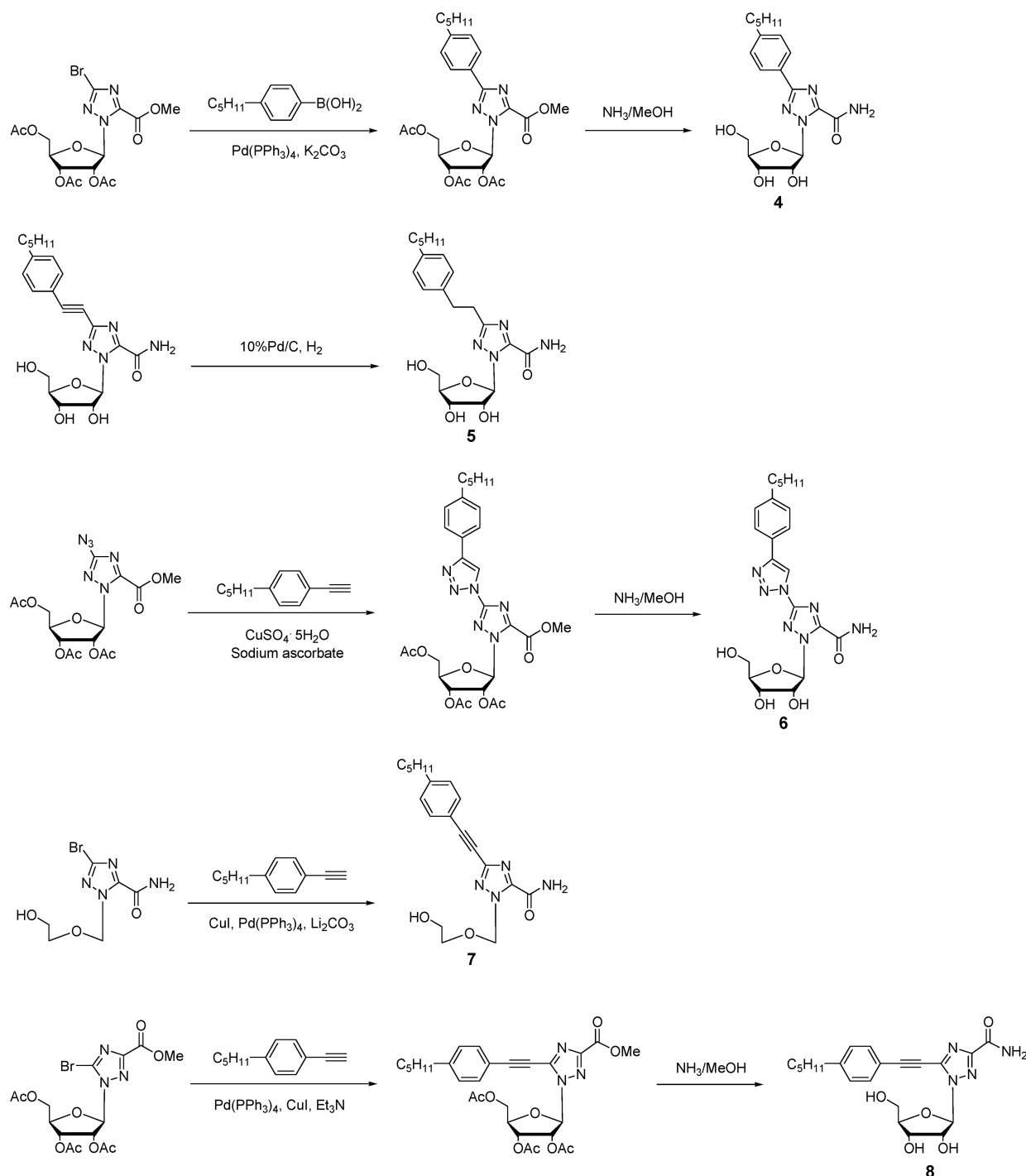
relationship analysis. Compounds **4**, **7**, and **8** were prepared using the synthetic procedures previously developed by our group (Scheme 1).<sup>11,13,15</sup> Molecule **5** was obtained by Pd/C-catalyzed hydrogenation of **3e**, and analogue **6** was obtained via the Huisgen reaction using 3-azidotriazole nucleoside<sup>19</sup> and 1-ethynyl-4-pentylbenzene followed by ammonolysis in  $\text{NH}_3/\text{MeOH}$  (Scheme 1).<sup>16a,17</sup>

**2. Biology. In Vitro Antiproliferative Activity Assay.** We used a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to evaluate the anticancer activity of all the synthesized nucleosides on the MiaPaCa-2 cells. One of the compounds, **3e**, led to a significant reduction in MiaPaCa-2 cell proliferation and/or viability by 80% 2 days following treatment (Figure 3). Under the same conditions, gemcitabine only moderately inhibited cell proliferation by less than 30% (Figure 3).

Since **3e** bears the alkyl chain in its structure, we further examined its analogues **3c** and **3d** (Figure 2), each bearing different alkyl chain lengths. These compounds were found to be inactive against MiaPaCa-2 cell proliferation. Moreover, we checked the activity of the analogue **3r**, which corresponds to **3e** with a phenyl group removed. Compound **3r** failed to significantly inhibit the growth of MiaPaCa-2 cells. Therefore, both the alkyl chain  $\text{C}_5\text{H}_{11}$  and the phenyl ring seem crucial for **3e** to retain its activity.

In addition, we found that removing the triple bond from **3e** or replacing it with either a flexible  $-\text{CH}_2\text{CH}_2-$  linker or a rigid triazole ring led to the inactive analogues **4**, **5**, and **6**, respectively (Figure 2). These data reflect the importance of the triple bond connection between the phenyl group and the triazole ring for the activity of **3e**. Moreover, neither the acyclic nucleoside analogue **7** nor the 5-isomeric analogue **8** (Figure 2) exhibited any considerable inhibition on cell

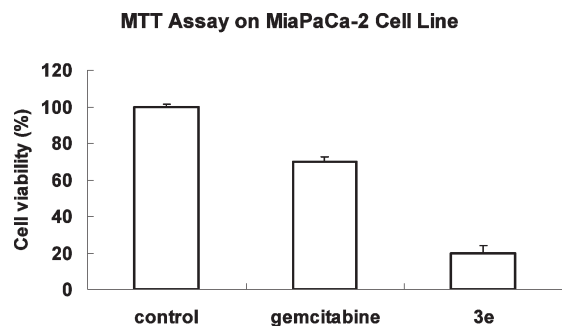
Scheme 1. Synthesis of Compounds 4–8 the Structural Analogues of 3e



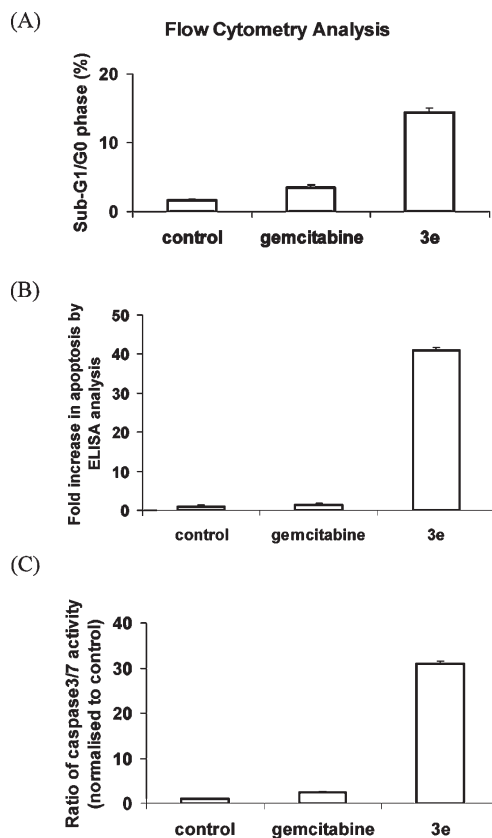
growth. It is interesting to note that **2e**, the corresponding protected form of **3e**, was devoid of any activity on MiaPa-Ca-2 cells. This may have resulted from the reduced H-bonding possibility and increased steric hindrance related to the acetyl protected ribose sugar moiety in **2e**. Furthermore, both **2j**, the structural isomer of our previously identified anticancer lead **1**, and **3j**, the deprotected form of **2j**, failed to yield any notable activity. All of these observations led us to conclude that the ribose component and the 3-isomeric structure contribute largely to the activity of **3e**. In our previous study with triazole ribonucleosides, only protected arylethynyltriazole nucleosides showed anticancer activity.<sup>11</sup> The identification of the active compound **3e** in this work

revealed that the sugar-deprotected alkynyltriazole ribonucleosides **3** may also constitute a new structural lead with anticancer activity.

**Caspase-Dependent Apoptosis-Inducing Properties and Anticancer Activity.** Our previous molecular lead **1** was shown to display caspase-dependent apoptosis-inducing properties and anticancer activity.<sup>11</sup> As for lead **1**, we studied the apoptosis-inducing activity of **3e** in MiaPaCa-2 cells using fluorescence-activated cell sorting (FACS) flow cytometry,<sup>20</sup> ELISA for DNA/histone release,<sup>21</sup> and a caspase-3/7 activity assay<sup>20</sup> (Figure 4). A significant 14-fold increase in the fraction of cells undergoing apoptosis (sub-G1/G0 fraction) was revealed by FACS flow analysis following treatment

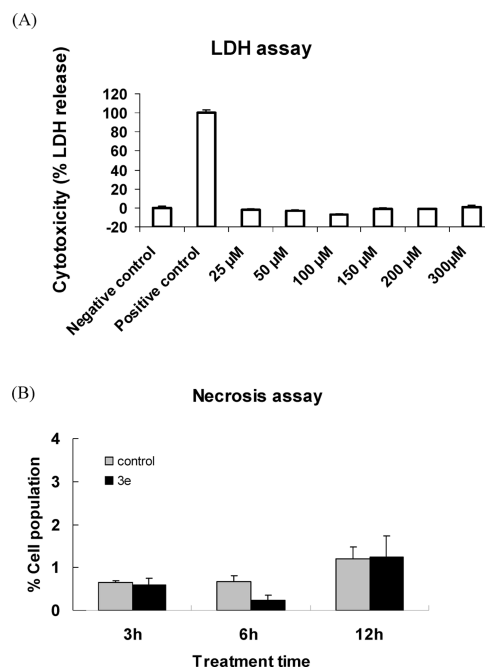


**Figure 3.** Inhibitory effect of compound **3e** on the pancreatic cancer MiaPaCa-2 cell growth. MiaPaCa-2 cells were incubated with compound **3e** for 48 h and then assayed by MTT, using no treatment and gemcitabine as controls. The values are the mean  $\pm$  SE of three independent experiments.



**Figure 4.** Apoptosis-induction in MiaPaCa-2 cells after 48 h of treatment with **3e** using gemcitabine and no treatment as control: (A) flow cytometry was used to quantify the percentage of cells undergoing apoptosis (cells in sub-G1/G0); (B) ELISA test for detection of DNA/histone nucleosome release; (C) caspase-3/7 activity measurement. All the experiments were done in triplicate.

with **3e** compared to nontreatment control (Figure 4A), indicating that induction of apoptosis represented the main mode of activity for **3e**. Further results from ELISA showed a spectacular 40-fold increase in DNA/histone nucleosome release following treatment with **3e** compared to nontreatment control (Figure 4B), in line with those obtained from FACS flow (Figure 4A), thus confirming the apoptosis-induced antiproliferative activity of **3e**. Moreover, using caspase-3/7 colorimetric assay, we revealed a similarly impressive 30-fold increase in caspase-3/7 activation following treatment with **3e** compared to nontreatment control and a



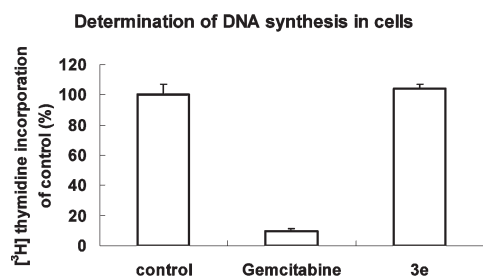
**Figure 5.** Necrosis induced by **3e** assessed by (A) LDH assay at different concentration. Negative control determines the LDH activity released from the untreated normal cells (= spontaneous LDH release), and positive control determines the maximum releasable LDH activity in the cells (= maximum LDH release). (B) Flow cytometry: the necrotic cells were determined by staining with 7-aminoactinomycin D (7AAD).

12-fold increase compared to gemcitabine control (Figure 4C), suggesting a caspase-3/7-dependent mechanism of apoptosis induction by **3e**.

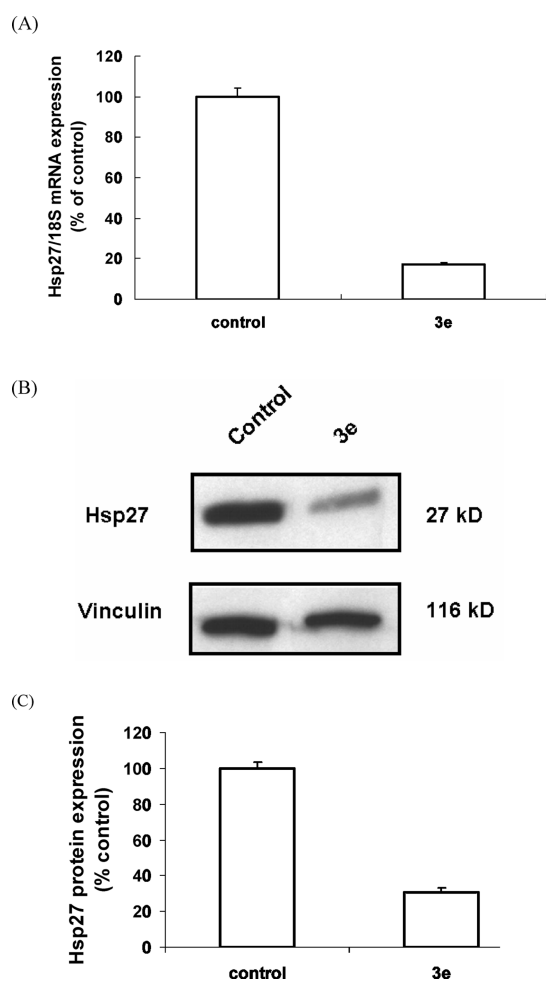
**Necrosis Effect.** Because of the presence of the long hydrophobic alkyl chain  $C_5H_{11}$ , we suspected that **3e** might execute its antiproliferative effect on MiaPaCa-2 cells additionally via necrosis. Necrosis is a passive, nonprogrammed form of cell death mainly occurring following the induction of cell membrane damage/leakage and the consequent rupture of the cell and its organelles. We therefore performed a lactate dehydrogenase (LDH) cytotoxicity assay to measure membrane damage and LDH release. Compound **3e** in DMSO of different concentrations was tested. Even at concentrations up to 300  $\mu$ M, no significant induction of LDH release ( $<2\%$ ) was observed (Figure 5A). We further used flow cytometry to assess necrosis by staining the cells treated by **3e** with 7-aminoactinomycin (7-AAD), a membrane-impermeable dye that binds to DNA. As shown in Figure 5B, no significant fraction of necrosis cells ( $<2\%$ ) was observed following up to 12 h of treatment with **3e**. Collectively, these results demonstrate that **3e** does not act as a membrane destabilizing/disrupting agent and is devoid of any toxicity leading to cell membrane damage.

**Effect on DNA Synthesis.** Since **3e** is a nucleoside analogue and may mimic natural nucleosides, one of its possible mechanisms of action could therefore be to interfere in the pathways of DNA synthesis and, consequently, inhibit DNA synthesis and cell growth. Gemcitabine, the current first-line drug in the treatment of pancreatic cancer, is able to inhibit DNA replication and subsequently induce apoptosis.<sup>22</sup> In order to examine whether **3e** also exerts its antiproliferative activity by inhibiting DNA synthesis, the action of **3e** on the metabolism of nucleic acids was evaluated in MiaPaCa-2



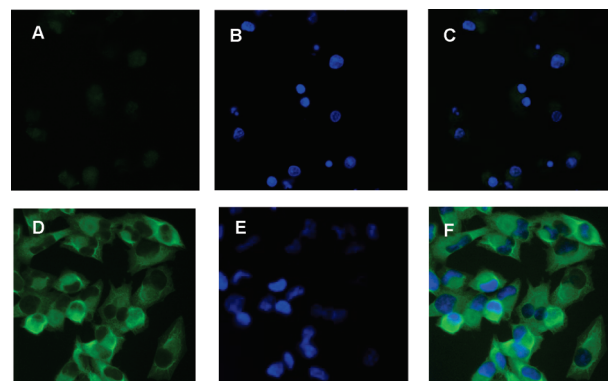


**Figure 6.** Inhibition of **3e** on DNA synthesis. Cells in exponential growth phase were incubated with gemcitabine and **3e**, respectively, for 4 h and then labeled with [<sup>3</sup>H]thymidine for 6 h. The incorporation of [<sup>3</sup>H]dTMP was determined as described in Experimental Section.



**Figure 7.** Effects of **3e** on Hsp27 mRNA (A) and protein (B) expression in MiaPaCa-2 cells. (A) MiaPaCa-2 cells were treated with **3e** for 24 h. Purified RNA was then analyzed by quantitative RT-PCR to monitor Hsp27 mRNA expression. Quantitative analysis of Hsp27 mRNA levels was normalized to 18S ribosomal mRNA levels in MiaPaCa-2 cells. (B) MiaPaCa-2 cells were treated with **3e**. At 48 h after the treatment, proteins were extracted from cultured cells and Hsp27 protein levels were analyzed by Western blotting using vinculin as reference. (C) Quantification of Western blotting analysis of Hsp27 protein expression with treatment of **3e** compared to no treatment as control.

cells by measuring the incorporation of [<sup>3</sup>H]thymidine into the DNA. As shown in Figure 6, no significant inhibition on DNA synthesis was observed with **3e**, whereas more than

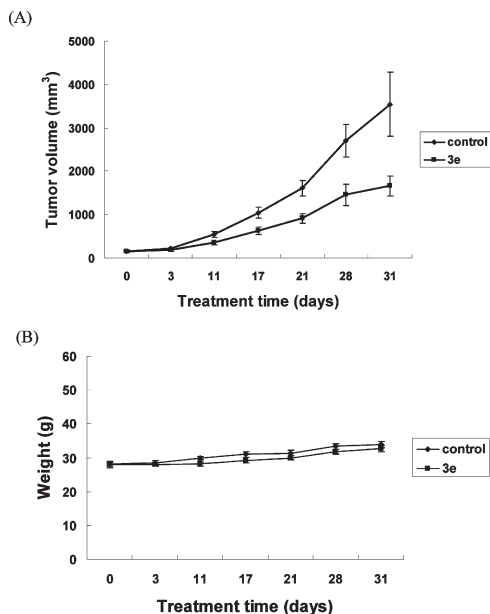


**Figure 8.** Immunofluorescence staining for Hsp27 protein in MiaPaCa-2 cells treated with **3e** (A–C) compared to untreated cells (D–F). Hsp27 protein was revealed by the antibody labeled with green fluorescent Alexa fluor 488 dye, and the nuclei were visualized by being stained with DAPI. All photomicrographs were taken with the same magnification: green channel image of cells treated with **3e** (A) and untreated cells (D); blue channel image of the nuclei of cells treated with **3e** (B) and untreated cells (E); (C) merged fluorescent images of (A) and (B); (F) merged fluorescent images of (D) and (E).

90% DNA inhibition was achieved with gemcitabine under the same conditions. This finding suggests different mechanisms of anticancer action between **3e** and gemcitabine.

**Effective Down-Regulation of Heat Shock Protein 27.** Since the identified active compound **3e** inhibits cell growth of drug-resistant pancreatic cancer MiaPaCa-2 cells, we reasoned that it may specifically target some apoptotic pathways related to drug resistance. As Hsp27 plays an important role in gemcitabine resistance in patients with pancreatic cancer,<sup>5</sup> we wondered whether **3e** exerted its anticancer activity by down-regulating Hsp27 expression. We then focused our attention on investigating the effect of **3e** on Hsp27 using quantitative real-time PCR and Western blotting analysis to assess the mRNA level and the protein level of Hsp27, respectively. Indeed, treatment with **3e** significantly down-regulated the level of Hsp27 mRNA expression by 80% after 24 h (Figure 7A) and similarly decreased the Hsp27 protein expression after 48 h (Figures 7B and Figure 7C). The efficient down-regulation of Hsp27 by **3e** was further revealed using immunofluorescence. As demonstrated in Figure 8, effective inhibition on Hsp27 expression after treatment with **3e** was revealed noticeably by the corresponding antibody labeled with green fluorescent Alexa fluor 488 (Figure 8A), compared to the nontreatment control (Figure 8D), and the resulting cell death was observed with cells treated with **3e** (Figures 8A–C) compared with untreated cells (Figures 8D–F). Overall, these results demonstrate the ability of **3e** to effectively inhibit the expression of Hsp27 and provide insight on the anticancer activity pathway of **3e** on drug-resistant pancreatic cancer MiaPaCa-2 cells. To our knowledge, **3e** is the first small molecule known to down-regulate Hsp27, leading to potent anticancer activity. It may thus constitute a new lead in the development of novel anticancer candidates, although the detailed mechanisms underlying the biological activity of **3e** require further investigation.

**In Vivo Tumor Growth Inhibition Assay.** We finally evaluated the antitumor effect of **3e** in nude mice bearing MiaPaCa-2-xenografted tumors (Figure 9). Each mouse was inoculated with 10<sup>7</sup> human pancreatic cancer MiaPaCa-2 cells on the shoulder and was allowed to grow for 7–10 days



**Figure 9.** (A) Effect of **3e** treatment on drug-resistant MiaPaCa-2 tumor growth in vivo. (B) Mice weight recording throughout the in vivo experiments. Nude mice bearing MiaPaCa-2 tumors of 100 mm<sup>3</sup> were randomly selected for treatment with **3e** and no treatment as control. **3e** was injected intraperitoneally (150 mg/kg/mice) every 3 days for 5 weeks, and tumor volume was measured once a week. All of the results were expressed as the mean  $\pm$  SE ( $n = 8$ ); points are the means values of analysis; bars are the standard errors.

to reach an average tumor volume of 100 mm<sup>3</sup>. The mice were then randomly selected for treatment with **3e** versus no treatment as a control. Compound **3e** was administrated intraperitoneally (ip) once every 3 days for 5 consecutive weeks. Tumor growth was measured every week and systemic toxicity evaluated by monitoring the weight loss of the mice throughout the in vivo experiments. Figure 9A shows that treatment with **3e** significantly reduced MiaPaCa-2 tumor volume by 50% after 3 weeks of treatment. In addition, no toxicity was observed, as demonstrated by the absence of weight loss (Figure 9B) and by the normal growth and behavior of all mice tested. These results clearly demonstrate the potent and efficacious antitumor activity of compound **3e** and the lack of adverse effects. This compound may therefore constitute a novel lead in the search for anticancer drug candidates with new mechanisms of action.

## Conclusion

On the basis of our earlier anticancer lead compound **1**, we further developed 3-ethynyltriazole ribonucleosides **2** and **3**, the structural isomers of 5-ethynyltriazole ribonucleosides previously synthesized in our group. The 3-ethynyltriazole ribonucleosides were prepared using a simple and efficient Sonogashira coupling reaction. Among the newly synthesized ribonucleosides, we have identified the lead compound **3e**, which displayed potent and effective anticancer activity in vitro and in vivo in the drug-resistant pancreatic cancer model MiaPaCa-2 with no adverse effects.

The preliminary structure–activity relationship study of **3e** demonstrated that both alkyl chain C<sub>5</sub>H<sub>11</sub> and the triple bond connection between the phenyl group and the triazole ring are critical for the anticancer activity. Despite the presence of a relative hydrophobic alkyl chain C<sub>5</sub>H<sub>11</sub> attached to the phenyl ring on the triazole nucleobase, no obvious membrane-desta-

bilizing activity or necrosis was observed for **3e**. We further confirmed that the caspase-dependent apoptosis induction represents the main mode of action for the anticancer effect of **3e**. Preliminary investigations on the mechanism of action revealed that **3e** did not inhibit DNA synthesis but significantly down-regulated Hsp27 expression. These observations collectively suggest that **3e** differs from gemcitabine and may have a novel mode of action involving the down-regulation of Hsp27 and consequent anticancer effect. It therefore is a novel anticancer lead, opening the door to new possibilities in the discovery of drugs targeting Hsp27.

To our knowledge, the arylethynyltriazole ribonucleoside **3e** represents the first small molecule found to down-regulate Hsp27 and induce potent caspase-dependent apoptosis with a resulting anticancer activity. This compound bears a simple and concise structural motif, which can be easily modulated for further structural optimization and the study of structure–activity relationships. It therefore emerges as a promising structural lead in the search for new anticancer candidates with novel mechanisms of action against pancreatic cancer. Future work is directed toward lead optimization with other types of triazole nucleosides and comprehensive investigations on structure–activity relationships as well as on the precise mechanisms of action related to Hsp27 down-regulation or other possible pathways.

## 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 <sup>1</sup>H NMR spectra were recorded at 300 or 600 MHz and the <sup>13</sup>C NMR spectra recorded at 75 or 150 MHz on Varian Mercury-VX300 and Varian Inova-600 spectrometers. 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 to be >95% by HPLC analysis using an analytical column of Hypersil C18 (ELITE) (4.6 mm  $\times$  250 mm) with an isocratic elution of CH<sub>3</sub>CN/H<sub>2</sub>O, 90/10 (method 1), and an analytical column of Nucleosil 120-5C8 (HICROM) (4.6 mm  $\times$  250 mm) with an isocratic elution of CH<sub>3</sub>CN/H<sub>2</sub>O, 90/10 (method 2).

**General Procedure for Preparing **2** via a Microwave Assisted Sonogashira Reaction.** 3-Bromo-1-[2,3,5-tri-*O*-acetyl- $\beta$ -D-ribofuranosyl]-1,2,4-triazole-5-carboxylate (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  $^{\circ}$ C for 30 min and then cooled to room temperature. The reaction mixture was concentrated under reduced pressure, and the crude residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate, 2:1). The purified material was dried in vacuo to afford the corresponding products **2a–t**.

**2a.** An amount of 74.7 mg (81%) of product was obtained, isolated as a colorless oil. HPLC:  $t = 6.62$  min, purity >99% (method 1);  $t = 6.65$  min, purity 98.8% (method 2). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  7.58–7.61 (m, 2H, phenyl-H), 7.37–7.42 (m, 3H, phenyl-H), 6.96 (d, 1H,  $J = 2.1$  Hz, H-1'), 5.86–5.89 (m, 1H, H-2'), 5.77–5.81 (m, 1H, H-3'), 4.45–4.49



(m, 2H, H-4' + H-5'), 4.17–4.22 (m, 1H, H-5'), 4.03 (s, 3H, –OCH<sub>3</sub>), 2.15 (s, 3H, –C(O)CH<sub>3</sub>), 2.13 (s, 3H, –C(O)CH<sub>3</sub>), 2.10 (s, 3H, –C(O)CH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 169.5, 168.5, 168.3, 156.4, 146.8, 143.9, 131.1, 128.6, 127.4, 120.0, 89.8, 88.5, 79.7, 78.0, 73.3, 69.7, 61.7, 52.5, 19.6, 19.4. Maldi-MS: *m/z* 508.1 [M + Na]<sup>+</sup>. HRMS: calcd for C<sub>23</sub>H<sub>23</sub>N<sub>3</sub>O<sub>9</sub>Na<sup>+</sup> 508.1326, found 508.1323. IR: 2230.8 cm<sup>−1</sup>.

**2b.** An amount of 77.2 mg (75%) of product was obtained, isolated as a colorless oil. HPLC: *t* = 6.62 min, purity >99% (method 1); *t* = 6.51 min, purity >99% (method 2). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.54 (d, 2H, *J* = 8.7 Hz, phenyl-H), 6.96 (d, 1H, *J* = 2.1 Hz, H-1'), 6.90 (d, 2H, *J* = 8.7 Hz, phenyl-H), 5.86–5.88 (m, 1H, H-2'), 5.78–5.82 (m, 1H, H-3'), 4.46–4.50 (m, 2H, H-4' + H-5'), 4.15–4.21 (m, 1H, H-5'), 4.03 (s, 3H, –OCH<sub>3</sub>), 3.84 (s, 3H, –OCH<sub>3</sub>), 2.16 (s, 3H, –C(O)CH<sub>3</sub>), 2.13 (s, 3H, –C(O)CH<sub>3</sub>), 2.10 (s, 3H, –C(O)CH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 171.2, 170.1, 170.0, 161.1, 158.1, 148.7, 145.3, 134.3, 114.7, 113.6, 91.7, 90.0, 81.2, 78.6, 74.9, 71.3, 63.3, 55.9, 54.1, 21.3, 21.1. Maldi-MS: *m/z* 576.1 [M + Na]<sup>+</sup>. HRMS: calcd for C<sub>24</sub>H<sub>22</sub>N<sub>3</sub>O<sub>9</sub>F<sub>3</sub>Na<sup>+</sup> 576.1200, found 576.1188. IR: 2232.2 cm<sup>−1</sup>.

**2c.** An amount of 62.9 mg (63%) of product was obtained, isolated as a colorless oil. HPLC: *t* = 6.72 min, purity >99% (method 1); *t* = 6.62 min, purity >99% (method 2). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.49 (d, 2H, *J* = 8.1 Hz, phenyl-H), 7.18 (d, 2H, *J* = 8.1 Hz, phenyl-H), 6.95 (d, 1H, *J* = 2.1 Hz, H-1'), 5.86–5.88 (m, 1H, H-2'), 5.77–5.81 (m, 1H, H-3'), 4.45–4.48 (m, 2H, H-4' + H-5'), 4.15–4.21 (m, 1H, H-5'), 4.03 (s, 3H, –OCH<sub>3</sub>), 2.38 (s, 3H, –CH<sub>3</sub>), 2.15 (s, 3H, –C(O)CH<sub>3</sub>), 2.13 (s, 3H, –C(O)CH<sub>3</sub>), 2.09 (s, 3H, –C(O)CH<sub>3</sub>). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>): δ 170.8, 169.7, 169.6, 157.7, 148.3, 145.1, 140.2, 132.3, 129.4, 118.2, 91.4, 89.7, 81.0, 78.8, 74.6, 71.0, 62.9, 53.7, 21.8, 20.9, 20.7. Maldi-MS: *m/z* 522.1 [M + Na]<sup>+</sup>. HRMS: calcd for C<sub>24</sub>H<sub>25</sub>N<sub>3</sub>O<sub>9</sub>Na<sup>+</sup> 522.1483, found 522.1481. IR: 2234.1 cm<sup>−1</sup>.

**2d.** An amount of 73.2 mg (69%) of product was obtained, isolated as a white solid. HPLC: *t* = 7.00 min, purity >98.3% (method 1); *t* = 6.88 min, purity >95.2% (method 2). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.51 (d, 2H, *J* = 8.1 Hz, phenyl-H), 7.19 (d, 2H, *J* = 8.1 Hz, phenyl-H), 6.96 (d, 1H, *J* = 1.5 Hz, H-1'), 5.86–5.89 (m, 1H, H-2'), 5.78–5.82 (m, 1H, H-3'), 4.46–4.49 (m, 2H, H-4' + H-5'), 4.16–4.21 (m, 1H, H-5'), 4.03 (s, 3H, –OCH<sub>3</sub>), 2.61 (t, 2H, *J* = 7.8 Hz, –CH<sub>2</sub>–), 2.16 (s, 3H, –C(O)CH<sub>3</sub>), 2.13 (s, 3H, –C(O)CH<sub>3</sub>), 2.10 (s, 3H, –C(O)CH<sub>3</sub>), 1.61–1.69 (m, 2H, –CH<sub>2</sub>–), 0.95 (t, 3H, *J* = 7.2 Hz, –CH<sub>3</sub>). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>): δ 170.6, 169.5, 169.4, 166.3, 157.5, 148.0, 144.8, 144.7, 132.0, 128.6, 118.2, 91.2, 89.4, 80.7, 78.5, 74.4, 70.7, 62.7, 53.5, 38.0, 24.2, 20.7, 20.5, 20.4, 13.7. Maldi-MS: *m/z* 550.2 [M + Na]<sup>+</sup>. HRMS: calcd for C<sub>26</sub>H<sub>29</sub>N<sub>3</sub>O<sub>9</sub>Na<sup>+</sup> 550.1796, found 550.1808. IR: 2229.4 cm<sup>−1</sup>.

**2e.** An amount of 81.0 mg (73%) of product was obtained, isolated as a colorless oil. HPLC: *t* = 7.49 min, purity 97.9% (method 1); *t* = 7.16 min, purity 96.5% (method 2). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.51 (d, 2H, *J* = 8.1 Hz, phenyl-H), 7.18 (d, 2H, *J* = 8.1 Hz, phenyl-H), 6.96 (d, 1H, *J* = 2.1 Hz, H-1'), 5.86–5.89 (m, 1H, H-2'), 5.78–5.81 (m, 1H, H-3'), 4.45–4.50 (m, 2H, H-4' + H-5'), 4.15–4.21 (m, 1H, H-5'), 4.03 (s, 3H, –OCH<sub>3</sub>), 2.62 (t, 2H, *J* = 7.7 Hz, –CH<sub>2</sub>–), 2.15 (s, 3H, –C(O)CH<sub>3</sub>), 2.13 (s, 3H, –C(O)CH<sub>3</sub>), 2.10 (s, 3H, –C(O)CH<sub>3</sub>), 1.60–1.67 (m, 2H, –CH<sub>2</sub>–), 1.31–1.38 (m, 4H, –CH<sub>2</sub>–), 0.90 (t, 3H, *J* = 6.6 Hz, –CH<sub>3</sub>). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>): δ 170.8, 169.7, 169.6, 157.8, 148.3, 145.2, 145.1, 132.3, 128.8, 118.4, 91.5, 89.7, 81.0, 78.7, 74.6, 71.0, 62.9, 53.7, 36.2, 31.6, 31.0, 22.7, 20.9, 20.7, 14.2. Maldi-MS: *m/z* 578.2 [M + Na]<sup>+</sup>. HRMS: calcd for C<sub>28</sub>H<sub>33</sub>N<sub>3</sub>O<sub>9</sub>Na<sup>+</sup> 578.2109, found 578.2101. IR: 2234.4 cm<sup>−1</sup>.

**2f.** An amount of 50.9 mg (49%) of product was obtained, isolated as a yellow solid. HPLC: *t* = 6.81 min, purity 97.2% (method 1); *t* = 6.54 min, purity 95.2% (method 2). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.53 (d, 2H, *J* = 8.1 Hz, phenyl-H), 7.36

(d, 2H, *J* = 8.1 Hz, phenyl-H), 6.97 (d, 1H, *J* = 2.1 Hz, H-1'), 5.84–5.87 (m, 1H, H-2'), 5.77–5.81 (m, 1H, H-3'), 4.44–4.48 (m, 2H, H-4' + H-5'), 4.15–4.21 (m, 1H, H-5'), 4.04 (s, 3H, –OCH<sub>3</sub>), 2.16 (s, 3H, –C(O)CH<sub>3</sub>), 2.14 (s, 3H, –C(O)CH<sub>3</sub>), 2.09 (s, 3H, –C(O)CH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 170.8, 169.8, 169.7, 157.7, 147.9, 145.2, 136.1, 133.6, 129.1, 119.8, 89.9, 89.8, 80.9, 80.2, 74.6, 71.0, 63.1, 53.8, 20.9, 20.8. Maldi-MS: *m/z* 542.1 [M + Na]<sup>+</sup>. HRMS: calcd for C<sub>23</sub>H<sub>22</sub>N<sub>3</sub>O<sub>9</sub>CiNa<sup>+</sup> 542.0937, found 542.0924. IR: 2230.6 cm<sup>−1</sup>.

**2g.** An amount of 73.4 mg (73%) of product was obtained, isolated as a colorless oil. HPLC: *t* = 6.61 min, purity >99% (method 1); *t* = 6.55 min, purity 98.6% (method 2). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.57–7.61 (m, 2H, phenyl-H), 7.08 (dd, 2H, *J*<sub>HH</sub> = *J*<sub>HF</sub> = 8.7 Hz, phenyl-H), 6.96 (d, 1H, *J* = 2.1 Hz, H-1'), 5.85–5.87 (m, 1H, H-2'), 5.77–5.80 (m, 1H, H-3'), 4.44–4.49 (m, 2H, H-4' + H-5'), 4.15–4.21 (m, 1H, H-5'), 4.03 (s, 3H, –OCH<sub>3</sub>), 2.15 (s, 3H, –C(O)CH<sub>3</sub>), 2.13 (s, 3H, –C(O)CH<sub>3</sub>), 2.09 (s, 3H, –C(O)CH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 170.8, 169.8, 169.6, 163.5 (d, <sup>1</sup>*J*<sub>CF</sub> = 250.5 Hz), 157.7, 148.0, 145.2, 134.4 (d, <sup>3</sup>*J*<sub>CF</sub> = 9.0 Hz), 117.4, 116.2 (d, <sup>2</sup>*J*<sub>CF</sub> = 21.4 Hz), 90.0, 89.8, 80.9, 79.1, 74.6, 71.0, 63.0, 53.8, 20.9, 20.7. Maldi-MS: *m/z* 526.1 [M + Na]<sup>+</sup>. HRMS: calcd for C<sub>23</sub>H<sub>22</sub>N<sub>3</sub>O<sub>9</sub>FNa<sup>+</sup> 526.1232, found 526.1222. IR: 2239.7 cm<sup>−1</sup>.

**2h.** An amount of 64.4 mg (64%) of product was obtained, isolated as a colorless oil. HPLC: *t* = 6.60 min, purity 99.0% (method 1); *t* = 6.54 min, purity 95.8% (method 2). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.30–7.40 (m, 3H, phenyl-H), 7.09–7.15 (m, 1H, phenyl-H), 6.96 (s, 1H, H-1'), 5.85–5.87 (m, 1H, H-2'), 5.76–5.80 (m, 1H, H-3'), 4.44–4.49 (m, 2H, H-4' + H-5'), 4.15–4.21 (m, 1H, H-5'), 4.04 (s, 3H, –OCH<sub>3</sub>), 2.15 (s, 3H, –C(O)CH<sub>3</sub>), 2.13 (s, 3H, –C(O)CH<sub>3</sub>), 2.09 (s, 3H, –C(O)CH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 170.8, 169.8, 169.6, 162.5 (d, <sup>1</sup>*J*<sub>CF</sub> = 246.0 Hz), 157.7, 147.8, 145.3, 130.4 (d, <sup>3</sup>*J*<sub>CF</sub> = 7.8 Hz), 128.3, 123.2, 119.1 (d, <sup>2</sup>*J*<sub>CF</sub> = 23.6 Hz), 117.3 (d, <sup>2</sup>*J*<sub>CF</sub> = 20.2 Hz), 89.8, 89.6, 81.0, 80.0, 74.6, 71.0, 63.0, 53.8, 20.9, 20.7. Maldi-MS: *m/z* 526.1 [M + Na]<sup>+</sup>. HRMS: calcd for C<sub>23</sub>H<sub>22</sub>N<sub>3</sub>O<sub>9</sub>FNa<sup>+</sup> 526.1232, found 526.1223. IR: 2240.8 cm<sup>−1</sup>.

**2i.** An amount of 64.4 mg (64%) of product was obtained, isolated as a pink solid. HPLC: *t* = 6.63 min, purity >99% (method 1); *t* = 6.61 min, purity 95.6% (method 2). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.58 (dd, 1H, *J*<sub>HH</sub> = *J*<sub>HF</sub> = 7.8 Hz, phenyl-H), 7.39–7.41 (m, 1H, phenyl-H), 7.09–7.18 (m, 2H, phenyl-H), 6.96 (d, 1H, *J* = 2.4 Hz, H-1'), 5.87–5.89 (m, 1H, H-2'), 5.76–5.80 (m, 1H, H-3'), 4.46–4.50 (m, 2H, H-4' + H-5'), 4.15–4.21 (m, 1H, H-5'), 4.04 (s, 3H, –OCH<sub>3</sub>), 2.15 (s, 3H, –C(O)CH<sub>3</sub>), 2.13 (s, 3H, –C(O)CH<sub>3</sub>), 2.11 (s, 3H, –C(O)CH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 170.9, 169.7, 169.6, 163.3 (d, <sup>1</sup>*J*<sub>CF</sub> = 252.8 Hz), 157.7, 147.8, 145.3, 134.2, 131.8 (d, <sup>3</sup>*J*<sub>CF</sub> = 7.9 Hz), 124.4 (d, <sup>4</sup>*J*<sub>CF</sub> = 3.4 Hz), 116.0 (d, <sup>2</sup>*J*<sub>CF</sub> = 20.3 Hz), 110.1 (d, <sup>2</sup>*J*<sub>CF</sub> = 14.6 Hz), 89.8, 84.6, 84.0, 81.1, 74.6, 71.0, 62.7, 53.8, 20.9, 20.7. Maldi-MS: *m/z* 526.1 [M + Na]<sup>+</sup>. HRMS: calcd for C<sub>23</sub>H<sub>22</sub>N<sub>3</sub>O<sub>9</sub>FNa<sup>+</sup> 526.1232, found 526.1227. IR: 2237.8 cm<sup>−1</sup>.

**2j.** An amount of 64.1 mg (58%) of product was obtained, isolated as a colorless oil. HPLC: *t* = 6.75 min, purity >99% (method 1); *t* = 6.63 min, purity 96.9% (method 2). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.63–7.73 (m, 4H, phenyl-H), 6.97 (s, 1H, H-1'), 5.85–5.86 (m, 1H, H-2'), 5.76–5.80 (m, 1H, H-3'), 4.45–4.48 (m, 2H, H-4' + H-5'), 4.15–4.21 (m, 1H, H-5'), 4.04 (s, 3H, –OCH<sub>3</sub>), 2.15 (s, 3H, –C(O)CH<sub>3</sub>), 2.13 (s, 3H, –C(O)CH<sub>3</sub>), 2.08 (s, 3H, –C(O)CH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 170.7, 169.8, 169.6, 157.6, 147.6, 145.3, 132.6, 131.2, 125.7, 125.1, 121.4 (q, <sup>1</sup>*J*<sub>CF</sub> = 272 Hz), 89.9, 89.3, 81.4, 81.0, 74.6, 71.0, 63.0, 53.8, 20.9, 20.7. Maldi-MS: *m/z* 576.1 [M + Na]<sup>+</sup>. HRMS: calcd for C<sub>24</sub>H<sub>22</sub>N<sub>3</sub>O<sub>9</sub>F<sub>3</sub>Na<sup>+</sup> 576.1200, found 576.1188. IR: 2235.8 cm<sup>−1</sup>.

**2k.** An amount of 70.8 mg (64%) of product was obtained, isolated as a colorless oil. HPLC: *t* = 6.75 min, purity 96.0% (method 1); *t* = 6.62 min, purity 95.3% (method 2). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.88 (s, 1H, phenyl-H), 7.76–7.79 (m, 1H, phenyl-H), 7.66–7.68 (m, 1H, phenyl-H), 7.49–7.55 (m, 1H,

phenyl-H), 6.97 (d, 1H,  $J = 1.5$  Hz, H-1'), 5.85–5.87 (m, 1H, H-2'), 5.77–5.81 (m, 1H, H-3'), 4.44–4.49 (m, 2H, H-4' + H-5'), 4.16–4.22 (m, 1H, H-5'), 4.04 (s, 3H,  $-\text{OCH}_3$ ), 2.16 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.14 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.09 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.7, 169.8, 169.6, 157.6, 147.6, 145.3, 135.4, 131.4 (q,  $^2J_{\text{CF}} = 32.9$  Hz), 129.3, 129.1, 126.4, 123.7 (q,  $^1J_{\text{CF}} = 271$  Hz), 122.3, 89.8, 89.2, 80.9, 80.6, 74.6, 70.9, 63.0, 53.8, 20.9, 20.7. Maldi-MS:  $m/z$  576.1  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{24}\text{H}_{22}\text{N}_3\text{O}_9\text{F}_3\text{Na}^+$  576.1200, found 576.1187. IR: 2235.7  $\text{cm}^{-1}$ .

**2l.** An amount of 60.8 mg (55%) of product was obtained, isolated as a colorless oil. HPLC:  $t = 6.70$  min, purity >99% (method 1);  $t = 6.60$  min, purity 96.9% (method 2).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.70–7.78 (m, 2H, phenyl-H), 7.51–7.58 (m, 2H, phenyl-H), 6.97 (d, 1H,  $J = 2.1$  Hz, H-1'), 5.88–5.91 (m, 1H, H-2'), 5.75–5.79 (m, 1H, H-3'), 4.46–4.51 (m, 2H, H-4' + H-5'), 4.16–4.21 (m, 1H, H-5'), 4.04 (s, 3H,  $-\text{OCH}_3$ ), 2.15 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.14 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.10 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.8, 169.7, 169.6, 157.6, 147.6, 145.4, 135.0, 132.1 (q,  $^2J_{\text{CF}} = 30.9$  Hz), 131.8, 129.7, 126.2, 123.4 (q,  $^1J_{\text{CF}} = 272$  Hz), 119.4, 89.7, 86.4, 84.2, 81.1, 74.5, 70.8, 62.5, 53.8, 20.8, 20.6. Maldi-MS:  $m/z$  576.1  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{24}\text{H}_{22}\text{N}_3\text{O}_9\text{F}_3\text{Na}^+$  576.1200, found 576.1190. IR: 2233.8  $\text{cm}^{-1}$ .

**2m.** An amount of 71.7 mg (73%) of product was obtained, isolated as a colorless oil. HPLC:  $t = 6.66$  min, purity >99% (method 1);  $t = 6.52$  min, purity 96.3% (method 2).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.38–7.43 (m, 2H, thiophenyl-H), 7.03–7.06 (m, 1H, thiophenyl-H), 6.96 (d, 1H,  $J = 2.1$  Hz, H-1'), 5.85–5.87 (m, 1H, H-2'), 5.76–5.80 (m, 1H, H-3'), 4.45–4.48 (m, 2H, H-4' + H-5'), 4.15–4.21 (m, 1H, H-5'), 4.03 (s, 3H,  $-\text{OCH}_3$ ), 2.16 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.13 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.10 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.9, 169.8, 169.6, 157.7, 148.0, 145.2, 134.4, 129.4, 127.5, 121.1, 89.8, 84.8, 83.0, 81.0, 74.6, 71.0, 63.0, 53.8, 20.9, 20.7. Maldi-MS:  $m/z$  514.1  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{21}\text{H}_{21}\text{N}_3\text{O}_9\text{SNa}^+$  514.0890, found 514.0889. IR: 2228.5  $\text{cm}^{-1}$ .

**2n.** An amount of 51.1 mg (52%) of product was obtained, isolated as a colorless oil. HPLC:  $t = 6.61$  min, purity >99% (method 1);  $t = 6.53$  min, purity >99% (method 2).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.67–7.68 (m, 1H, thiophenyl-H), 7.32–7.34 (m, 1H, thiophenyl-H), 7.24–7.27 (m, 1H, thiophenyl-H), 6.96 (d, 1H,  $J = 1.5$  Hz, H-1'), 5.85–5.87 (m, 1H, H-2'), 5.77–5.81 (m, 1H, H-3'), 4.45–4.49 (m, 2H, H-4' + H-5'), 4.14–4.21 (m, 1H, H-5'), 4.03 (s, 3H,  $-\text{OCH}_3$ ), 2.16 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.13 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.09 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.8, 169.8, 169.6, 157.7, 148.1, 145.1, 131.5, 130.1, 126.0, 120.5, 89.7, 86.5, 80.9, 79.0, 74.6, 71.0, 63.0, 53.8, 20.9, 20.7. Maldi-MS:  $m/z$  514.1  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{21}\text{H}_{21}\text{N}_3\text{O}_9\text{SNa}^+$  514.0890, found 514.0876. IR: 2228.8  $\text{cm}^{-1}$ .

**2o.** An amount of 59.6 mg (61%) of product was obtained, isolated as a colorless oil. HPLC:  $t = 7.60$  min, purity 98.0% (method 1);  $t = 7.15$  min, purity 98.1% (method 2).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.53 (s, 1H, imidazole-H), 7.49 (s, 1H, imidazole-H), 6.97 (d, 1H,  $J = 1.2$  Hz, H-1'), 5.84–5.86 (m, 1H, H-2'), 5.74–5.78 (m, 1H, H-3'), 4.44–4.49 (m, 2H, H-4' + H-5'), 4.15–4.21 (m, 1H, H-5'), 4.04 (s, 3H,  $-\text{OCH}_3$ ), 3.77 (s, 3H,  $-\text{NCH}_3$ ), 2.16 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.14 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.08 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.7, 169.8, 169.6, 157.6, 147.7, 145.3, 139.4, 137.2, 114.7, 89.8, 86.7, 80.9, 79.3, 74.6, 70.9, 63.0, 53.8, 32.6, 20.9, 20.71, 20.67. Maldi-MS:  $m/z$  490.2  $[\text{M} + \text{H}]^+$ . HRMS: calcd for  $\text{C}_{21}\text{H}_{24}\text{N}_5\text{O}_9^+$  490.1567, found 490.1548. IR: 2228.4  $\text{cm}^{-1}$ .

**2p.** An amount of 60.6 mg (62%) of product was obtained, isolated as a colorless oil. HPLC:  $t = 6.80$  min, purity 96.5% (method 1);  $t = 6.54$  min, purity 98.3% (method 2).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.92 (s, 1H, H-1'), 6.37 (s, 1H, cyclohexenyl-H), 5.82–5.85 (m, 1H, H-2'), 5.74–5.78 (m, 1H, H-3'), 4.43–4.47 (m, 2H, H-4' + H-5'), 4.14–4.18 (m, 1H, H-5'),

4.01 (s, 3H,  $-\text{OCH}_3$ ), 2.05–2.21 (m, 13H, cyclohexane-H +  $-\text{C}(\text{O})\text{CH}_3$ ), 1.60–1.67 (m, 4H, cyclohexane-H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.6, 169.5, 169.3, 157.6, 148.4, 144.8, 138.8, 119.4, 92.9, 89.4, 80.8, 74.5, 70.9, 62.8, 53.4, 28.4, 25.8, 22.1, 20.7, 20.5, 20.4. Maldi-MS:  $m/z$  512.2  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}_9\text{Na}^+$  512.1639, found 512.1631. IR: 2219.9  $\text{cm}^{-1}$ .

**2q.** An amount of 72.7 mg (75%) of product was obtained, isolated as a colorless oil. HPLC:  $t = 6.58$  min, purity 97.1% (method 1);  $t = 6.51$  min, purity >99% (method 2).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.91 (s, 1H, H-1'), 5.80–5.82 (m, 1H, H-2'), 5.72–5.76 (m, 1H, H-3'), 4.43–4.46 (m, 2H, H-4' + H-5'), 4.13–4.19 (m, 1H, H-5'), 4.01 (s, 3H,  $-\text{OCH}_3$ ), 3.70 (t, 2H,  $J = 6.3$  Hz,  $-\text{CH}_2-$ ), 2.64 (t, 2H,  $J = 6.5$  Hz,  $-\text{CH}_2-$ ), 2.14 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.12 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.07 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.7, 169.7, 169.5, 157.6, 147.9, 144.9, 91.1, 89.6, 80.8, 74.5, 71.8, 70.9, 63.0, 53.7, 43.6, 30.8, 20.8, 20.6, 16.7. Maldi-MS:  $m/z$  508.1  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{20}\text{H}_{24}\text{N}_3\text{O}_9\text{ClNa}^+$  508.1093, found 508.1088. IR: 2227.8  $\text{cm}^{-1}$ .

**2r.** An amount of 62.6 mg (65%) of product was obtained, isolated as a colorless oil. HPLC:  $t = 6.81$  min, purity >99% (method 1);  $t = 6.70$  min, purity 95.6% (method 2).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.91 (s, 1H, H-1'), 5.82–5.83 (m, 1H, H-2'), 5.74–5.78 (m, 1H, H-3'), 4.43–4.46 (m, 2H, H-4' + H-5'), 4.12–4.18 (m, 1H, H-5'), 4.01 (s, 3H,  $-\text{OCH}_3$ ), 2.41 (t, 2H,  $J = 7.1$  Hz,  $-\text{CH}_2-$ ), 2.14 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.12 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.08 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 1.57–1.67 (m, 4H,  $-\text{CH}_2-$ ), 1.30–1.47 (m, 4H,  $-\text{CH}_2-$ ), 0.91 (t, 3H,  $J = 7.1$  Hz,  $-\text{CH}_3$ ).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.5, 169.4, 169.2, 157.4, 147.9, 144.5, 93.1, 89.2, 80.5, 74.2, 70.6, 62.6, 53.3, 30.9, 27.5, 22.0, 20.6, 20.4, 20.3, 18.9, 13.8. Maldi-MS:  $m/z$  512.2  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{22}\text{H}_{29}\text{N}_3\text{O}_9\text{Na}^+$  502.1796, found 502.1809. IR: 2249.8  $\text{cm}^{-1}$ .

**2s.** An amount of 77.9 mg (79%) of product was obtained, isolated as a colorless oil. HPLC:  $t = 6.61$  min, purity >99% (method 1);  $t = 6.55$  min, purity >99% (method 2).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.92 (d, 1H,  $J = 2.4$  Hz, H-1'), 5.80–5.83 (m, 1H, H-2'), 5.72–5.76 (m, 1H, H-3'), 4.43–4.46 (m, 2H, H-4' + H-5'), 4.12–4.19 (m, 1H, H-5'), 4.01 (s, 3H,  $-\text{OCH}_3$ ), 2.47 (br, 1H,  $-\text{OH}$ ), 2.14 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.12 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.08 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 1.99–2.05 (m, 4H, cyclopentany-H), 1.75–1.87 (m, 4H, cyclopentany-H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.9, 169.8, 169.6, 157.6, 147.7, 145.1, 95.5, 89.7, 80.9, 74.6, 74.4, 73.2, 71.0, 63.0, 53.8, 42.3, 23.7, 20.9, 20.7. Maldi-MS:  $m/z$  516.2  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{22}\text{H}_{27}\text{N}_3\text{O}_{10}\text{Na}^+$  516.1589, found 516.1581. IR: 2242.8  $\text{cm}^{-1}$ .

**2t.** An amount of 80.1 mg (79%) of product was obtained, isolated as a colorless oil. HPLC:  $t = 6.62$  min, purity >99% (method 1);  $t = 6.39$  min, purity 95.3% (method 2).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.93 (d, 1H,  $J = 1.5$  Hz, H-1'), 5.80–5.83 (m, 1H, H-2'), 5.74–5.77 (m, 1H, H-3'), 4.43–4.47 (m, 2H, H-4' + H-5'), 4.13–4.19 (m, 1H, H-5'), 4.02 (s, 3H,  $-\text{OCH}_3$ ), 2.24 (br, 1H,  $-\text{OH}$ ), 2.14 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.12 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.08 (s, 3H,  $-\text{C}(\text{O})\text{CH}_3$ ), 2.02–2.05 (m, 2H, cyclohexany-H), 1.65–1.78 (m, 8H, cyclohexane-H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.9, 169.8, 169.6, 157.6, 147.7, 145.0, 95.4, 89.6, 80.9, 74.5, 71.0, 68.8, 63.0, 62.9, 53.8, 42.3, 39.5, 25.2, 23.7, 23.2, 20.9, 20.7. Maldi-MS:  $m/z$  530.2  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{23}\text{H}_{29}\text{N}_3\text{O}_{10}\text{Na}^+$  530.1745, found 530.1740. IR: 2236.7  $\text{cm}^{-1}$ .

**General Procedure for Preparing 3. 2** was dissolved in 10 mL of saturated  $\text{NH}_3/\text{MeOH}$  and stirred at room temperature for 2 days. Then the solvent was removed and the residue was washed with  $\text{CH}_2\text{Cl}_2$ . The washed residual was dried in vacuo to afford the corresponding products **3a–t**.

**3a.** An amount of 87.8 mg (0.18 mmol) of **2a** was used for the reaction. An amount of 50.4 mg (81%) of product was obtained, isolated as a white solid. HPLC:  $t = 6.57$  min, purity 97.2% (method 1);  $t = 6.21$  min, purity >99% (method 2).  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  8.51 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.18 (br s,



1H, -C(O)NH, 7.64–7.67 (m, 2H, phenyl-H), 7.48–7.53 (m, 3H, phenyl-H), 6.77 (d, 1H,  $J = 3.0$  Hz, H-1'), 5.51 (d, 1H,  $J = 5.1$  Hz, -OH), 5.20 (d, 1H,  $J = 6.0$  Hz, -OH), 4.78 (t, 1H,  $J = 6.0$  Hz, -OH), 4.40–4.44 (m, 1H, H-2'), 4.19–4.23 (m, 1H, H-3'), 3.90–3.92 (m, 1H, H-4'), 3.41–3.57 (m, 2H, H-5').  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  161.3, 158.5, 149.0, 145.8, 132.5, 130.8, 129.7, 120.9, 91.4, 90.1, 86.1, 80.7, 74.9, 71.3, 62.7. Maldi-MS:  $m/z$  367.1 [M + Na] $^+$ . HRMS: calcd for  $\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_5\text{Na}^+$  367.1013, found 367.1017. IR: 2228.4  $\text{cm}^{-1}$ .

**3b.** An amount of 93.8 mg (0.18 mmol) of **2b** was used for the reaction. An amount of 48.3 mg (72%) of product was obtained, isolated as a white solid. HPLC:  $t = 6.68$  min, purity 98.6% (method 1);  $t = 6.27$  min, purity 96.3% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.44 (br s, 1H, -C(O)NH), 8.12 (br s, 1H, -C(O)NH), 7.57 (d, 2H,  $J = 8.4$  Hz, phenyl-H), 7.01 (d, 2H,  $J = 9.0$  Hz, phenyl-H), 6.74 (d, 1H,  $J = 2.7$  Hz, H-1'), 5.47 (d, 1H,  $J = 5.7$  Hz, -OH), 5.15 (d, 1H,  $J = 6.3$  Hz, -OH), 4.74 (t, 1H,  $J = 5.7$  Hz, -OH), 4.37–4.42 (m, 1H, H-2'), 4.19–4.22 (m, 1H, H-3'), 3.88–3.91 (m, 1H, H-4'), 3.80 (s, 3H, -OCH $_3$ ), 3.37–3.57 (m, 2H, H-5').  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  163.3, 160.9, 150.7, 148.4, 136.4, 117.5, 114.5, 93.5, 93.1, 87.8, 81.2, 76.9, 73.2, 64.6, 58.1. Maldi-MS:  $m/z$  397.1 [M + Na] $^+$ . HRMS: calcd for  $\text{C}_{17}\text{H}_{18}\text{N}_4\text{O}_6\text{Na}^+$  397.1119, found 397.1130. IR: 2233.5  $\text{cm}^{-1}$ .

**3c.** An amount of 75.7 mg (0.15 mmol) of **2c** was used for the reaction. An amount of 42.4 mg (78%) of product was obtained, isolated as a white solid. HPLC:  $t = 6.82$  min, purity 98.5% (method 1);  $t = 6.31$  min, purity >99% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.49 (br s, 1H, -C(O)NH), 8.16 (br s, 1H, -C(O)NH), 7.54 (d, 2H,  $J = 7.8$  Hz, phenyl-H), 7.29 (d, 2H,  $J = 7.8$  Hz, phenyl-H), 6.75 (d, 1H,  $J = 3.0$  Hz, H-1'), 5.51 (d, 1H,  $J = 5.4$  Hz, -OH), 5.19 (d, 1H,  $J = 5.4$  Hz, -OH), 4.77 (t, 1H,  $J = 5.4$  Hz, -OH), 4.41–4.42 (m, 1H, H-2'), 4.19–4.22 (m, 1H, H-3'), 3.90–3.91 (m, 1H, H-4'), 3.41–3.57 (m, 2H, H-5'), 2.36 (s, 3H, -OCH $_3$ ).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  158.6, 148.9, 146.0, 140.8, 132.4, 130.3, 117.9, 91.4, 90.3, 86.1, 80.2, 74.9, 71.3, 62.7, 21.8. Maldi-MS:  $m/z$  381.1 [M + Na] $^+$ . HRMS: calcd for  $\text{C}_{17}\text{H}_{18}\text{N}_4\text{O}_5\text{Na}^+$  381.1169, found 381.1177. IR: 2230.6  $\text{cm}^{-1}$ .

**3d.** An amount of 62.7 mg (0.12 mmol) of **2d** was used for the reaction. An amount of 38.2 mg (83%) of product was obtained, isolated as a white solid. HPLC:  $t = 7.04$  min, purity 96.4% (method 1);  $t = 6.51$  min, purity 97.7% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.51 (br s, 1H, -C(O)NH), 8.18 (br s, 1H, -C(O)NH), 7.56 (d, 2H,  $J = 7.8$  Hz, phenyl-H), 7.31 (d, 2H,  $J = 7.8$  Hz, phenyl-H), 6.78 (d, 1H,  $J = 3.9$  Hz, H-1'), 5.56 (d, 1H,  $J = 5.7$  Hz, -OH), 5.24 (d, 1H,  $J = 4.8$  Hz, -OH), 4.84 (t, 1H,  $J = 5.4$  Hz, -OH), 4.42–4.46 (m, 1H, H-2'), 4.21–4.26 (m, 1H, H-3'), 3.90–3.95 (m, 1H, H-4'), 3.55–3.63 (m, 2H, H-5'), 2.61 (t, 2H,  $J = 7.4$  Hz, -CH $_2$ -), 1.57–1.64 (m, 2H, -CH $_2$ -), 0.90 (t, 3H,  $J = 7.4$  Hz, -CH $_3$ ).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  157.3, 147.7, 144.8, 144.1, 131.2, 131.2, 128.5, 128.4, 117.0, 90.2, 89.1, 85.0, 84.9, 79.0, 73.7, 73.6, 70.1, 61.5, 36.6, 23.2, 13.05, 12.96. Maldi-MS:  $m/z$  409.1 [M + Na] $^+$ . HRMS: calcd for  $\text{C}_{19}\text{H}_{22}\text{N}_4\text{O}_5\text{Na}^+$  409.1482, found 409.1485. IR: 2231.0  $\text{cm}^{-1}$ .

**3e.** An amount of 110.1 mg (0.20 mmol) of **2e** was used for the reaction. An amount of 58.3 mg (71%) of product was obtained, isolated as a yellow solid. HPLC:  $t = 7.20$  min, purity >99% (method 1);  $t = 6.83$  min, purity >99% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.50 (br s, 1H, -C(O)NH), 8.17 (br s, 1H, -C(O)NH), 7.55 (d, 1H,  $J = 7.8$  Hz, phenyl-H), 7.30 (d, 1H,  $J = 8.1$  Hz, phenyl-H), 6.76 (d, 1H,  $J = 3.0$  Hz, H-1'), 5.51 (d, 1H,  $J = 5.1$  Hz, -OH), 5.19 (d, 1H,  $J = 5.7$  Hz, -OH), 4.78 (t, 1H,  $J = 5.6$  Hz, -OH), 4.40–4.45 (m, 1H, H-2'), 4.19–4.24 (m, 1H, H-3'), 3.89–3.94 (m, 1H, H-4'), 3.42–3.59 (m, 2H, H-5'), 2.62 (t, 2H,  $J = 7.2$  Hz, -CH $_2$ -), 1.56–1.61 (m, 2H, -CH $_2$ -), 1.25–1.35 (m, 4H, -CH $_2$ -), 0.86 (t, 3H,  $J = 6.9$  Hz, -CH $_3$ ).  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  158.6, 148.9, 146.0, 145.6, 132.5, 129.6, 118.2, 91.4, 90.3, 86.1, 80.2, 74.9, 71.3, 62.7, 35.7, 31.5, 31.0, 22.6, 14.6. Maldi-MS:  $m/z$  437.2 [M + Na] $^+$ . HRMS:

calcd for  $\text{C}_{21}\text{H}_{26}\text{N}_4\text{O}_5\text{Na}^+$  437.1795, found 437.1804. IR: 2228.0  $\text{cm}^{-1}$ .

**3f.** An amount of 60.0 mg (0.12 mmol) of **2f** was used for the reaction. An amount of 33.6 mg (77%) of product was obtained, isolated as a yellow solid. HPLC:  $t = 6.81$  min, purity 97.1% (method 1);  $t = 6.32$  min, purity 98.4% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.48 (br s, 1H, -C(O)NH), 8.17 (br s, 1H, -C(O)NH), 7.68 (d, 2H,  $J = 8.1$  Hz, phenyl-H), 7.55 (d, 2H,  $J = 8.1$  Hz, phenyl-H), 6.76 (d, 1H,  $J = 3.6$  Hz, H-1'), 5.52 (d, 1H,  $J = 6.0$  Hz, OH), 5.19 (d, 1H,  $J = 6.0$  Hz, OH), 4.78 (t, 1H,  $J = 5.9$  Hz, OH), 4.40–4.45 (m, 1H, H-2'), 4.19–4.23 (m, 1H, H-3'), 3.90–3.92 (m, 1H, H-4'), 3.40–3.59 (m, 2H, H-5').  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  163.3, 153.8, 150.4, 140.4, 139.0, 134.6, 124.6, 96.2, 93.7, 90.9, 86.4, 79.7, 76.0, 67.5. Maldi-MS:  $m/z$  401.1 [M + Na] $^+$ . HRMS: calcd for  $\text{C}_{16}\text{H}_{15}\text{N}_4\text{O}_5\text{ClNa}^+$  401.0623, found 401.0625. IR: 2234.9  $\text{cm}^{-1}$ .

**3g.** An amount of 86.4 mg (0.17 mmol) of **2g** was used for the reaction. An amount of 49.0 mg (80%) of product was obtained, isolated as a yellow solid. HPLC:  $t = 6.66$  min, purity 98.6% (method 1);  $t = 6.16$  min, purity 98.2% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.48 (br s, 1H, -C(O)NH), 8.16 (br s, 1H, -C(O)NH), 7.69–7.74 (m, 2H, phenyl-H), 7.32 (dd, 2H,  $J_{\text{HH}} = J_{\text{HF}} = 9.2$  Hz, phenyl-H), 6.74 (d, 1H,  $J = 3.0$  Hz, H-1'), 5.51 (d, 1H,  $J = 5.1$  Hz, -OH), 5.19 (d, 1H,  $J = 5.7$  Hz, -OH), 4.77 (t, 1H,  $J = 5.4$  Hz, -OH), 4.38–4.42 (m, 1H, H-2'), 4.17–4.22 (m, 1H, H-3'), 3.86–3.91 (m, 1H, H-4'), 3.38–3.57 (m, 2H, H-5').  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  163.5 (d,  $^1J_{\text{CF}} = 255$  Hz), 158.5, 149.0, 145.8, 135.1 (d,  $^3J_{\text{CF}} = 8.55$  Hz), 117.3, 117.1 (d,  $^2J_{\text{CF}} = 21.3$  Hz), 91.4, 89.1, 86.1, 80.4, 74.9, 71.3, 62.7. Maldi-MS:  $m/z$  385.1 [M + Na] $^+$ . HRMS: calcd for  $\text{C}_{16}\text{H}_{15}\text{N}_4\text{O}_5\text{FNa}^+$  385.0919, found 385.0923. IR: 2233.1  $\text{cm}^{-1}$ .

**3h.** An amount of 66.1 mg (0.14 mmol) of **2h** was used for the reaction. An amount of 36.3 mg (77%) of product was obtained, isolated as a yellow solid. HPLC:  $t = 6.64$  min, purity 96.6% (method 1);  $t = 6.19$  min, purity 96.5% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.49 (br s, 1H, -C(O)NH), 8.17 (br s, 1H, -C(O)NH), 7.49–7.55 (m, 3H, phenyl-H), 7.35–7.40 (m, 1H, phenyl-H), 6.74 (d, 1H,  $J = 3.6$  Hz, H-1'), 5.52 (d, 1H,  $J = 5.1$  Hz, -OH), 5.20 (d, 1H,  $J = 5.1$  Hz, -OH), 4.79 (t, 1H,  $J = 5.6$  Hz, -OH), 4.38–4.42 (m, 1H, H-2'), 4.17–4.22 (m, 1H, H-3'), 3.87–3.92 (m, 1H, H-4'), 3.38–3.58 (m, 2H, H-5').  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  158.5, 149.1, 145.5, 131.8, 129.0, 122.8, 119.1 (d,  $^2J_{\text{CF}} = 22.7$  Hz), 118.1 (d,  $^2J_{\text{CF}} = 20.4$  Hz), 91.5, 88.6, 86.2, 81.5, 74.9, 71.3, 62.7. Maldi-MS:  $m/z$  385.1 [M + Na] $^+$ . HRMS: calcd for  $\text{C}_{16}\text{H}_{15}\text{N}_4\text{O}_5\text{FNa}^+$  385.0919, found 385.0920. IR: 2235.5  $\text{cm}^{-1}$ .

**3i.** An amount of 69.8 mg (0.14 mmol) of **2i** was used for the reaction. An amount of 38.0 mg (77%) of product was obtained, isolated as a yellow solid. HPLC:  $t = 6.63$  min, purity 98.3% (method 1);  $t = 6.19$  min, purity 97.9% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.53 (br s, 1H, -C(O)NH), 8.19 (br s, 1H, -C(O)NH), 7.74 (dd, 1H,  $J_{\text{HH}} = J_{\text{HF}} = 7.2$  Hz, phenyl-H), 7.55–7.60 (m, 1H, phenyl-H), 7.30–7.44 (m, 2H, phenyl-H), 6.77 (d, 1H,  $J = 3.0$  Hz, H-1'), 5.53 (d, 1H,  $J = 5.1$  Hz, -OH), 5.20 (d, 1H,  $J = 6.0$  Hz, -OH), 4.78 (t, 1H,  $J = 5.9$  Hz, -OH), 4.42–4.43 (m, 1H, H-2'), 4.19–4.24 (m, 1H, H-3'), 3.88–3.93 (m, 1H, H-4'), 3.41–3.59 (m, 2H, H-5').  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  158.5, 149.1, 145.5, 134.5, 133.2, 125.8, 116.7 (d,  $^2J_{\text{CF}} = 19.4$  Hz), 109.4, 91.5, 86.2, 85.5, 83.5, 74.9, 71.3, 62.7. Maldi-MS:  $m/z$  385.1 [M + Na] $^+$ . HRMS: calcd for  $\text{C}_{16}\text{H}_{15}\text{N}_4\text{O}_5\text{FNa}^+$  385.0919, found 385.0917. IR: 2235.2  $\text{cm}^{-1}$ .

**3j.** An amount of 120.8 mg (0.22 mmol) of **2j** was used for the reaction. An amount of 67.4 mg (74%) of product was obtained, isolated as a yellow solid. HPLC:  $t = 6.65$  min, purity 98.5% (method 1);  $t = 6.16$  min, purity 98.8% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.66 (br s, 1H, -C(O)NH), 8.34 (br s, 1H, -C(O)NH), 7.96–8.04 (m, 4H, phenyl-H), 6.77 (d, 1H,  $J = 3.6$  Hz, H-1'), 5.69 (d, 1H,  $J = 5.4$  Hz, -OH), 5.36 (d, 1H,  $J = 5.7$  Hz, -OH), 4.93 (t, 1H,  $J = 5.7$  Hz, -OH), 4.40–4.45 (m, 1H, H-2'), 4.20–4.25 (m, 1H, H-3'), 4.04–4.05 (m, 1H, H-4'),

3.40–3.59 (m, 2H, H-5').  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  158.5, 149.1, 145.4, 133.4, 130.5 (q,  $^2J_{\text{CF}} = 31$  Hz), 126.5, 125.2, 123.9 (q,  $^1J_{\text{CF}} = 192$  Hz), 91.5, 88.5, 86.2, 82.8, 74.9, 71.4, 62.7. Maldi-MS:  $m/z$  435.1  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{17}\text{H}_{15}\text{N}_4\text{O}_5\text{F}_3\text{Na}^+$  435.0887, found 435.0892. IR: 2237.0  $\text{cm}^{-1}$ .

**3k.** An amount of 62.2 mg (0.11 mmol) of **2k** was used for the reaction. An amount of 35.3 mg (76%) of product was obtained, isolated as a white solid. HPLC:  $t = 6.66$  min, purity 98.8% (method 1);  $t = 6.25$  min, purity 97.7% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.47 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.19 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.02 (s, 1H, phenyl-H), 7.97 (d, 1H,  $J = 7.8$  Hz, phenyl-H), 7.89 (d, 1H,  $J = 7.8$  Hz, phenyl-H), 7.73–7.76 (m, 1H, phenyl-H), 6.77 (d, 1H,  $J = 3.0$  Hz, H-1'), 5.52 (d, 1H,  $J = 4.8$  Hz,  $-\text{OH}$ ), 5.20 (d, 1H,  $J = 5.7$  Hz,  $-\text{OH}$ ), 4.78 (t, 1H,  $J = 5.9$  Hz,  $-\text{OH}$ ), 4.41–4.43 (m, 1H, H-2'), 4.21–4.23 (m, 1H, H-3'), 3.91–3.93 (m, 1H, H-4'), 3.44–3.57 (m, 2H, H-5').  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  158.5, 149.1, 145.5, 136.5, 131.0, 128.9, 127.3, 122.1, 91.6, 88.3, 86.2, 82.0, 74.9, 71.3, 62.7. Maldi-MS:  $m/z$  435.1  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{17}\text{H}_{15}\text{N}_4\text{O}_5\text{F}_3\text{Na}^+$  435.0886, found 435.0890. IR: 2238.4  $\text{cm}^{-1}$ .

**3l.** An amount of 50.9 mg (0.10 mmol) of **2l** was used for the reaction. An amount of 30.1 mg (73%) of product was obtained, isolated as a white solid. HPLC:  $t = 6.66$  min, purity 98.4% (method 1);  $t = 6.23$  min, purity 98.6% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.50 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.19 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 7.89–7.95 (m, 2H, phenyl-H), 7.70–7.82 (m, 2H, phenyl-H), 6.77 (d, 1H,  $J = 3.6$  Hz, H-1'), 5.52 (d, 1H,  $J = 5.7$  Hz,  $-\text{OH}$ ), 5.20 (d, 1H,  $J = 6.0$  Hz,  $-\text{OH}$ ), 4.78 (t, 1H,  $J = 5.6$  Hz,  $-\text{OH}$ ), 4.42–4.47 (m, 1H, H-2'), 4.20–4.25 (m, 1H, H-3'), 3.91–3.96 (m, 1H, H-4'), 3.42–3.62 (m, 2H, H-5').  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  158.5, 149.2, 145.4, 135.5, 133.6, 131.2, 130.9 (q,  $^2J_{\text{CF}} = 30.3$  Hz), 127.0, 124.1 (q,  $^1J_{\text{CF}} = 271.7$  Hz), 118.8, 91.5, 86.2, 85.6, 85.4, 74.9, 71.3, 62.7. Maldi-MS:  $m/z$  435.1  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{17}\text{H}_{15}\text{N}_4\text{O}_5\text{F}_3\text{Na}^+$  435.0886, found 435.0877. IR: 2234.6  $\text{cm}^{-1}$ .

**3m.** An amount of 64.9 mg (0.13 mmol) of **2m** was used for the reaction. An amount of 37.3 mg (81%) of product was obtained, isolated as a white solid. HPLC:  $t = 6.60$  min, purity 98.9% (method 1);  $t = 6.13$  min, purity 95.5% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.46 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.16 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 7.80 (d, 1H,  $J = 5.1$  Hz, thiophenyl-H), 7.61–7.62 (m, 1H, thiophenyl-H), 7.18–7.21 (m, 1H, thiophenyl-H), 6.77 (d, 1H,  $J = 3.6$  Hz, H-1'), 5.50 (d, 1H,  $J = 5.1$  Hz,  $-\text{OH}$ ), 5.18 (d, 1H,  $J = 5.7$  Hz,  $-\text{OH}$ ), 4.77 (t, 1H,  $J = 6.0$  Hz,  $-\text{OH}$ ), 4.40–4.44 (m, 1H, H-2'), 4.20–4.25 (m, 1H, H-3'), 3.89–3.94 (m, 1H, H-4'), 3.42–3.61 (m, 2H, H-5').  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  158.5, 149.0, 145.7, 135.4, 131.4, 128.8, 120.2, 91.5, 86.1, 84.3, 83.8, 74.9, 71.3, 62.7. Maldi-MS:  $m/z$  373.1  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{14}\text{H}_{14}\text{N}_4\text{O}_5\text{SNa}^+$  373.0577, found 373.0580. IR: 2221.8  $\text{cm}^{-1}$ .

**3n.** An amount of 90.1 mg (0.18 mmol) of **2n** was used for the reaction. An amount of 40.5 mg (76%) of product was obtained, isolated as a white solid. HPLC:  $t = 6.62$  min, purity 98.3% (method 1);  $t = 6.05$  min, purity 97.5% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.48 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.17 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.13–8.14 (m, 1H, thiophenyl-H), 7.72–7.74 (m, 1H, thiophenyl-H), 7.38 (d, 1H,  $J = 4.8$  Hz, thiophenyl-H), 6.77 (d, 1H,  $J = 3.0$  Hz, H-1'), 5.51 (d, 1H,  $J = 5.1$  Hz,  $-\text{OH}$ ), 5.19 (d, 1H,  $J = 6.0$  Hz,  $-\text{OH}$ ), 4.78 (t, 1H,  $J = 5.4$  Hz,  $-\text{OH}$ ), 4.41–4.45 (m, 1H, H-2'), 4.21–4.26 (m, 1H, H-3'), 3.90–3.95 (m, 1H, H-4'), 3.43–3.63 (m, 2H, H-5').  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ ):  $\delta$  158.6, 148.9, 146.0, 133.1, 130.3, 128.2, 119.8, 91.4, 86.1, 85.9, 80.2, 74.9, 71.3, 62.7. Maldi-MS:  $m/z$  373.1  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{14}\text{H}_{14}\text{N}_4\text{O}_5\text{SNa}^+$  373.0577, found 373.0587. IR: 2234.0  $\text{cm}^{-1}$ .

**3o.** An amount of 54.8 mg (0.11 mmol) of **2o** was used for the reaction. An amount of 33.5 mg (86%) of product was obtained, isolated as a white solid. HPLC:  $t = 7.18$  min, purity 98.7% (method 1);  $t = 6.07$  min, purity 97.2% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.48 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.16 (br s,

1H,  $-\text{C}(\text{O})\text{NH}$ ), 7.87 (br s, 1H, imidazole-H), 7.53 (br s, 1H, imidazole-H), 6.76 (d, 1H,  $J = 3.6$  Hz, H-1'), 5.51 (d, 1H,  $J = 5.1$  Hz,  $-\text{OH}$ ), 5.19 (d, 1H,  $J = 5.7$  Hz,  $-\text{OH}$ ), 4.77 (t, 1H,  $J = 5.9$  Hz,  $-\text{OH}$ ), 4.40–4.45 (m, 1H, H-2'), 4.19–4.24 (m, 1H, H-3'), 3.89–3.94 (m, 1H, H-4'), 3.72 (s, 3H,  $-\text{NCH}_3$ ), 3.42–3.61 (m, 2H, H-5').  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  158.5, 149.1, 145.7, 141.1, 137.0, 114.2, 91.5, 87.6, 86.1, 79.0, 74.9, 71.3, 62.7, 32.6. Maldi-MS:  $m/z$  349.1  $[\text{M} + \text{H}]^+$ . HRMS: calcd for  $\text{C}_{14}\text{H}_{17}\text{N}_6\text{O}_5^+$  349.1255, found 349.1251. IR: 2235.1  $\text{cm}^{-1}$ .

**3p.** An amount of 66.1 mg (0.14 mmol) of **2p** was used for the reaction. An amount of 36.2 mg (74%) of product was obtained, isolated as a white solid. HPLC:  $t = 6.65$  min, purity 98.5% (method 1);  $t = 6.53$  min, purity 96.5% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.45 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.12 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 6.22 (d, 1H,  $J = 3.3$  Hz, H-1'), 6.37 (s, 1H,  $-\text{C}=\text{CH}$ ), 5.48 (d, 1H,  $J = 5.4$  Hz,  $-\text{OH}$ ), 5.17 (d, 1H,  $J = 5.7$  Hz,  $-\text{OH}$ ), 4.76 (t, 1H,  $J = 5.9$  Hz,  $-\text{OH}$ ), 4.37–4.39 (m, 1H, H-2'), 4.17–4.19 (m, 1H, H-3'), 3.87–3.89 (m, 1H, H-4'), 3.40–3.54 (m, 2H, H-5'), 2.14–2.17 (m, 4H, cyclohexenylethynyl-H), 1.56–1.61 (m, 4H, cyclohexenylethynyl-H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  158.6, 148.8, 146.2, 139.3, 119.3, 92.1, 91.3, 86.0, 78.3, 74.8, 71.3, 62.7, 28.7, 26.0, 22.3, 21.4. Maldi-MS:  $m/z$  371.1  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{16}\text{H}_{20}\text{N}_4\text{O}_5\text{Na}^+$  371.1326, found 371.1335. IR: 2220.0  $\text{cm}^{-1}$ .

**3q.** An amount of 88.4 mg (0.18 mmol) of **2q** was used for the reaction. An amount of 46.0 mg (74%) of product was obtained, isolated as a white solid. HPLC:  $t = 6.58$  min, purity 98.2% (method 1);  $t = 6.08$  min, purity 97.5% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.41 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.12 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 6.71 (d, 1H,  $J = 3.3$  Hz, H-1'), 5.47 (d, 1H,  $J = 5.4$  Hz,  $-\text{OH}$ ), 5.16 (d, 1H,  $J = 5.7$  Hz,  $-\text{OH}$ ), 4.75 (t, 1H,  $J = 5.7$  Hz,  $-\text{OH}$ ), 4.34–4.38 (m, 1H, H-2'), 4.15–4.19 (m, 1H, H-2'), 3.86–3.90 (m, 1H, H-3'), 4.74 (t, 2H,  $J = 6.5$  Hz,  $-\text{CH}_2-$ ), 3.40–3.60 (m, 2H, H-5'), 2.63 (t, 2H,  $J = 7.1$  Hz,  $-\text{CH}_2-$ ), 1.99–2.03 (m, 2H,  $-\text{CH}_2-$ ).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  158.6, 148.6, 146.0, 91.23, 91.16, 86.0, 74.8, 72.8, 71.3, 62.7, 44.7, 31.0, 16.5. Maldi-MS:  $m/z$  367.1  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{13}\text{H}_{17}\text{N}_4\text{O}_5\text{ClNa}^+$  367.0780, found 367.0782. IR: 2251.4  $\text{cm}^{-1}$ .

**3r.** An amount of 51.4 mg (0.11 mmol) of **2r** was used for the reaction. An amount of 28.7 mg (79%) of product was obtained, isolated as a white solid. HPLC:  $t = 6.72$  min, purity 98.7% (method 1);  $t = 6.32$  min, purity 97.8% (method 2).  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ ):  $\delta$  8.38 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.08 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 6.69 (d, 1H,  $J = 3.0$  Hz, H-1'), 5.46 (d,  $J = 4.8$  Hz,  $-\text{OH}$ ), 5.15 (d, 1H,  $J = 6.0$  Hz,  $-\text{OH}$ ), 4.74 (t, 1H,  $J = 5.9$  Hz,  $-\text{OH}$ ), 4.32–4.35 (m, 1H, H-2'), 4.14–4.16 (m, 1H, H-3'), 3.84–3.87 (m, 1H, H-4'), 3.39–3.53 (m, 2H, H-5'), 2.44 (t, 2H,  $J = 6.9$  Hz,  $-\text{CH}_2-$ ), 1.50–1.54 (m, 2H,  $-\text{CH}_2-$ ), 1.27–1.36 (m, 4H,  $-\text{CH}_2-$ ), 0.86 (t, 3H,  $J = 7.5$  Hz,  $-\text{CH}_3$ ).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  158.7, 148.6, 146.2, 92.8, 91.2, 86.0, 74.9, 72.4, 71.3, 62.8, 31.2, 27.9, 22.3, 18.8, 14.6. Maldi-MS:  $m/z$  361.1  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{19}\text{H}_{22}\text{N}_4\text{O}_5\text{Na}^+$  361.1482, found 361.1490. IR: 2250.0  $\text{cm}^{-1}$ .

**3s.** An amount of 90.1 mg (0.18 mmol) of **2s** was used for the reaction. An amount of 40.5 mg (63%) of product was obtained, isolated as a white solid. HPLC:  $t = 6.57$  min, purity 98.6% (method 1);  $t = 5.99$  min, purity 98.4% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.43 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.13 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 6.73 (d, 1H,  $J = 3.0$  Hz, H-1'), 5.56 (s, 1H,  $-\text{OH}$ ), 5.49 (d, 1H,  $J = 5.1$  Hz,  $-\text{OH}$ ), 5.17 (d, 1H,  $J = 5.1$  Hz,  $-\text{OH}$ ), 4.76 (t, 1H,  $J = 5.4$  Hz,  $-\text{OH}$ ), 4.35–4.40 (m, 1H, H-2'), 4.16–4.21 (m, 1H, H-3'), 3.86–3.91 (m, 1H, H-4'), 3.38–3.57 (m, 2H, H-5'), 1.80–1.96 (m, 4H, cyclopentanylnyl-H), 1.68–1.76 (m, 4H, cyclopentanylnyl-H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  158.6, 148.7, 146.0, 96.2, 91.3, 86.0, 74.9, 73.4, 73.2, 71.3, 62.7, 42.3, 23.7. Maldi-MS:  $m/z$  375.1  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{15}\text{H}_{20}\text{N}_4\text{O}_6\text{Na}^+$  375.1275, found 375.1275. IR: 2243.9  $\text{cm}^{-1}$ .

**3t.** An amount of 76.9 mg (0.15 mmol) of **2t** was used for the reaction. An amount of 32.9 mg (60%) of product was obtained,



isolated as a white solid. HPLC:  $t = 6.58$  min, purity 98.6% (method 1);  $t = 6.04$  min, purity 97.6% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.55 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.23 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 6.82 (d, 1H,  $J = 3.6$  Hz, H-1'), 5.82 (s, 1H,  $-\text{OH}$ ), 5.61 (d, 1H,  $J = 5.1$  Hz,  $-\text{OH}$ ), 5.31 (d, 1H,  $J = 5.1$  Hz,  $-\text{OH}$ ), 4.89 (t, 1H,  $J = 5.1$  Hz,  $-\text{OH}$ ), 4.46–4.49 (m, 1H, H-2'), 4.25–4.31 (m, 1H, H-3'), 3.96–4.01 (m, 1H, H-4'), 3.46–3.67 (m, 2H, H-5'), 1.53–2.01 (m, 10H, cyclohexanyl-H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  158.6, 148.7, 145.9, 96.2, 91.2, 86.0, 74.8, 73.4, 73.2, 71.2, 67.5, 62.7, 42.2, 25.3, 23.7, 23.6, 23.1. Maldi-MS:  $m/z$  389.1  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{16}\text{H}_{22}\text{N}_4\text{O}_6\text{Na}^+$  389.1432, found 389.1439. IR: 2249.9  $\text{cm}^{-1}$ .

**4.** Compounds **4** was synthesized according to the procedures described in ref 15a. HPLC:  $t = 7.08$  min, purity >99% (method 1);  $t = 6.55$  min, purity 98.7% (method 2).  $^1\text{H}$  NMR (600 MHz, DMSO- $d_6$ ):  $\delta$  8.31 (br, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.14 (br, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 7.95 (d, 2H,  $J = 8.4$  Hz, phenyl-H), 7.34 (d, 2H,  $J = 8.4$  Hz, phenyl-H), 6.81 (d, 1H,  $J = 2.4$  Hz, H-1'), 5.52 (d, 1H,  $J = 5.4$  Hz,  $-\text{OH}$ ), 5.18 (d, 1H,  $J = 4.8$  Hz,  $-\text{OH}$ ), 4.80 (t, 1H,  $J = 5.4$  Hz,  $-\text{OH}$ ), 4.47–4.78 (m, 1H, H-2'), 4.36–4.37 (m, 1H, H-3'), 3.95–3.98 (m, 1H, H-4'), 3.51–3.67 (m, 2H, H-5'), 2.62 (t, 2H,  $J = 7.5$  Hz,  $-\text{CH}_2-$ ), 1.58–1.63 (m, 2H,  $-\text{CH}_2-$ ), 1.27–1.33 (m, 4H,  $-\text{CH}_2-$ ), 0.87 (t, 3H,  $J = 6.9$  Hz,  $-\text{CH}_3$ ).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  160.3, 159.3, 149.2, 145.0, 129.4, 128.1, 126.7, 90.9, 86.1, 75.2, 71.6, 63.0, 35.6, 31.5, 31.1, 22.6, 14.6. Maldi-MS:  $m/z$  413.2  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{19}\text{H}_{26}\text{N}_4\text{O}_5\text{Na}^+$  413.1801, found 413.1820.

**5.** An amount of 40.9 mg (0.099 mmol) of **3e** was dissolved in 5 mL of MeOH and 4.0 mg of 10% Pd/C added.  $\text{H}_2$  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 40.7 mg (98.6%) of product **10** as a white solid. HPLC:  $t = 7.14$  min, purity >99% (method 1);  $t = 6.49$  min, purity 95.1% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.21 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.00 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 7.14 (d, 2H,  $J = 7.8$  Hz, phenyl-H), 7.09 (d, 2H,  $J = 7.8$  Hz, phenyl-H), 6.69 (d, 1H,  $J = 2.7$  Hz, H-1'), 5.40 (d, 1H,  $J = 6.0$  Hz,  $-\text{OH}$ ), 5.10 (d, 1H,  $J = 6.0$  Hz,  $-\text{OH}$ ), 4.73 (t, 1H,  $J = 6.0$  Hz,  $-\text{OH}$ ), 4.32–3.35 (m, 1H, H-2'), 4.17–4.23 (m, 1H, H-3'), 3.85–3.88 (m, 1H, H-4'), 3.43–3.63 (m, 2H, H-5'), 2.94 (br, 4H,  $-\text{CH}_2-$ ), 2.51–2.54 (m, 2H,  $-\text{CH}_2-$ ), 1.51–1.59 (m, 2H,  $-\text{CH}_2-$ ), 1.23–1.28 (m, 4H,  $-\text{CH}_2-$ ), 0.86 (t, 3H,  $J = 6.9$  Hz,  $-\text{CH}_3$ ).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  162.4, 159.3, 148.5, 140.6, 138.7, 128.9, 128.8, 90.9, 85.5, 75.9, 71.5, 63.1, 35.4, 33.6, 31.6, 31.3, 30.3, 22.6, 14.6. Maldi-MS: 441  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{21}\text{H}_{30}\text{N}_4\text{O}_5\text{Na}^+$  441.2108, found 441.2099.

**6.** The protected precursor of **6** was synthesized according to the procedures described in ref 16a. The obtained protected compound was dissolved in 10 mL of saturated  $\text{NH}_3/\text{MeOH}$  and stirred at room temperature for 2 days. Then the solvent was removed and the residue was purified by flash chromatography on silica gel ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 6:1). The purified product was dried in vacuo to afford **6** as a white solid (85.4%). HPLC:  $t = 7.11$  min, purity >99% (method 1);  $t = 6.42$  min, purity >99% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  9.15 (s, 1H, triazole-H), 8.52 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.31 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 7.90 (d, 2H,  $J = 7.8$  Hz, phenyl-H), 7.30 (d, 2H,  $J = 8.1$  Hz, phenyl-H), 6.80 (d, 1H,  $J = 3.3$  Hz, H-1'), 5.59 (d, 1H,  $J = 5.4$  Hz,  $-\text{OH}$ ), 5.18 (d, 1H,  $J = 6.0$  Hz,  $-\text{OH}$ ), 4.75 (t, 1H,  $J = 5.7$  Hz,  $-\text{OH}$ ), 4.44–4.47 (m, 1H, H-2'), 4.25–4.31 (m, 1H, H-3'), 3.93–3.94 (m, 1H, H-4'), 3.46–3.60 (m, 2H, H-5'), 2.60 (t,  $J = 7.5$  Hz, 2H,  $-\text{CH}_2-$ ), 1.56–1.61 (m, 2H,  $-\text{CH}_2-$ ), 1.28–1.29 (m, 4H,  $-\text{CH}_2-$ ), 0.85 (t, 3H,  $J = 6.8$  Hz,  $-\text{CH}_3$ ).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  158.3, 153.5, 149.4, 147.8, 143.7, 129.6, 127.6, 126.3, 120.8, 91.9, 86.4, 75.0, 71.3, 62.8, 35.5, 31.5, 31.2, 22.6, 14.6. ESI-MS:  $m/z$  479.9  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{21}\text{H}_{27}\text{N}_5\text{O}_5\text{Na}^+$  480.1971, found 480.1985.

**7.** Compounds **7** was synthesized according to the procedures described in ref 13. The obtained product is a white solid with a

yield of 95%. HPLC:  $t = 7.43$  min, purity >99% (method 1);  $t = 6.93$  min, purity 95.4% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.51 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 8.14 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 7.54 (d, 2H,  $J = 7.8$  Hz, phenyl-H), 7.30 (d, 2H,  $J = 7.8$  Hz, phenyl-H), 5.93 (s, 2H, H-1'), 4.73 (t, 1H,  $J = 5.4$  Hz,  $-\text{OH}$ ), 3.58 (t, 2H,  $J = 5.0$  Hz, H-2'), 3.44–3.49 (m, 2H, H-3'), 2.62 (t, 2H,  $J = 7.4$  Hz,  $-\text{CH}_2-$ ), 1.56–1.60 (m, 2H,  $-\text{CH}_2-$ ), 1.21–1.28 (m, 4H,  $-\text{CH}_2-$ ), 0.87 (t, 3H,  $J = 6.8$  Hz,  $-\text{CH}_3$ ).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  157.8, 146.8, 146.4, 145.1, 132.1, 128.6, 118.1, 91.1, 79.5, 78.3, 71.7, 61.5, 36.0, 31.4, 30.8, 22.5, 14.0. FAB-MS:  $m/z$  357  $[\text{M} + \text{H}]^+$ , 379  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{19}\text{H}_{25}\text{N}_4\text{O}_3^+$  357.1921, found 357.1911. IR: 2227.35  $\text{cm}^{-1}$ .

**8.** The protected precursor of **8** was synthesized according to the procedures described in ref 11. The obtained protected compound was dissolved in 10 mL of saturated  $\text{NH}_3/\text{MeOH}$  and stirred at room temperature for 2 days. Then the solvent was removed and the residue was washed with  $\text{CH}_2\text{Cl}_2$ . The washed residue was dried in vacuo to afford the corresponding product **8** as white solid (71%). HPLC:  $t = 7.28$  min, purity >99% (method 1);  $t = 6.55$  min, purity 95.4% (method 2).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  8.03 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 7.76 (br s, 1H,  $-\text{C}(\text{O})\text{NH}$ ), 7.62 (d, 2H,  $J = 7.8$  Hz, phenyl-H), 7.35 (d, 2H,  $J = 8.1$  Hz, phenyl-H), 5.99 (d, 1H,  $J = 4.2$  Hz, H-1'), 5.63 (d, 1H,  $J = 5.7$  Hz,  $-\text{OH}$ ), 5.28 (d, 1H,  $J = 6.0$  Hz,  $-\text{OH}$ ), 4.80 (t, 1H,  $J = 6.0$  Hz,  $-\text{OH}$ ), 4.49–4.54 (m, 1H, H-2'), 4.21–4.26 (m, 1H, H-3'), 3.95–4.00 (m, 1H, H-4'), 3.33–3.62 (m, 2H, H-5'), 2.64 (t, 2H,  $J = 7.5$  Hz,  $-\text{CH}_2-$ ), 1.54–1.64 (m, 2H,  $-\text{CH}_2-$ ), 1.25–1.35 (m, 4H,  $-\text{CH}_2-$ ), 0.86 (t, 3H,  $J = 6.9$  Hz,  $-\text{CH}_3$ ).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ ):  $\delta$  160.4, 157.7, 146.6, 140.8, 132.7, 129.8, 117.1, 98.2, 91.1, 86.9, 74.9, 74.7, 71.2, 62.7, 35.7, 31.5, 30.9, 22.6, 14.6. Maldi-MS:  $m/z$  437  $[\text{M} + \text{Na}]^+$ . HRMS: calcd for  $\text{C}_{21}\text{H}_{26}\text{N}_4\text{O}_5\text{Na}^+$  437.1795, found 437.1798. IR: 2226.2  $\text{cm}^{-1}$ .

**In Vitro Cell Growth Inhibition Assay.** Pancreatic cancer chemoresistant MiaPaCa-2 cells were cultured in DMEM medium (Gibco) supplemented with 10% fetal bovine serum (FBS). Cells were seeded at a density of 15 000 cells per well in 96-well View Plate (Packard) in 250  $\mu\text{L}$  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 medium alone as control or containing different concentrations of compounds. Plates were further incubated at 37  $^\circ\text{C}$  and 5%  $\text{CO}_2$  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.

**FACS Flow Cytometry.** Cells were seeded in 10 cm dishes at the 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 ethanol, 70%, overnight at 4  $^\circ\text{C}$ . After a wash with phosphate–citrate buffer, cells were treated with 200  $\mu\text{L}$  of RNase (500  $\mu\text{g}/\text{mL}$ ), labeled with 1 mL of propidium iodide (50  $\mu\text{g}/\text{mL}$ ), 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.

**ELISA Assay for DNA/Histone Release.** 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<sup>Plus</sup>, Roche). The 96-well plates were centrifuged (200g) for 10 min, the supernatant was discarded, and lysis buffer was added. After lysis, the samples were centrifuged and an amount of 20  $\mu\text{L}$  of the supernatant was



transferred to a streptavidin-coated microtiter plate. Biotin-labeled anti-histone 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 nm in a microplate reader. The enrichment in histone-DNA fragments is expressed as a fold increase in absorbance compared with control.

**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  $\mu$ L of Apo-ONE homogeneous caspase-3/7 reagent was added to each well of a black 96-well plate containing 100  $\mu$ L 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 measuring the fluorescence of each well.

**Lactate Dehydrogenase (LDH) Assay.** MiaPaCa-2 cells were seeded 15 000 cells per well in 96-well plates. Twenty-four hours later, cells were treated with the test compound for 1 h. Then the LDH concentration was measured by using commercial LDH kit (cytotoxicity detection kit, Roche). The LDH reaction mixture was freshly prepared according to the manufacturer's protocol (Roche Diagnostics), 100  $\mu$ L added to each well of a 96-well plate containing 100  $\mu$ L of blank, control, or cells in culture, and the plate incubated for 10 min at 25 °C. Control was performed with lysis buffer and medium and set as 100% and 0% LDH release, respectively. The relative LDH release is defined by the ratio of LDH released over total LDH in the intact cells. All samples were performed in triplicate.

**Necrosis Assay on Flow Cytometry.** MiaPaCa-2 cells were seeded in 6 cm dishes at a density of 500 000 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 3, 6, and 12 h of treatment, the cells were trypsinized and the collected cell pellet was washed with cold PBS twice. The samples were pelleted through centrifugation at 3000 rpm for 5 min and resuspended in 100  $\mu$ L of binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM  $\text{CaCl}_2$ , pH 7.4). The nuclear stain 7-AAD (7-aminoactinomycin D) was added to the samples, which were then incubated for 15 min at 25 °C. Necrotic cells, as well as those cells in the advanced stages of apoptosis, have permeabilized membranes and allow 7-AAD to stain the cell nucleus. Immediately after staining, flow cytometry was performed on fluorescence activated cell sorting (FACS Calibur, Becton Dickinson, Le Pont-De-Claix, France). Each sample was performed in triplicate.

**Determination of DNA Synthesis in Whole Cells.** MiaPaCa-2 Cells were seeded at a density of 15 000 cells per well in 96-well View Plate. Then the cells in exponential growth phase were treated with the compounds for 4 h and then labeled with [ $^3\text{H}$ ]thymidine (10  $\mu\text{Ci}/\text{mL}$ ) for 6 h at 37 °C and 5%  $\text{CO}_2$ . Then the cells were harvested and DNA synthesis activity was determined according to the radioactivity by using liquid scintillation counting.

**Quantitative Real-Time PCR (qRT-PCR).** The expression of the Hsp27 mRNAs was analyzed by quantitative real-time PCR amplification analysis. Total RNAs were isolated using TRIzol method (Invitrogen). Then 1  $\mu\text{g}$  of RNAs were reverse-transcribed into cDNA by using the ImProm-II reverse transcription system (Promega) (0.5  $\mu\text{g}$  of oligo dT primer, 1  $\mu\text{L}$  of ImProm-II reverse transcriptase, 0.5  $\mu\text{L}$  of RNase inhibitor, 1  $\mu\text{L}$  of 10 mM

dNTP mix, 4.8  $\mu\text{L}$  of 25 mM  $\text{MgCl}_2$ , 4  $\mu\text{L}$  of 5  $\times$  RT buffer) in a final volume of 20  $\mu\text{L}$ . The thermal cycling protocol employed included two steps (step 1 consisting of 70 °C for 5 min, 4 °C for 5 min; step 2 consisting of 25 °C for 5 min, 42 °C for 1 h, 70 °C for 15 min, and 4 °C forever). The real time PCR was conducted using the LightCycler 2.0 instrument (Roche Diagnostics GmbH, Mannheim, Germany). Reaction mixtures contained a total volume of 20  $\mu\text{L}$  consisting of 5  $\mu\text{L}$  of each diluted cDNA (1:10), 10  $\mu\text{L}$  of SYBR Premix Ex Taq (2 $\times$ ) (TaKaRa Bio. Inc., Japan), 0.4  $\mu\text{L}$  of primers forward and primers reverse (10  $\mu\text{M}$ ) of target gene Hsp27 and internal control 18S, and 4.2  $\mu\text{L}$  of  $\text{H}_2\text{O}$ . The sequences of primers are as follows: Hsp27 primer F, 5'-TCCCTGGATGTCAACCACTTC-3', and Hsp27 primer R, 5'-TCTCCACCACGCCATCCT-3'; 18S primer F, 5'-CTAC-CACATCCAAGGAAGGC-3', and 18S primer R, 5'-TTTTC-GTCACTACCTCCCCG-3 (Eurogentec S. A., Seraing, Belgium). The PCR conditions were as follows: an initial denaturation step at 95 °C for 10 s, then 45 cycles of 95 °C for 5 s, 57 °C for 6 s, and 72 °C for 12 s. A melting curve was carried out after the amplification program by heating at temperatures from 65 to 95 °C in 1.5 min. And a final cooling step at 40 °C for 1 min was performed. Each sample was analyzed in triplicate in the PCR reaction to estimate the reproducibility of data. The data were acquired by using Roche Molecular Biochemicals LightCycler software, version 3.5, and statistical analysis was done with RelQuant (Roche).

**Western Blotting Analysis.** Samples containing equal amounts of protein (15  $\mu\text{g}$ ) from lysates of cultured MiaPaCa-2 cells were analyzed by Western blot analysis as described previously<sup>7,8</sup> with 1:5000 diluted antihuman Hsp27 rabbit polyclonal antibody (Stressgen Assay Designs Inc., MI) and 1:2000 diluted antihuman vinculin mouse monoclonal antibody (Sigma Chemical Co., St. Louis, MO). Specific proteins were detected using an enhanced chemiluminescence Western blotting analysis system (Amersham Life Science, Arlington Heights, IL).

**Immunofluorescence Detection of Hsp27 Protein Expression.** For immunofluorescence detection of Hsp27 protein in MiaPaCa-2 cells, the cells after different treatments were fixed with paraformaldehyde (Sigma-Aldrich, Lyon, France) (4% aqueous solution) and permeabilized with Triton 100 $\times$  1%. Then the cells were incubated with 1:500 diluted antimouse Hsp27 monoclonal antibody (Stressgen Assay Designs Inc., MI) at 4 °C overnight. After saturation in BSA/PBS, the cells were incubated with 1:750 diluted secondary green-fluorescent labeled antimouse antibody (Invitrogen Ltd., Paisley, U.K.). Then the cells were mounted with Prolong Gold antifade reagent containing DAPI (4, 6-diamidino-2-phenylindole) (Invitrogen Ltd., Paisley, U.K.). The samples were visualized using a Nikon Eclipse 90i microscope equipped with a Nikon digital sight DS-1QM/H digital camera (Nikon Instruments Inc., Japan). Images were acquired using NIS-Elements AR 2.30 software (Nikon Instruments Inc.).

**Assessment of in Vivo Tumor Growth.** Institutional guidelines for the proper and humane use of animals in research were followed. Approximately  $1 \times 10^7$  MiaPaCa-2 cells were inoculated subcutaneously with 0.1 mL of Matrigel (BD Biosciences Discovery Labware) to 5-week-old male xenografted nude mice. When MiaPaCa-2 tumors reached 100  $\text{mm}^3$ , mice were randomly selected for treatment with test compound and no treated mice were used as control. Each experimental group consisted of eight mice. After randomization, 150 mg/kg test compound was injected twice a week by intraperitoneal (ip) injection for 5 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).

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