

A Novel Bitriazolyl Acyclonucleoside Endowed with Dual Antiproliferative and Immunomodulatory Activity

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S Supporting Information

ABSTRACT: A novel bitriazolyl acyclonucleoside was discovered to exhibit powerful antiproliferative effects on different cancer cell lines through caspase-dependent apoptosis and at the same time stimulate the immune response in dendritic cells via Toll-like receptor 7 (TLR7) signaling. This promising compound with dual anticancer and immunomodulatory activity may represent a new generation of highly efficacious drug candidates for use in cancer therapy.

INTRODUCTION

Cancer is one of the most difficult known diseases to treat because of its high genetic heterogeneity and fast developing drug resistance. The development of cancer therapies over the past few decades has principally concerned chemotherapy combined with other treatment modalities such as surgery, radiotherapy, and targeted therapy. However, there is still an urgent need for novel anticancer drug candidates able to induce a more durable response and further facilitate tumor eradication in cancer patients. Cancer immunotherapy has gained interest based on the hypothesis that the progression of spontaneous malignancies can be suppressed by the immune system.^{1,2} A growing body of recent evidence suggests that the combination of immunotherapy and chemotherapy holds enormous potential to enhance clinical benefits due to its targeting of different tumor-cell survival pathways and overcoming drug resistance in advanced-stage cancers.^{3,4} At present, this combined modality goal is mainly achieved by combining cancer vaccines with chemotherapeutic drugs.^{3,4} However, anticancer agents able to simultaneously stimulate tumor-specific immune responses have attracted much attention recently considering the potential that harnessing such a synergic effect may have on more effectively combating tumor progression.⁵

Nucleoside analogues represent a significant class of drugs that elicit antimetabolic⁶ and immunostimulatory activity.⁷ Ribavirin (Figure 1), a triazole nucleoside currently recommended in the treatment of the infection caused by hepatitis C virus,⁸ is known to promote host T-cell-mediated immunity against viral infection,⁹ while certain guanine ribonucleoside analogues (isatoribine in Figure 1) have been reported to potentiate immune responses via the activation of Toll-like receptor 7 (TLR7).⁷ In our continuing efforts to develop novel aryltriazole nucleosides imparted with biologically interesting activities,^{10–12} we have focused much attention on nucleoside analogues bearing a bitriazolyl moiety (1 and 2 in Figure 1).

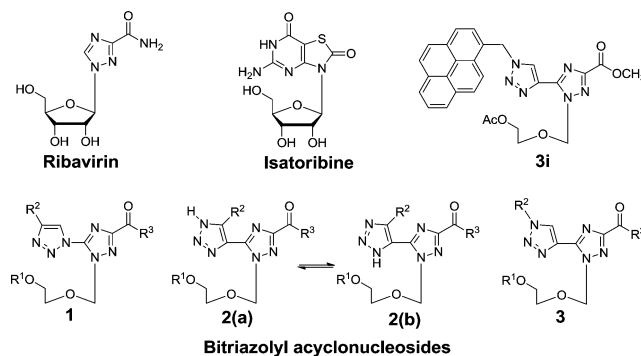


Figure 1. Structures of ribavirin, isatoribine, and bitriazolyl acyclonucleosides 1, 2, and 3.

This is because their more expanded aromatic surface and increased rigidity of the nucleobase may favor interactions with biological targets to induce potent anticancer and antiviral activities.^{10–19} Here, we disclose a novel bitriazolyl acyclonucleoside (3i in Figure 1) endowed with dual anticancer and immunomodulatory activity. This compound belongs to a new family of bitriazolyl acyclonucleosides (3 in Figure 1) which were synthesized via the Cu(I) catalyzed Click process starting from terminal alkyne-containing triazole acyclonucleoside and various arylazides. 3i displayed potent antiproliferative activity against different cancer cell lines and was able to activate caspase-dependent apoptosis in the drug-resistant pancreatic cancer MiaPaCa-2 cell line. Most importantly, this compound was also able to stimulate the immune response in mouse dendritic cells through the activation of TLR7. This dual-acting anticancer and immunomodulating compound may constitute a potentially interesting drug candidate for cancer therapy. We present below the synthesis of this novel bitriazolyl

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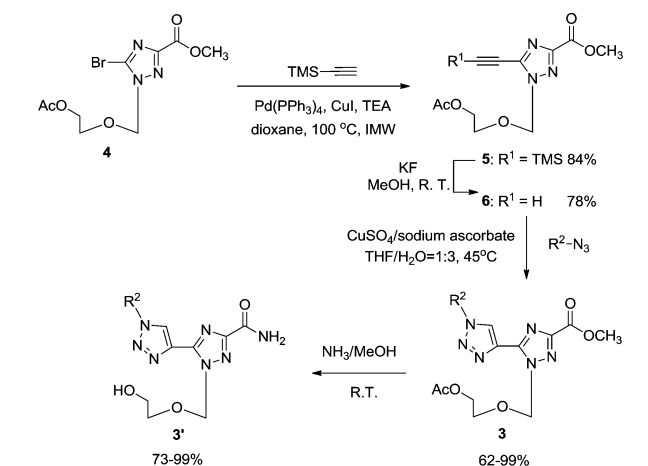
acyclonucleoside and the evaluation of its anticancer and immunomodulatory activity.

CHEMISTRY

The motivation behind developing the bitriazolyl acyclonucleosides **3** was based on our previous results obtained with the bitriazolyl acyclonucleosides **1** and **2**.^{15,16} Though compounds **1** have potent antiviral and anticancer activities,¹⁰ the linkage between the two triazole units via a C–N bond makes this family of compounds unstable and difficult to synthesize in good yields at large quantities,¹⁵ thus limiting their potential future application. By bridging of the 1,2,3-triazole ring to the 1,2,4-triazole system via a C–C bond, the stability of the bitriazolyl acyclonucleosides **2** is significantly increased.¹⁶ However, the synthetic strategy does not afford this family of compounds in their pure form of a **2(a)** or **2(b)** isomer but rather a mixture of both. We therefore envisioned the novel bitriazolyl acyclonucleosides **3**, which could be conveniently prepared via Huisgen reaction and obtained in straightforward isomeric pure form with precise structure.

The synthesis of **3** was adapted from that previously reported^{15,16} and comprised a Cu(I)-catalyzed Huisgen reaction by coupling the terminal alkyne bearing acyclonucleoside **6** with various arylazides followed by the deprotection (scheme in Table 1). **6** was produced by a two-step reaction

Table 1. Synthesis of Compounds **3** and **3'**



R ²	3	yield, %	3'	yield, %
Ph	3a	73	3'a	99
4-Me-Ph	3b	98	3'b	85
4-OMe-Ph	3c	98	3'c	99
4-CF ₃ -Ph	3d	76	3'd	91
4-NO ₂ -Ph	3e	62	3'e	96
CH ₃ (CH ₂) ₁₁	3f	83	3'f	89
benzyl	3g	78	3'g	94
1-naphthalenyl	3h	83, 97 ^a	3'h	92
1-methylpyrenyl	3i	44, 99 ^a	3'i	73

^aThe experiment was performed in THF/H₂O = 3/1.

sequence including the Sonogashira reaction of 5-bromotriazole acyclonucleoside **4** with trimethylsilylacetylene (TMSA) and the subsequent deprotection of the trimethylsilyl (TMS) group in the presence of KF (scheme in Table 1).^{17,20} The synthesis of **3** involved the Huisgen reaction of **6** with arylazides in the presence of CuSO₄/sodium ascorbate using THF/H₂O as solvent, which gave good to excellent yields (Table 1).

Investigation of the scope of substrate demonstrated that the reaction between **6** and arylazides bearing electron-donating groups usually gave quantitative yields (**3b** and **3c** in Table 1), while that concerning the electron-withdrawing azides was associated with slightly lower yields (**3d** and **3e** in Table 1). This can be explained by the fact that the electron-withdrawing group may impair the nucleophilicity of the azides and lead to an unfavorable cycloaddition reaction. Noteworthy is that since the pyrenyl group is relatively hydrophobic, increasing the ratio of organic solvent in the reaction dramatically ameliorated the yield of **3i** (Table 1). Finally, the subsequent ammonolysis of **3** in NH₃/MeOH furnished the desired products **3'** favorably (Table 1).

EVALUATION OF ANTIPROLIFERATION ACTIVITY

The initial biological screening of the bitriazolyl acyclonucleosides **3** and **3'** was performed in human drug-resistant pancreatic cancer MiaPaCa-2 cells by measuring their antiproliferation activity using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Of all the novel compounds synthesized, **3i** showed exceptional cell growth suppressing activity that was superior to the currently recommended anticancer drug gemcitabine (Figure 2A). To

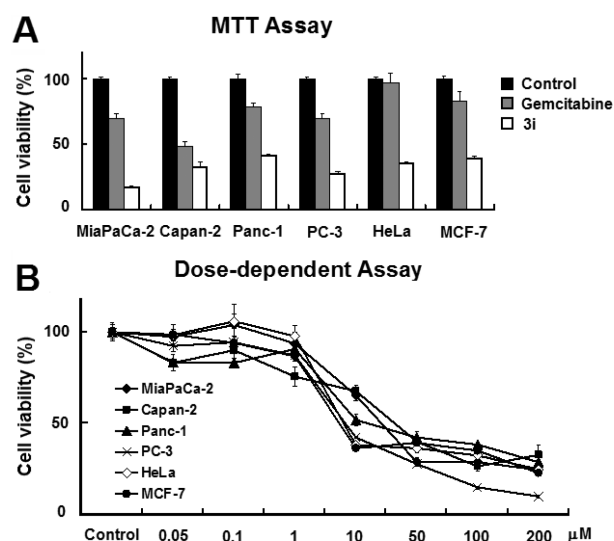


Figure 2. Inhibitory effect of compound **3i** on different cancer cell lines. (A) The indicated cell lines were left untreated (control) or incubated with 50 μ M **3i** or 50 μ M gemcitabine, and cell viability was assessed 48 h later by MTT. (B) The indicated cell lines were incubated with various concentrations of **3i**, and cell viability was assessed 48 h later by MTT. The values are the mean \pm SE of three independent experiments.

further assess its activity against other cancers, **3i** was investigated across other cancer types using gemcitabine as the reference control. As shown in Figure 2A, in addition to its growth inhibitory effect on MiaPaCa-2 cells, **3i** was able to inhibit the proliferation of the human pancreatic cancer Capan-2 and Panc-1, prostate cancer PC-3, cervical cancer HeLa, and breast cancer MCF-7 cell lines. Moreover, a dose-dependent assay with **3i** in the above-mentioned cell lines conducted at 50 nM to 200 μ M revealed a 50% growth inhibition (IC₅₀) of most cell lines at a dose of 10 μ M or below (Figure 2B).

Considering the potent anticancer activity shown by **3i**, we performed a structure–activity relationship analysis (Figure 3

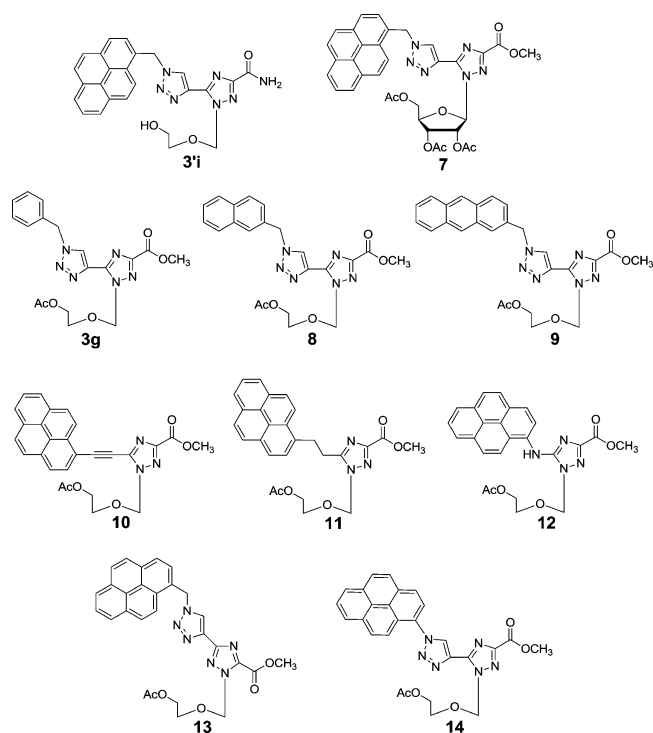


Figure 3. Structural analogues of 3i.

and Supporting Information Figure S1) using pancreatic cancer MiaPaCa-2 cells as the test model. Compound 3i (Figure 3), the deprotected form of 3i, showed significantly decreased activity, and the ribonucleoside analogue 7 (Figure 3) was devoid of any notable activity. This implies that alteration of the sugar part of 3i was detrimental to anticancer activity. We then turned our attention to the contribution of the pyrene group to the anticancer activity of 3i and prepared compounds 8 and 9 bearing naphthalenyl and anthracenyl groups (Figure 3), both of which are less bulky comparatively. Both 8 and 9 as well as 3g containing a benzyl group (Figure 3) showed reduced anticancer activity, indicating the importance of the pyrene system. To verify the contribution of the bitriazolyl motif, we further synthesized the non-bitriazolyl compounds 10–12 (Figure 3 and Scheme S1), employing an alkynyl group, alkyl chain, or amine functionality²¹ to replace the 1,2,3-triazole ring and connect the 1,2,4-triazole with the pyrenyl moiety. The considerably reduced activity exhibited by all these analogues compared with 3i confirmed the pivotal role of the bitriazolyl motif in the anticancer activity displayed by 3i. Finally, 13 (the constitutional isomer of 3i) and 14, in which the CH₂ group between the pyrenyl group and the 1,2,3-triazole ring has been removed (Figure 3), failed to elicit any notable activity, indicating the detrimental effect of even minor structural variation in 3i.

■ APOPTOSIS-INDUCED ANTIPROLIFERATION ACTIVITY

Altogether the obtained results highlighted 3i as possessing the highest antiproliferative activity among all the newly synthesized compounds described in this work. We therefore evaluated its ability to induce apoptosis in the drug-resistant pancreatic cancer cell line MiaPaCa-2. We first assessed the proapoptosis effect of 3i using fluorescence-activated cell sorting (FACS) flow cytometry. The FACS analysis based on

propidium iodide (PI) staining of the cellular DNA represents an efficient method to monitor cell cycle distribution. As can be seen in Figure 4A, in contrast to the untreated or gemcitabine-

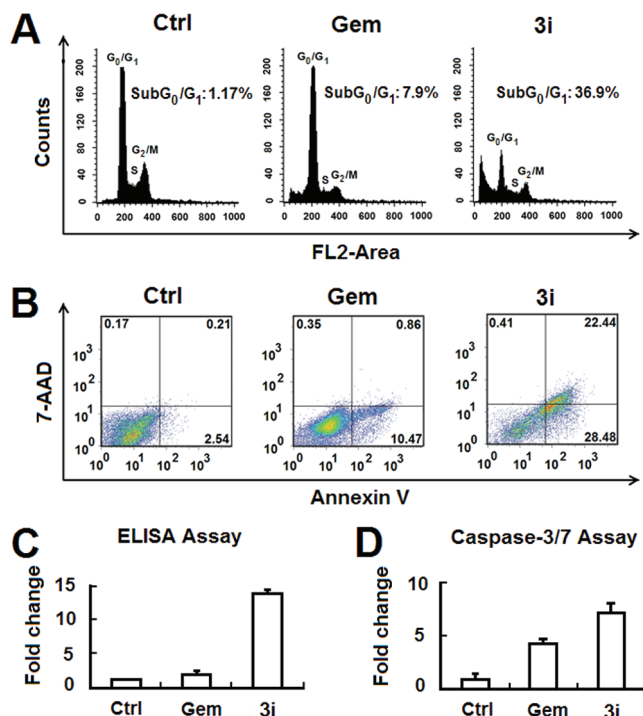


Figure 4. Apoptosis induction in pancreatic cancer MiaPaCa-2 cells after 48 h treatment with 3i using gemcitabine (Gem) and no treatment (Ctrl) as reference control. (A) DNA histograms from control and treated cells. Proportion of cells in sub-G₀/G₁ phase represents cells undergoing apoptosis. (B) Apoptosis but not necrosis was induced following treatment of 3i, using flow cytometry to quantify the cells undergoing apoptosis (annexin V positive cells) and necrosis (7-AAD positive cells). (C) ELISA test for detection of DNA/histone release. (D) Caspase-3/7 activity measurement. All the experiments were done in triplicate.

treated condition, 3i treatment caused a much stronger accumulation of apoptotic cells (the cells in sub-G₀/G₁ phase), indicating that the main mode of antiproliferative activity shown by 3i was the induction of apoptosis. We went on to test whether the 3i-treated cells underwent apoptosis and necrosis by flow cytometry analysis using annexin V-FITC and 7-aminoactinomycin (7AAD) staining, respectively. In 3i-treated cells, the percentage of apoptotic cells was increased by more than 20-fold while no significant fraction of necrotic cells (<0.5%) was observed even after 48 h of treatment, suggesting the predominant role of apoptosis in 3i induced cell death (Figure 4B). Early events in apoptosis can also be revealed by DNA fragmentation and the subsequent release of DNA/histone nucleosomes into the cytoplasm. We therefore used an enzyme linked immunosorbent assay (ELISA) to detect cytoplasmic nucleosomes, and a remarkable 15-fold increase in DNA/histone nucleosome release was observed after treatment with 3i compared with nontreated cells, which was significantly greater than that caused by gemcitabine (Figure 4C). We also examined the ability of 3i to induce caspase-3/7 activation, the final molecular step leading to apoptosis execution. Our data demonstrate that 3i treatment could promote a significant increase in caspase-3/7 activity compared with untreated or gemcitabine treated cells (Figure

4D). Taken together, these results suggest that **3i** has the ability to induce apoptosis and may therefore constitute a potential anticancer drug lead.

■ IMMUNOMODULATORY ACTIVITY

It is known that the triazole nucleoside ribavirin and the guanine derivative isatoribine are the representative nucleoside compounds capable of stimulating immune response in cells, some of which act through TLR7 signaling.^{7,9} Since **3i** is a bitriazolyl nucleoside and triazole nucleosides are reported to structurally mimic guanine nucleosides,^{9,22} we wanted to test whether **3i** also possessed immunomodulatory activity in addition to its anticancer activity. To do so, bone marrow dendritic cells derived from wild-type mice were stimulated with two doses of **3i**, and the production of interleukin-6 (IL-6) in culture supernatants was measured 24 h later by ELISA. As shown in Figure 5A, **3i** induced the production of IL-6 in a dose-dependent manner, indicating that **3i** is able to induce an immune response.

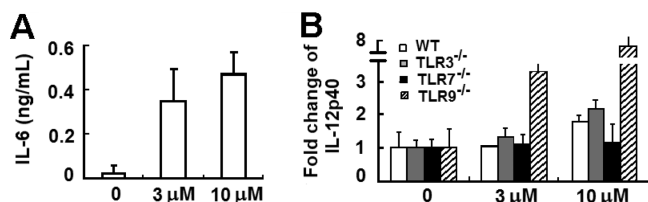


Figure 5. Compound **3i** exhibited immunostimulatory activity through TLR7 signaling. (A) Wild-type bone marrow dendritic cells were left untreated or stimulated with two doses of **3i**, and 24 h later the production of IL-6 in culture supernatants was measured by ELISA. Data are the mean \pm SD of four mice per group. (B) Bone marrow dendritic cells from wild-type (WT), TLR3^{-/-}, TLR7^{-/-}, and TLR9^{-/-} mice were left untreated or stimulated with different doses of **3i**, and 24 h later the production of IL-12p40 in culture supernatants was measured by ELISA.

Since guanine derivatives such as isatoribine act as agonists of TLR7,⁹ we also explored whether **3i** could induce the TLR-mediated immune response, an answer to which would allow us to better understand the mechanism behind the **3i**-induced immune response. To do so, we focused on TLR7, TLR3, and TLR9, since all three TLRs are located in endosomes, detect microbial nucleic acids, and are involved in viral recognition that has also been implicated in cancer development.²⁵ Bone marrow dendritic cells derived from wild-type, TLR3^{-/-}, TLR7^{-/-}, and TLR9^{-/-} mice were left untreated or stimulated with **3i**. After 24 h, the culture supernatants were collected and the protein levels of IL-12p40 assessed by ELISA. The production of IL-12p40 by wild-type, TLR3^{-/-}, and TLR9^{-/-} cells increased upon **3i** treatment compared with untreated cells (Figure 5B). Interestingly, TLR7^{-/-} cells were totally unresponsive even upon stimulation with 10 μ M **3i** (Figure 5B), suggesting that **3i** acts through TLR7 signaling. Although TLRs are best known for their ability to control host defense upon infection, emerging evidence suggests that TLRs can serve as negative regulators of cancer through various mechanisms including the stimulation of adaptive immune system.²⁴ Among the different TLRs, TLR7 has received the most attention in cancer biology because of the robust anticancer activity of small-molecule agonists that act through TLR7.²⁵ Thus, the fact that **3i** can signal through TLR7 to

induce an immune response further highlights the promising role of **3i** as a lead of drug candidate for cancer therapy.

■ CONCLUSION

We have synthesized a novel family of bitriazolyl acyclonucleosides **3** via a Huisgen reaction with remarkably improved yields in mild reaction conditions. Among the synthesized compounds, **3i** exhibited the most powerful antiproliferative effects on numerous cancer cell lines. This anticancer activity was shown to be principally the result of caspase-dependent apoptosis. Most interestingly, **3i** also has the capacity to stimulate the immune response in dendritic cells via TLR7 signaling, demonstrating the dual anticancer and immunomodulatory activity of this lead compound. We believe our discovery of the dual-acting **3i** will provide a new perspective and advancement of our program on triazole nucleosides in the continuing quest for more potent candidates to treat cancer or other life-threatening diseases in which the immune system has an impact on the therapeutic outcome.

■ EXPERIMENTAL SECTION

All compounds were determined to be >95% pure by HPLC.

Synthesis of **3i.** Compound **6** (26.7 mg, 0.10 mmol), CuSO₄·5H₂O (1.3 mg, 0.005 mmol), and sodium ascorbate (9.9 mg, 0.05 mmol) were dissolved in a mixed solvent system (THF/H₂O, 3:1, 4 mL) under argon. The 1-(azidomethyl)pyrene (30.8 mg, 0.12 mmol) was added. The yellow mixture was stirred at 45 °C until complete consumption of **6**. Column chromatographic purification (petroleum ether/ethyl acetate, 1:1) afforded the product **3i** in a yield of 99% (52.2 mg).

■ ASSOCIATED CONTENT

Supporting Information

Synthesis of **3–14**, their NMR spectra, HPLC analysis, and biological assays. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Mellman, I.; Coukos, G.; Dranoff, G. Cancer immunotherapy comes of age. *Nature* **2011**, *480*, 480–489.
- (2) Sharma, P.; Wagner, K.; Wolchok, J. D.; Allison, J. P. Novel cancer immunotherapy agents with survival benefit: recent successes and next steps. *Nat. Rev. Cancer* **2011**, *11*, 805–812.

- (3) Ramakrishnan, R.; Antonia, S.; Gabrilovich, D. Combined modality immunotherapy and chemotherapy: a new perspective. *Cancer Immunol. Immunother.* **2008**, *57*, 1523–1529.
- (4) Proietti, E.; Moschella, F.; Capone, I.; Belardelli, F. Exploitation of the propulsive force of chemotherapy for improving the response to cancer immunotherapy. *Mol. Oncol.* **2012**, *6*, 1–14.
- (5) Galluzzi, L.; Senovilla, L.; Zitvogel, L.; Kroemer, G. The secret ally: immunostimulation by anticancer drugs. *Nat. Rev. Drug Discovery* **2012**, *11*, 215–233.
- (6) Herdewijn, P. *Modified Nucleosides in Biochemistry, Biotechnology and Medicine*; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2008.
- (7) Lee, J.; Chuang, T. H.; Redecke, V.; She, L.; Pitha, P. M.; Carson, D. A.; Raz, E.; Cottam, H. B. Molecular basis for the immunostimulatory activity of guanine nucleoside analogs: activation of Toll-like receptor 7. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6646–6651.
- (8) Clercq, E. D. The design of drugs for HIV and HCV. *Nat. Rev. Drug Discovery* **2007**, *6*, 1001–1018.
- (9) Ogawa, K.; Hige, S.; Nakanishi, M.; Yamamoto, Y.; Chuma, M.; Nagasaka, A.; Asaka, M. Immunological and mutagenic actions of ribavirin monotherapy preceding combination therapy with interferon for patients with chronic hepatitis C. *Antiviral Ther.* **2009**, *14*, 513–522.
- (10) Xia, Y.; Qu, F.; Peng, L. Triazole nucleoside derivatives bearing aryl functionalities on the nucleobases show antiviral and anticancer activity. *Mini-Rev. Med. Chem.* **2010**, *10*, 806–821.
- (11) Wan, J.; Xia, Y.; Liu, Y.; Wang, M.; Rocchi, P.; Yao, J.; Qu, F.; Neyts, J.; Iovanna, J. L.; Peng, L. Discovery of novel arylethynyltriazole ribonucleosides with selective and effective antiviral and antiproliferative activity. *J. Med. Chem.* **2009**, *52*, 1144–1155.
- (12) Xia, Y.; Liu, Y.; Wan, J.; Wang, M.; Rocchi, P.; Qu, F.; Iovanna, J. L.; Peng, L. Novel triazole ribonucleoside down-regulates heat shock protein 27 and induces potent anticancer activity on drug-resistant pancreatic cancer. *J. Med. Chem.* **2009**, *52*, 6083–6096.
- (13) Wang, M.; Xia, Y.; Qu, F.; Peng, L. Novel triazole nucleoside analogs with anticancer activity. *Chin. J. Med. Chem.* **2010**, *20*, 562–370.
- (14) Xia, Y.; Li, W.; Qu, F.; Fan, Z.; Liu, X.; Berro, C.; Rauzy, E.; Peng, L. Synthesis of bitriazolyl nucleosides and unexpectedly different reactivity of azidotriazole nucleoside isomers in the Huisgen reaction. *Org. Biomol. Chem.* **2007**, *5*, 1695–1701.
- (15) Li, W.; Xia, Y.; Fan, Z.; Qu, F.; Wu, Q.; Peng, L. Bitriazolyl acyclonucleosides with antiviral activity against tobacco mosaic virus. *Tetrahedron Lett.* **2008**, *49*, 2804–2809.
- (16) Wang, M.; Zhu, R.; Fan, Z.; Fu, Y.; Feng, L.; Yao, J.; Maggiani, A.; Xia, Y.; Qu, F.; Peng, L. Bitriazolyl acyclonucleosides synthesized via Huisgen reaction using internal alkynes show antiviral activity against tobacco mosaic virus. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 354–357.
- (17) Zhu, R.; Wang, M.; Xia, Y.; Qu, F.; Neyts, J.; Peng, L. Arylethynyltriazole acyclonucleosides inhibit hepatitis C virus replication. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3321–3327.
- (18) Liu, Y.; Xia, Y.; Li, W.; Cong, M.; Maggiani, A.; Leyssen, P.; Qu, F.; Neyts, J.; Peng, L. S-Aryltriazole acyclonucleosides: synthesis and biological evaluation against hepatitis C virus. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3610–3613.
- (19) Wang, M.; Xia, Y.; Fan, Y.; Rocchi, P.; Qu, F.; Iovanna, J. L.; Peng, L. A novel arylethynyltriazole acyclonucleoside inhibits proliferation of drug-resistant pancreatic cancer cells. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5979–5983.
- (20) Dyrager, C.; Börjesson, K.; Dinér, P.; Elf, A.; Albinsson, B.; Wilhelmsson, L. M.; Gröthli, M. Synthesis and photophysical characterisation of fluorescent 8-(1H-1,2,3-triazol-4-yl)adenosine derivatives. *Eur. J. Org. Chem.* **2009**, *2009*, 1515–1521.
- (21) Fan, Y.; Xia, Y.; Tang, J.; Rocchi, P.; Qu, F.; Iovanna, J.; Peng, L. Ligand-mediated highly effective and selective C–N coupling for synthesizing bioactive N-aryltriazole acyclonucleosides. *Org. Lett.* **2010**, *12*, 5712–5715.
- (22) Wu, J. Z.; Lin, C.-c.; Hong, Z. Ribavirin, viramidine and adenosine-deaminase-catalysed drug activation: implication for nucleoside prodrug design. *J. Antimicrob. Chemother.* **2003**, *52*, 543–546.
- (23) Morris, J. D. H.; Eddleston, A. L. W. F.; Crook, T. Viral infection and cancer. *Lancet* **1995**, *346*, 754–758.
- (24) Rakoff-Nahoum, S.; Medzhitov, R. Toll-like receptors and cancer. *Nat. Rev. Cancer* **2009**, *9*, 57–63.
- (25) Schon, M. P.; Schon, M. TLR7 and TLR8 as targets in cancer therapy. *Oncogene* **2008**, *27*, 190–199.