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N-Aryltriazole ribonucleosides with potent antiproliferative activity against drug-resistant pancreatic cancer

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ABSTRACT

Novel *N*-aryltriazole nucleosides were synthesized via Cu-mediated C–N cross-coupling reaction starting with 3-aminotriazole ribonucleoside and various boronic acids. Two of them exhibited potent apoptosis-related antiproliferative activity against the drug-resistant pancreatic cancer cell line MiaPaCa-2, with an increased potency compared to gemcitabine, the reference treatment for pancreatic cancer. A preliminary SAR study suggests that the appended *N*-aryl moiety and the substituent at its *para*-position, as well as the ribose sugar component, contribute considerably to the observed antiproliferative activity.

Synthetic nucleoside analogs are of considerable importance in the search for new structural leads with biologically interesting activity.[1] Over the last few years, our research program has aimed to develop novel triazole nucleoside analogs bearing aromatic systems on the nucleobase,[2–9] with a view to identifying new drug candidates against various cancers, particularly drug-resistant pancreatic cancer.[2,3] Pancreatic cancer represents one of the most lethal forms of human cancer, and drug resistance develops extremely fast.[10] The current first-line treatment for pancreatic cancer is based on gemcitabine (Fig. 1), a nucleoside drug which is only moderately effective yielding a mere 12% response rate, with a median survival period of five months and a five-year survival rate as low as 3%.[11] Consequently, there is an urgent need to develop new and more efficacious drug candidates to treat pancreatic cancer.[12]

We have previously developed a series of triazole nucleosides with appended aromatic moieties on the triazole ring, such as arylethynyltriazolyl,[2–4] bitriazolyl,[5,6] aryltriazolyl,[7,8] and *N*-aryl nucleosides,[9] with the rationale to combine the special properties of triazole heterocycles[13] with aromatic systems as enlarged nucleobases. Such triazole nucleoside analogs may exhibit favorable binding properties with the corresponding biological targets via greater and stronger interactions[14] and in turn offer novel mechanisms of action. Some of these triazole nucleosides (**I** and **II** in Fig. 1) indeed demonstrated potent anticancer activity against drug-resistant pancreatic cancer in vitro and in vivo,[2,3] with entirely new mechanisms of action responsible for the down-regulation of the heat shock protein Hsp27,[2] a novel target for anticancer therapy which plays an important role in the drug-resistance observed in certain cancers.[15–17]

In our continuing efforts to develop triazole nucleosides as novel anticancer drug candidates, we became interested in *N*-aryltriazole ribonucleosides (**A** and **B** in Fig. 1). The frequent occurrence of the *N*-aryl motif in many natural products and synthetic drugs[18] justifies the interest in their presence within the nucleobases of nucleoside analogs with respect to designing new nucleoside mimics. Additionally, we postulated that the relatively flexible amine linkage between the triazole ring and aryl moiety may confer an adaptive binding flexibility to the corresponding biological targets. This would be an advantage over our previously identified lead compounds **I** and **II** (Fig. 1), which have rather rigid triple bond bridge between the aryl moiety and triazole ring of the nucleobase.[2,3]

We have previously established the synthesis of *N*-aryltriazole acyclonucleoside analogs (**A** in Fig. 1) using *Chan-Lam* modified Cu-mediated cross-coupling reaction.[9] However, none of the synthesized acyclonucleoside analogs demonstrated any notable anticancer activity. Here, we report the synthesis of novel *N*-aryltriazole ribonucleoside analogs (**B** in Fig. 1) using the *Chan-Lam* cross-coupling reaction and the evaluation of their antiproliferative activity against pancreatic cancer cells. Two of the novel

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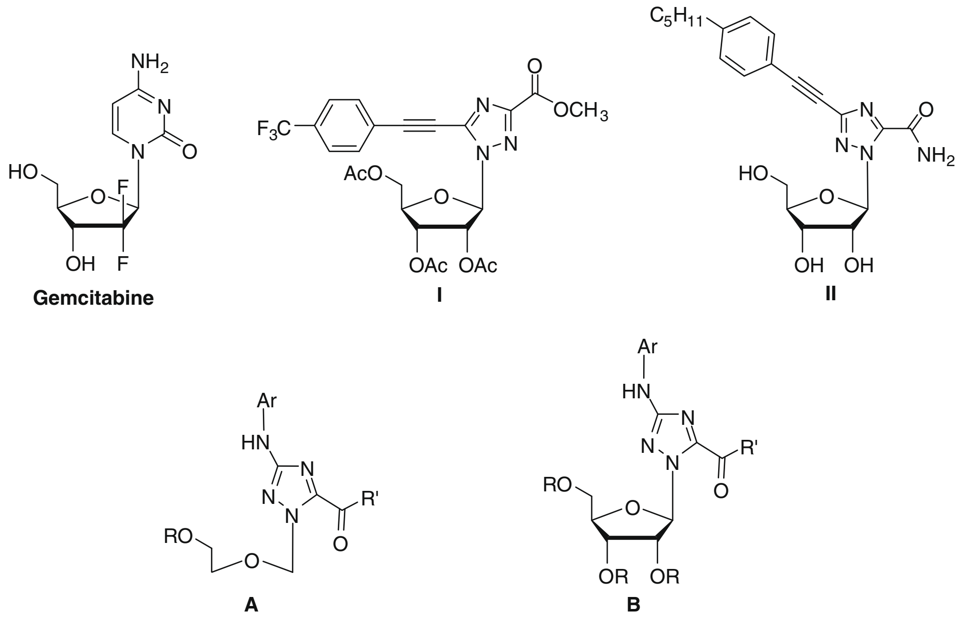
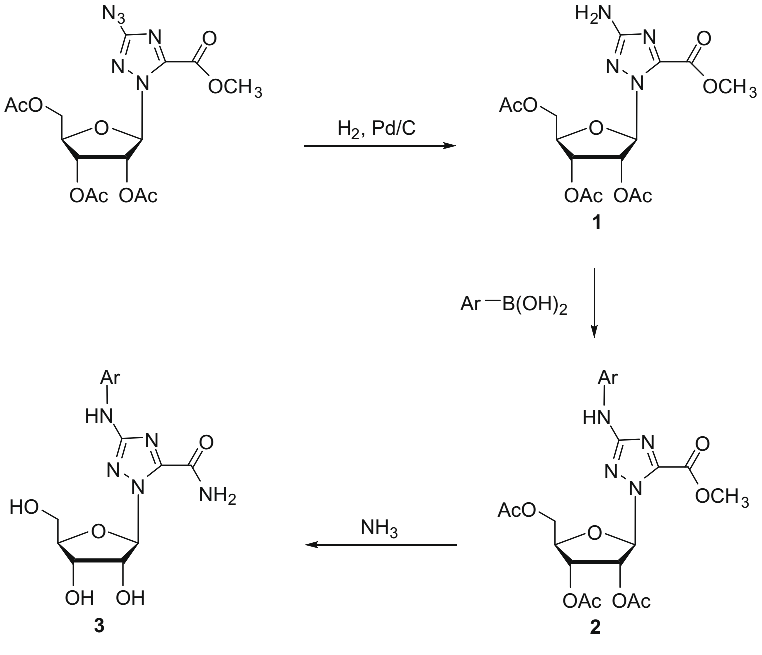


Figure 1. Gemcitabine, identified anticancer triazole nucleoside leads **I** and **II**, as well as *N*-aryltriazole acyclonucleosides (A) and ribonucleosides (B).

N-aryltriazole ribonucleoside analogs exhibited potent antiproliferative activity against the drug-resistant pancreatic cancer cell line MiaPaCa-2, presumably via a mechanism of caspase-dependent apoptosis induction.

The synthesis of *N*-aryltriazole ribonucleosides **A**, specifically **2** and **3** in Scheme 1, was achieved using our previously developed protocol of Cu-mediated C–N cross coupling (Scheme 1, Table 1).[9] The starting material 3-aminotriazole ribonucleoside **1** was obtained via reductive hydrogenation of the corresponding 3-azidotriazole ribonucleoside.[19,20] The N-arylation was performed with various boronic acids in the presence of $Cu(OAc)_2$ and pyridine with freshly prepared molecular sieves in CH_2Cl_2 at room temperature and open air for 3 days.[21] It should be noted that in many cases we were unable to obtain the corresponding products in reasonably good yields (Table 1). In particular, the Cu-mediated N-arylation reaction was not at all effective with sterically demanding arylboronic reagents (Table 1, entries 4 and 13). This could mainly be ascribed to the steric congestion created by the substituent at the *ortho*-position, which may in turn strongly impede C–N coupling. In addition, no clear-cut electronic effect was observed for the products bearing either electron-donating (Table 1, entries 7 and 8) or electron-withdrawing substituents (Table 1, entries 9–12) on the phenylamine. After further treatment in NH_3/MeOH at room temperature, the nucleosides **2** were transformed to the corresponding deprotected form **3** with good to excellent yields (Table 1).[22]



Scheme 1. Synthesis of *N*-aryltriazole ribonucleosides **2** and **3** starting from aminotriazole nucleoside **1** via Cu-mediated N-arylation followed by subsequent ammonolysis.

Table 1
N-Aryltriazole ribonucleosides **2** and **3** synthesized via Cu-mediated N-arylation and subsequent ammonolysis

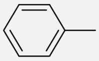
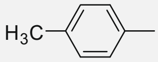
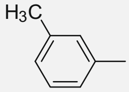
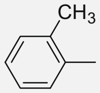
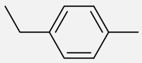
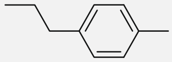
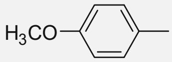
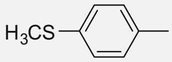
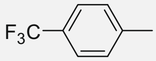
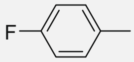
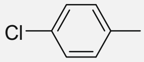
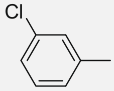
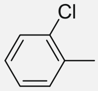
Entry	Ar	Product **2**	Yield (%)	Product **3**	Yield (%)
1		**2a**	44	**3a**	98
2		**2b**	44	**3b**	98
3		**2c**	60	**3c**	81
4		**2d**	0	**3d**	–
5		**2e**	43	**3e**	94
6		**2f**	46	**3f**	82
7		**2g**	34	**3g**	95
8		**2h**	29	**3h**	86
9		**2i**	21	**3i**	96
10		**2j**	50	**3j**	94
11		**2k**	42	**3k**	98
12		**2l**	40	**3l**	90
13		**2m**	0	**3m**	–

Table 2
The proliferation of human pancreatic cancer MiaPaCa-2 and Capan-2 cells as well as human prostate cancer PC-3 cells was assessed using MTT assay after treatment with 50 μM compound **2** or **3**, in comparison with gemcitabine and taxol, the clinical drugs for pancreatic cancer and prostate cancer, respectively

Entry	Compound	Cell proliferation (%)		
		MiaPaCa-2	Capan-2	PC-3
1	**2a**	137.7 ± 5.6	96.2 ± 2.2	91.0 ± 5.2
2	**2b**	58.6 ± 1.7	47.1 ± 1.4	68.6 ± 4.8
3	**2c**	99.9 ± 0.04	84.9 ± 6.0	94.2 ± 2.0
4	**2d**	–	–	–
5	**2e**	99.0 ± 1.8	90.0 ± 5.2	96.1 ± 1.3
6	**2f**	102.8 ± 1.9	95.9 ± 6.5	90.2 ± 2.9
7	**2g**	96.4 ± 1.0	72.0 ± 2.4	85.6 ± 4.5
8	**2h**	85.4 ± 2.5	88.1 ± 4.8	105.6 ± 1.9
9	**2i**	98.6 ± 3.6	64.4 ± 4.1	88.4 ± 2.3
10	**2j**	80.2 ± 6.4	79.8 ± 3.6	84.7 ± 2.4
11	**2k**	43.8 ± 1.6	59.6 ± 2.2	60.2 ± 1.2
12	**2l**	72.4 ± 6.4	65.8 ± 3.5	69.8 ± 4.3
13	**2m**	–	–	–
14	**3a**	68.0 ± 1.5	46.6 ± 2.0	73.9 ± 3.8
15	**3b**	85.6 ± 4.0	63.9 ± 3.0	107.5 ± 4.3
16	**3c**	100.1 ± 3.3	76.3 ± 2.8	96.2 ± 1.8
17	**3d**	–	–	–
18	**3e**	99.8 ± 3.7	66.0 ± 3.1	91.4 ± 0.7
19	**3f**	103.1 ± 2.8	71.5 ± 1.4	90.3 ± 1.5
20	**3g**	126.4 ± 1.0	126.2 ± 7.2	102.2 ± 2.3
21	**3h**	98.6 ± 2.8	71.1 ± 2.9	92.1 ± 2.7
22	**3i**	100.3 ± 0.4	121.9 ± 2.3	89.4 ± 3.9
23	**3j**	68.1 ± 4.7	62.9 ± 3.1	67.0 ± 5.4
24	**3k**	95.9 ± 1.5	72.8 ± 3.5	70.2 ± 4.2
25	**3l**	88.7 ± 1.5	66.2 ± 1.5	77.4 ± 1.8
26	**3m**	–	–	–
27	Gemcitabine	70.4 ± 3.4	32.7 ± 1.0	–
28	Taxol	–	–	41.3 ± 0.2

We then assessed the anticancer activity of the synthesized *N*-aryltriazole ribonucleosides **2** and **3** against the drug-resistant human pancreatic cancer cell line MiaPaCa-2, drug-sensitive human pancreatic cancer cell line Capan-2 and hormone-refractory human prostate cancer cell line PC-3.[23] Cells were treated with 50 μM compound for 48 h in comparison with non-treatment control. The number of viable cells remaining after treatment was determined by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTT) colorimetric assay (Table 2). To our great delight, two compounds **2b** and **2k** displayed interesting dose–responsive inhibitory activity on cell growth of MiaPaCa-2 cells, with a much better potency than the control reference drug gemcitabine (Fig. 2A), and an equivalent activity compared to the previously identified two triazole nucleoside leads **I** and **II** (Fig. 2B). In addition, the inhibitory effect of **2b** and **2k** on cell growth seems to be general, because both of them elicited also some inhibition on the drug-sensitive pancreatic cancer Capan-2 cells and hormone-refractory prostate cancer PC-3 cells (Table 2). However, their inhibitory effects on Capan-2 and PC-3 cells are much less effective than the corresponding clinical references, gemcitabine and taxol, respectively. We thus focused our attention only on their activity on drug-resistant pancreatic cancer MiaPaCa-2 cells.

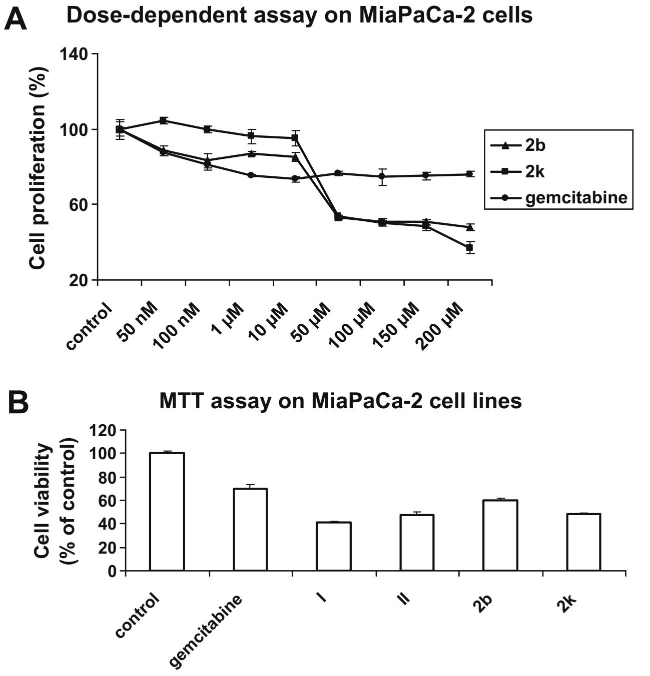


Figure 2. Inhibitory effect of compounds **2b** and **2k** on the pancreatic cancer MiaPaCa-2 cell growth, assayed by MTT test. (A) Dose–responsive effect of **2b** and **2k** on the growth of MiaPaCa-2 cells compared to gemcitabine. (B) Antiproliferative activity of **2b** and **2k** on the MiaPaCa-2 cells in comparison with non-treatment control, gemcitabine control and the previously identified two triazole nucleoside leads **I** and **II**.

A preliminary delineation of the structural features behind the antiproliferative activity shown by compounds **2b** and **2k** on MiaPaCa-2 cells was obtained, based on comparing the analogs with different substituents on the phenyl ring. Compounds bearing strong electron-donating (**2g** and **2h**) and electron-withdrawing groups (**2i**) on the phenyl ring elicited no considerable inhibitory effect on cell proliferation. This may be due to their unfavorable electronic properties. In addition, replacing the methyl functionality and the Cl atom on the phenyl moiety in **2b** and **2k** with either smaller atoms such as H and F (**2a** and **2j**) or larger groups such as ethyl and propyl groups (**2e** and **2f**), led to a significant reduction in activity. Further analogs harboring a substitution at the *meta*-position (**2c** and **2l**) displayed considerably decreased activity. Consequently, the size and the position, as well as the electronic properties of the substituent on the phenyl ring all contribute to the observed antiproliferative activity of **2b** and **2k**.

We next examined the importance of the sugar component based on the structural analogs of **2b**. Removing the protecting groups at the ribose sugar led to an inactive analog **3b**. The reason that the acetyl protected analog **2b** elicited activity may be ascribed to the increased lipophilicity brought by the acetyl protection, which may facilitate the cell uptake and result in effective biological activity; whereas the unprotected analog is more polar, which may lead to an inefficient cell uptake and thus no activity. Further substituting the ribose sugar by an acyclic sugar component (**4** and **5** in Scheme 2)[9,24] significantly decreased the activity. These findings indicate the importance of the acetyl protection of ribose sugar. It is worthy to mention that **2b** and **2k** may have completed different mechanisms of action from gemcitabine, which may require detailed investigation in this direction.

We also assessed the role of the amine bridge between the aryl group and the triazole ring in **2b** (Scheme 2). Either removing the amine linkage (**6** in Scheme 2)[25] or replacing the amine functionality in **2b** with a rigid triple bond connection (**7** in Scheme 2)[2] or triazolyl ring (**8** in Scheme 2)[5a] led to a considerable reduction in activity (data not shown). We therefore concluded that both the flexible structure and the balanced polarity of amine linkage may contribute collectively to the observed antiproliferative activity.

We further studied the apoptosis-inducing activity of **2b** and **2k** in MiaPaCa-2 cells using fluorescence-activated cell sorting (FACS) flow cytometry.[26] Cells were treated with 50 μM of either **2b** or **2k**, using non-treatment as control. After 48 h of treatment, the cells were labeled with propidium iodide and immediately analyzed. An increase in the fraction of cells undergoing apoptosis (sub-G1/G0 fraction) was revealed following treatment with **2b** and **2k** compared to non-treatment control (Fig. 3A), indicating that the induction of apoptosis represented the main mode of activity. Interestingly, both **2b** and **2k** elicited similar apoptotic cell population compared to lead **I**, whereas much more important apoptosis than lead **II** at 50 μM. It is to note that lead **II** showed very significant apoptosis induction only at an elevated concentration of 100 μM.[2]

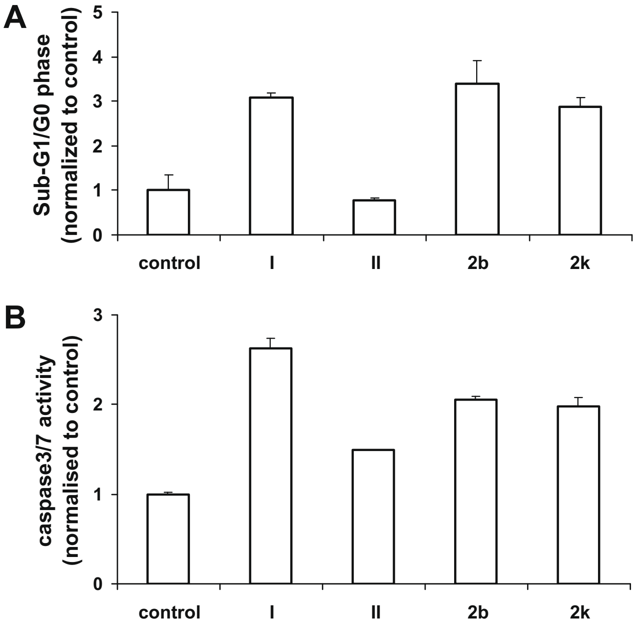
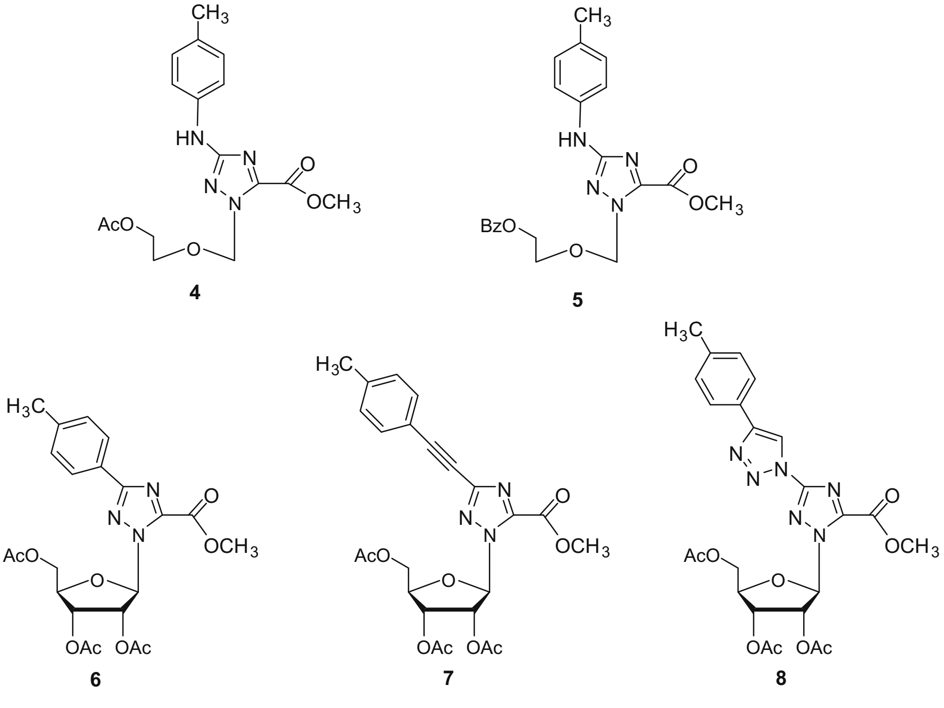


Figure 3. Apoptosis-induction in MiaPaCa-2 cells after treatment with the compounds **I**, **II**, **2b** and **2k**, using non-treatment as control. (A) Flow cytometry was used to quantify the percentage of cells undergoing apoptosis (cells in sub-G1/G0). (B) Caspase-3/7 activity was measured using Caspase-Glo luminescent assay.



Scheme 2. Structural analogs for SAR analysis of **2b**.

To further confirm the apoptosis-inducing activity of **2b** and **2k** in MiaPaCa-2 cells, we determined caspase-3/7 activity using a luminescent assay.[27] MiaPaCa-2 cells were treated with the test compound at 50 μM and the caspase-3/7 activity was measured by the cleavage of the luminogenic substrate containing the tetrapeptide sequence DEVD according to the instructions.[27] The obtained results revealed a similarly increase in caspase-3/7 activation following treatment with **2b** and **2k** compared to non-treatment control and positive control with previous leads **I** and **II** (Fig. 3B). Altogether, these findings suggest a caspase-3/7-dependent mechanism of apoptosis-induction.

In conclusion, we have synthesized a novel family of *N*-aryl-triazole ribonucleoside analogs via Cu-mediated C–N cross-coupling reaction. Although the N-arylation reaction could not deliver the corresponding products in good yields, the yield was sufficient to allow a preliminary in vitro assay to investigate their antiproliferative activity against drug-resistant human pancreatic cancer MiaPaCa-2 cells, drug-sensitive human pancreatic cancer Capan-2 cells and hormone-refractory human prostate cancer PC-3 cells. Two compounds elicited potent anticancer activity against the drug-resistant human pancreatic cancer cell line MiaPaCa-2, with a better potency than gemcitabine, the reference treatment for pancreatic cancer. This finding suggests that these two *N*-aryl-triazole nucleosides may represent novel structural leads in the search for new anticancer candidate drugs. We are currently pursuing the chemistry of N-arylation on triazole nucleosides with a view to significantly improving the reaction yield and broadening the substrate scope. Not only would we then have the necessary quantity of products for further in vivo and mechanistic studies but also have access to a large diversity of molecular structures for future structure/activity relationship analysis, essential to the search for novel anticancer drug candidates.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.02.104.

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20. *Preparation of 3-aminotriazole ribonucleoside **1**:* To a solution of 1.00 g of 3-azido-1-[2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl]-1,2,4-triazole-5-carboxylate (2.3 mmol) in 15 mL CH_2Cl_2, 0.10 g Pd/C catalyst (5 wt % of Pd, 25.6 mmol) was added. The reaction was then carried out with H_2 at a pressure of 1 atm. After the complete consumption of the starting material (detected with TLC), the mixture was filtered over Celite, the filtrate was concentrated under reduced pressure, giving 0.92 g of **1** as white powder in the yield of 98%.
21. *General procedure for preparing **2** via copper-mediated N-arylation with arylboronic acid:* To a mixture of 3-amino-1-[2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl]-1,2,4-triazole-5-carboxylate (100.0 mg, 0.25 mmol), 6 equiv arylboronic acid (1.50 mmol) and 68.2 mg anhydrous copper acetate (0.38 mmol) was added 5 mL dichloromethane (distilled freshly over calcium hydride). Then, 40.2 μL freshly distilled pyridine (0.50 mmol) and ca. 20 mg powder of 4 Å molecular sieve (activated at 500 °C for 5 h) were added rapidly. The mixture was stirred at room temperature for 3 days, and then filtered over Celite. The filtration was concentrated under reduced pressure, and the obtained residue was purified on silica gel with petroleum ether/ethyl acetate (1:1, v/v), giving the corresponding product **2** as powder.
22. *General procedure for preparing **3**:* The corresponding **2** was dissolved in 12 mL of a saturated NH_3/MeOH solution and stirred at room temperature for 2 days. Then the solvent was removed and the residue was washed three times with CH_2Cl_2. The washed residue was dried in vacuo to afford the corresponding product **3**.
23. *Anti-cancer assay in MiaPaCa-2:* Pancreatic cancer 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 μL of medium containing the same components as described above. Cells were allowed to attached overnight and then culture medium was removed and replace with fresh media alone as control or containing different compounds. Plates were further incubated at 37 °C and 5% CO_2 for 48 h. The number of viable cells remaining after the appropriate treatment with 50 μM compound was determined by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTT) colorimetric assay.
24. Compound **4** was synthesized following the protocol described in Ref. 9.
25. Compound **6** was synthesized following the protocol described in Ref. 8.
26. *FACS flow cytometry:* Cells were seeded in 10 cm dishes at the density of 10^6 cells/dish and allowed to adhere and proliferate overnight. Culture medium was then removed and fresh media containing the 50 μM 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 °C. After a wash with phosphate–citrate buffer, cells were treated with 200 μL RNase (500 μg/mL), labeled with 1 mL propidium iodide (50 μg/mL), and immediately analyzed by fluorescence-activated cell sorting (FACS Calibur, Becton Dickinson, Le Pont-De-Claix, France). Each sample was performed in triplicate.
27. *Caspase-3/7 cleavage assay:* Caspase-3/7 activity was measured using the Caspase-Glo 3/7 Assay 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 at 50 μM for 24 h. Next 100 μL of Caspase-Glo 3/7 Assay Reagent was added to each well of a white 96-well plate containing 100 μL of blank, control or cells in culture. The caspase-3/7 activity was measured by the cleavage of the luminogenic substrate containing the tetrapeptide sequence DEVD according to the instructions of the manufacturer (Promega). The plate was incubated at room temperature for 1 h before measuring the luminescence of each well. Each experiment was performed in triplicate.