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Bitriazolyl acyclonucleosides with antiviral activity against tobacco mosaic virus

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Abstract

Bitriazolyl acyclonucleosides were synthesized via the Huisgen reaction and then subjected to ammonolysis. The antiviral activity of these nucleosides against tobacco mosaic virus (TMV) was assessed. Like the previously described bitriazolyl compounds, these new bitriazolyl acyclonucleosides were found to show anti-TMV activity. This suggests that the bitriazolyl moieties are important structural features involved in the antiviral activity of these compounds.

Keywords: Triazole nucleosides; Acyclonucleosides; Bitriazoly compounds; Tobacco mosaic virus; Anti-TMV

Triazole heterocycles are important structural motifs with applications in the fields of medicinal chemistry and agrochemistry. Ribavirin (Scheme 1), a triazole nucleoside, which was the first synthetic nucleoside found to show antiviral activity against many viruses,[1] is the only small-molecular-weight drug available so far for treating viral infections caused by hepatitis C virus (HCV).[2] Fluconazole (Scheme 1), the structure of which comprises two 1,2,4-triazole units, is a powerful antifungal agent.[3] In our search for novel triazole compounds with potential medicinal and agrochemical applications,[4–8] we discovered that bitriazolyl compounds (**A** in Scheme 1) as well as bitriazolyl ribonucleosides (**B** in Scheme 1) have promising antiviral effects on the tobacco mosaic virus (TMV), an agricultural plant virus.[7,8] It is worth noting that the two triazole rings in the bitriazolyl motif show quasi co-planarity,[7,8] which results in an expanded aromatic system. This feature may promote the binding of bitriazolyl compounds to their biological targets by providing a larger aromatic binding surface, broader scope for H-bonding and greater polarity due to the triazole structural motifs.

Our ongoing search for further novel triazole compounds included a project for developing bitriazolyl acyclonucleosides (**C** in Scheme 1). Acyclic nucleosides belong to an important class of biologically active nucleosides. Acyclovir[9] (Scheme 1), which was the first acyclic nucleoside to be successfully developed as an antiviral drug, has become the gold standard for the treatment of herpes simplex viral infections.[2] HEPT (Scheme 1), a base-modified acyclic nucleoside derivative, efficiently inhibits HIV replication by acting as a non-nucleoside reverse transcriptase inhibitor while being much less toxic than the clinical drug AZT.[10] The bitriazolyl acyclonucleosides **C** can be classified as base-modified acyclonucleoside derivatives. Here we report on the synthesis and characterization of this new series of bitriazolyl acyclonucleoside derivatives and their antiviral activity against TMV.

Bitriazolyl acyclonucleosides were synthesized using the strategy previously developed for bitriazolyl

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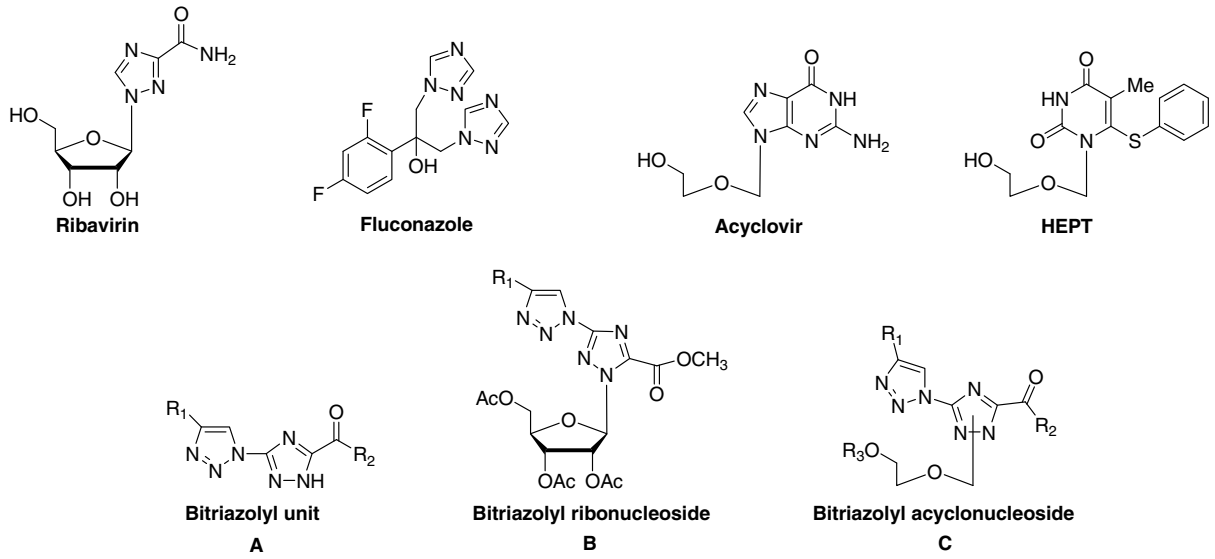


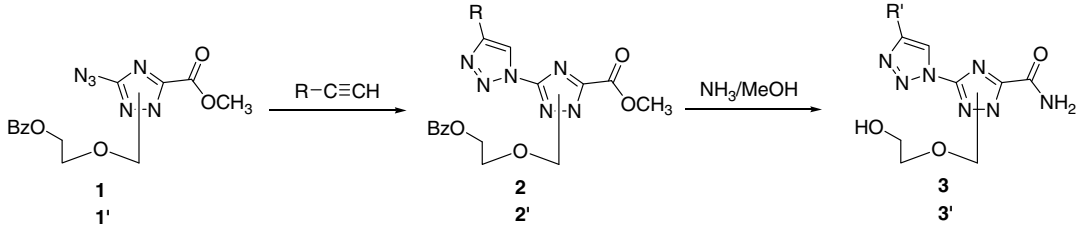
Scheme 1. Ribavirin, fluconazole, acyclovir, HEPT, bitriazolyl units (**A**), bitriazolyl ribonucleoside (**B**), and bitriazolyl acyclonucleoside (**C**).

ribonucleosides,[8] namely, via the Huisgen reaction[11] between azidotriazole acyclonucleosides and various terminal alkynes, and then subjected to ammonolysis in NH_3/MeOH (Scheme 2).

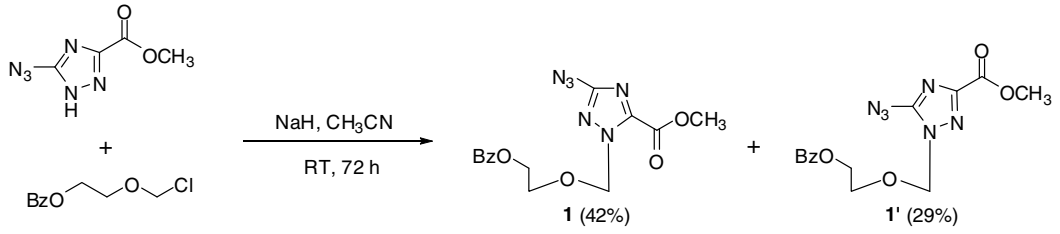
The starting materials for the Huisgen reaction, 3-azidotriazole acyclonucleoside (**1**) and 5-azidotriazole acyclonucleoside (**1′**), were prepared in yields of 42% and 29%, respectively, by alkylating the azidotriazole[6] with 2-(chloromethoxy)ethyl benzoate[12] in the presence of NaH (Scheme 3).[13] The reason why a more favorable yield was produced for **1** was mainly that much less steric congestion occurred between the azido group and the sugar moiety in **1**. Similar results were also observed when synthesizing the azidotriazole ribonucleosides, the photolabeling probes of ribavirin.[14]

Bitriazolyl acyclonucleosides **2** were formed in good to excellent yields[15] (Scheme 4, Table 1) via the copper(I)-catalyzed Huisgen reaction under similar conditions to those previously described.[6–8] In the case of products **2**, which were obtained in yields of less than 80%, the Cu(I) catalyst loading was increased from 5% to 30%. In most cases, this improved the product yields to 90% or more.

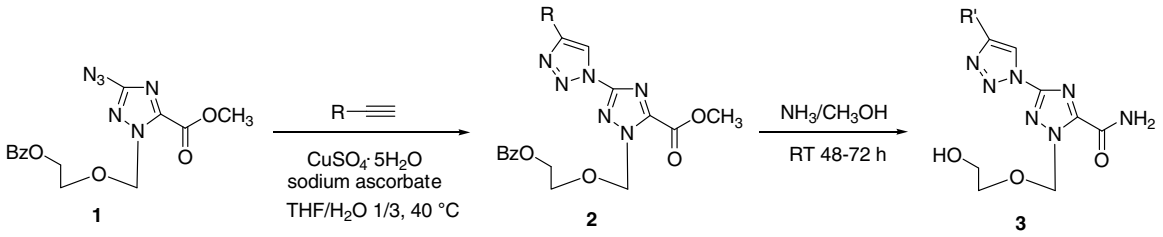
Further treatment of **2** in NH_3/MeOH at room temperature resulted in debenzoylation of the acyclic sugar moiety and amination of the carboxylester group on the triazole ring (Scheme 4 and Table 1), yielding products **3** in the form of white solids,[16] which could be directly precipitated out of the reaction solution. Compounds **3** were therefore purified by simply filtering and washing the precipitate. Although the ammonolysis reaction in NH_3/MeOH



Scheme 2. Synthesis of bitriazolyl acyclonucleosides.

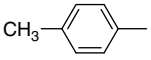
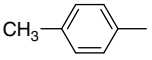
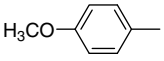
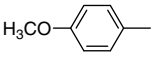
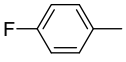
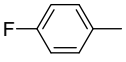
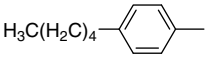
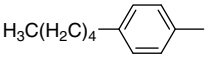
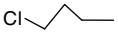
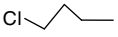
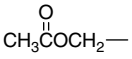
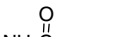


Scheme 3. Synthesis of azidotriazole acyclonucleosides **1** and **1′**.



Scheme 4. Bitriazolyl compounds **2** and **3** synthesized from **1.**

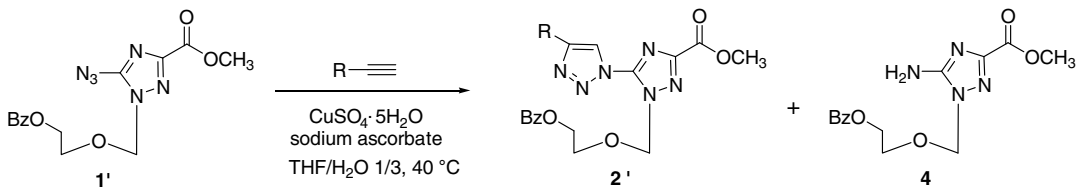
Table 1
Bitriazolyl compounds **2** and **3** synthesized from **1**

Entry	R	Product	Yield (%)	R′	Product	Yield (%)
1		**2a**	93[a]		**3a**	76
2		**2b**	91[b]		**3b**	66
3		**2c**	92[b]		**3c**	87
4		**2d**	79[a]		**3d**	86
5		**2e**	91[b]		**3e**	74
6		**2f**	90[b]		**3f**	74
7		**2g**	84[a]		**3g**	65
8		**2h**	81[a]		**3h**	63
9		**2i**	86[a]		**3i**	89
10		**2j**	82[a]		**3j**	94

[a] 5 mol % Copper(I) as catalyst at 40 °C.
[b] 30 mol % Copper(I) as catalyst at 40 °C.

worked very well, as indicated by TLC analysis, only moderate yields of **3** were observed in some cases, presumably due to the high solubility of these compounds in CH_2Cl_2 and MeOH, which resulted in considerable loss of product during the precipitation and filtration work-up procedures.

Synthesis of bitriazolyl compounds **2′** and **3′** proved to be problematic. Huisgen reaction with **1′** gave low to moderate yields of the desired products **2′**,[17] along with considerable amounts of 5-aminotriazole acyclonucleoside **4**, the reduction product of **1′** (Scheme 5 and Table 2).



Scheme 5. Synthesis of **2′** via Huisgen reaction starting with **1′**.

Table 2
Synthesis of **2′** via Huisgen reaction starting with **1′**

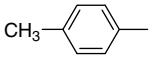
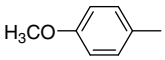
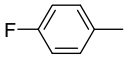
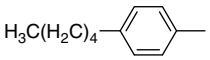
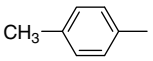
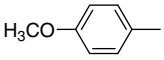
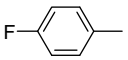
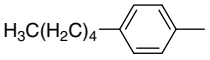
Entry	R	**2′** (%)	**4** (%)
1		**2a′** (44.6)	31.9
2		**2b′** (48.0)	28.8
3		**2c′** (41.8)	30.6
4		**2d′** (41.6)	45.4
5		**2e′** (36.1)	30.0

Table 3
Synthesis of **3′** via ammonolysis of **2′**

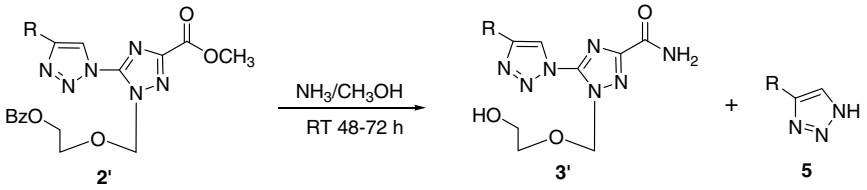
Entry	R	Product	Yield (%)	Product	Yield (%)
1		**3a′**	38.5	**5a**	61.4
2		**3b′**	45.6	**5b**	54.2
3		**3c′**	26.0	**5c**	71.3
4		**3d′**	46.2	**5d**	42.3
5		**3e′**	51.7	**5e**	47.1

In some cases, **2′** could not be isolated in pure form due to the formation of numerous side products (data not shown). As we know from our previous study, since the azido group in the 5-azidotriazole nucleoside is more electron-deficient than its counterpart in the 3-azidotriazole nucleoside isomer, it undergoes copper-catalyzed reduction more readily than the Huisgen reaction in the presence of sodium ascorbate.[8] Contrary to what occurred with the 5-azidotriazole ribonucleoside, which yielded no Huisgen adduct in our previous study,[8] the present Huisgen reaction with 5-azidotriazole acyclonucleoside **1′** gave low to moderate yields of products **2′**, which suggests that the steric hindrance exerted by the sugar moiety may also be one of the factors inhibiting the Huisgen reaction with azidotriazole nucleoside isomers. All in all, the electron-deficient nature of the azido group and the steric hindrance to which it is subjected both make 5-azidotriazole acyclonucleoside **1′** rather unsuitable for the Huisgen reaction.

The subsequent ammonolysis of **2′** was not a straightforward procedure either. Only low to moderate yields of the expected products **3′** were obtained:[18] considerable amounts of **2′** were decomposed and by-product **5** was formed (Scheme 6 and Table 3).[19] The reason for this outcome was probably that the 1,2,3-triazolyl group is a good leaving group, which is ready to be displaced when connected to an electron-deficient aromatic system. It has been reported by Robins et al. that the 1,2,4-triazolyl group is a good leaving group for S_NAr reactions and metal-catalyzed cross-coupling reactions.[20] The present finding suggests that 1,2,3-triazolyl might retain similar reactivity as 1,2,4-triazolyl in chemical reactions when acting as a leaving group. Since the 1,2,3-triazolyl group can be easily introduced via the Huisgen reaction, this finding may open new avenues for the chemical synthesis of nucleosides and heterocycles.

The bitriazolyl acyclonucleosides **2**, **2′**, **3**, and **3′** synthesized were tested to assess their antiviral activity against TMV, using the previously described[7] conventional half-leaf juice rubbing method[8,21] on fresh tobacco plant leaves. The results obtained are listed in Table 4, in comparison with ribavirin (the control substance). Compounds **2b**, **2f**, **3e**, **3i**, and **3j** showed similar levels of antiviral activity to ribavirin (Table 4). These active compounds have chemically and structurally diverse substituents acting on the bitriazolyl unit, which suggests that anti-TMV activities in this family of compounds may be compatible with some structural diversity in this position. It is also worth noting that the active acyclonucleoside **2b** shows exactly the same bitriazolyl structural motif as the corresponding active ribonucleoside.[8] These results suggest on the whole that the bitriazolyl scaffold may be a potentially useful structural motif for designing candidates with antiviral activity against TMV, regardless of whether the sugar moiety is acyclic or cyclic ribosyl.

In conclusion, a new series of bitriazolyl acyclonucleosides was synthesized via the Cu(I)-catalyzed Huisgen reaction using azidotriazole acyclonucleosides and various terminal alkynes. Huisgen reactions with 5-azidotriazole acyclonucleoside proved to be much less straightforward than with 3-azidotriazole acyclonucleoside, giving lower product yields and considerable levels of by-product formation. This is in line with our previous findings on



Scheme 6. Synthesis of **3′** via ammonolysis of **2′**.

Table 4
Antiviral activity of bitriazolyl acyclonucleosides against TMV

Compound	Anti-TMV[a] activity (%)	Compound	Anti-TMV[a] activity (%)
2a	33 ± 4	**3a**	34 ± 6
2b	49 ± 1	**3b**	0
2c	16 ± 4	**3c**	0
3d	22 ± 11	**3d**	21 ± 2
2e	36 ± 7	**3e**	42 ± 2
2f	47 ± 3	**3f**	29 ± 4
2g	10 ± 1	**3g**	0
2h	21 ± 4	**3h**	27 ± 13
2i	6 ± 5	**3i**	46 ± 4
2j	15 ± 5	**3j**	42 ± 8
2a′	0	**3a′**	14 ± 7
2b′	0	**3b′**	0
2c′	0	**3c′**	21 ± 7
2d′	18 ± 6	**3d′**	34 ± 2
2e′	6 ± 3	**3e′**	17 ± 5
Ribavirin	49 ± 8		

[a] Antiviral inhibition percentage (%) calculated by comparing the average numbers of the viral inflammations on the two half of leaves with and without treating of the active compounds.[7,8,21]

the synthesis of bitriazolyl ribonucleosides:[8] the electron-deficient nature of the azido component in the triazole ring of 5-azidotriazole acyclonucleoside and steric congestion make azide reduction easier to perform than Huisgen cycloaddition. Like the previously synthesized bitriazolyl compounds **A** and **B**, some of the newly synthesized bitriazolyl acyclonucleosides **C** showed anti-TMV activity. This finding further confirms that the bitriazolyl motif is involved in anti-TMV activity. We are currently studying structure/activity relationships in this family of compounds and screening bitriazolyl leads against other viruses.

Experimental: ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra at 75 MHz 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 internal reference. FAB and ESI MS were determined using ZAB-HF-3F or Finnigan LCQ Advantage mass spectrometer. High resolution mass spectra were obtained by Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) using an IonSpec 4.7 Tesla fourier transform mass spectrometer. Flash chromatography was performed using silica gel (200–300 mesh) from Qingdao Ocean Chemicals in China.

Acknowledgments

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Supplementary data

Analytical data, ^{1}H NMR and ^{13}C NMR spectra of all the new compounds described. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.02.139.

References and notes

1. Sidwell, R. W.; Huffman, J. H.; Khare, G. P.; Allen, L. B.; Witkowski, J. T.; Robins, R. K. *Science* **1972**, *177*, 705–706.
2. De Clercq, E. *Nature Rev. Drug Discovery* **2002**, *1*, 13–25.
3. Al-Soud, Y. A.; Al-Dweri, M. N.; Al-Masoudi, N. A. *IL FARMACO* **2004**, *59*, 775–783.
4. Wan, J. Q.; Zhu, R. Z.; Xia, Y.; Qu, F. Q.; Wu, Q. Y.; Yang, G. F.; Neyts, J.; Peng, L. *Tetrahedron Lett.* **2006**, *47*, 6727–6731.
5. Zhu, R. Z.; Qu, F. Q.; Quéléver, G.; Peng, L. *Tetrahedron Lett.* **2007**, *48*, 2389–2393.
6. Xia, Y.; Qu, F. Q.; Li, W.; Wu, Q. Y.; Peng, L. *Heterocycles* **2005**, *65*, 345–352.
7. Xia, Y.; Fan, Z. J.; Yao, J. H.; Liao, Q.; Li, W.; Qu, F. Q.; Peng, L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2693–2698.
8. Xia, Y.; Li, W.; Qu, F. Q.; Fan, Z. J.; Liu, X. F.; Berro, C.; Rauzy, E.; Peng, L. *Org. Biomol. Chem.* **2007**, *5*, 1695–1701.
9. (a) Schaeffer, H. J.; Beauchamp, L.; De Miranda, P.; Elion, G. B.; Bauer, D. J.; Collins, P. *Nature* **1978**, *272*, 583–585; (b) Elion, G. B.; Furman, P. A.; Fyfe, J. A.; De Miranda, P.; Beauchamp, L.; Schaeffer, H. J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5716–5720.
10. (a) Miyasaka, T.; Tanaka, H.; Baba, M.; Hayakawa, H.; Walker, R. T.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1989**, *32*, 2507–2509; (b) Tanaka, H.; Takashima, H.; Ubasawa, M.; Sekiya, K.; Nitta, I.; Baba, M.; Shigeta, S.; Walker, R. T.; De Clercq, E.; Miyasaka, T. *J. Med. Chem.* **1992**, *35*, 4713–4719; (c) Ren, J.; Esnouf, R.; Garman, E.; Somers, D.; Ross, C.; Kirby, I.; Keeling, J.; Darby, G.; Jones, Y.; Stuart, D.; Stammers, D. *Nature Struct. Biol.* **1995**, *2*, 293–302.
11. (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021; (b) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128–1137; (c) Bock, V. D.; Hiemstra, H.; Maarseveen, J. H. *Eur. J. Org. Chem.* **2006**, 51–68; (d) Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* **2007**, *36*, 1249–1262.
12. (a) Keyser, G. E.; Bryant, J. D.; Barrio, J. R. *Tetrahedron Lett.* **1979**, *20*, 3263–3264; (b) Robins, M. J.; Hatfield, P. W. *Can. J. Chem.* **1982**, *60*, 547–553.
13. *Preparation of* **1** *and* **1′**: In a solution of methyl 5-azide-1,2,4-triazole-3-carboxylate (1.25 g, 7.4 mmol) in 40 mL anhydrous acetonitrile, 0.28 g of sodium hydride (70% in mineral oil, 8.2 mmol) was added portionwise under strong stirring. When sodium hydride was completely dissolved, 1.58 mL (8.9 mmol) of 2-(chloromethoxy)ethyl benzoate was added dropwise, and the mixture was stirred at room temperature for 72 h. The reaction mixture was filtrated and the filtrate was concentrated under reduced pressure. The residue was purified on silica gel with petroleum ether/ethyl acetate (2:1, v/v), affording **1** as a white solid (1.07 g, 41.8%) and **1′** as a waxy solid (0.75 g, 29.2%).
14. Wu, Q. Y.; Qu, F. Q.; Wan, J. Q.; Zhu, X.; Xia, Y.; Peng, L. *Helv. Chim. Acta* **2004**, *87*, 811–819.
15. *General procedure for preparing* **2** *via copper(I)-catalyzed Huisgen reaction*: Azide (**1**) (ca. 0.10 mmol) and the corresponding alkyne (1.2 equiv, 0.12 mmol) were dissolved in 8 mL THF/H_2O (1:3, v/v). A freshly prepared aqueous solution of sodium ascorbate (0.5 equiv, 0.05 mmol, in 0.1 mL water) was added, followed by a freshly prepared aqueous solution of $CuSO_4{\cdot}5H_2O$ (0.05 equiv or 0.3 equiv in 0.1 mL water). The reaction mixture was stirred at 40 °C until

complete consumption of **1**. The reaction mixture was extracted with ethyl acetate (3 × 10 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, and concentrated. The obtained residue was purified on silica gel with petroleum ether/ethyl acetate (2:1, v/v), giving the corresponding product **2** as a white powder.

16. *General procedure for preparing* **3**: Compound **2** (ca. 100 mg) was suspended in approximately 15 mL of NH_3/CH_3OH solution and stirred at room temperature for 2–3 days. The solvent was removed under reduced pressure and the residue was diluted with CH_2Cl_2. The white precipitate of **3** was obtained by filtration, washed with fresh CH_2Cl_2, and dried *in vacuo*.
17. *General procedure for preparing* **2′**: Similar as that for preparing **2**. **4** was obtained by further eluting the column, after elution of **2′**, with CH_2Cl_2/CH_3OH (30/:1, v/v).
18. *General procedure for preparing* **3′**: Similar as that for preparing **3**. The reaction residue was purified on silica gel with petroleum ether/ethyl acetate (1:1, v/v), leading to the corresponding triazole by-product **5**. **3′** were obtained then by further elution of the column with CH_2Cl_2/CH_3OH (30:1, v/v).
19. Kallander, L. S.; Lu, Q.; Chen, W.; Tomaszek, T.; Yang, G.; Tew, D.; Meek, T. D.; Hofmann, G. A.; Schulz-Pritchard, C. K.; Smith, W. W.; Janson, C. A.; Ryan, M. D.; Zhang, G.; Johanson, K. O.; Kirkpatrick, R. B.; Ho, T. F.; Fisher, P. W.; Mattern, M. R.; Johnson, R. K.; Hansbury, M. J.; Winkler, J. D.; Ward, K. W.; Veber, D. F.; Thompson, S. K. *J. Med. Chem.* **2005**, *48*, 5644–5647.
20. (a) Nowak, I.; Robins, M. J. *J. Org. Chem.* **2006**, *71*, 8876–8883; (b) Liu, J.; Robins, M. J. *Org. Lett.* **2004**, *6*, 3421–3423; (c) Robins, M. J.; Miles, R. W.; Samano, M. C.; Kaspar, R. L. *J. Org. Chem.* **2001**, *66*, 8204–8210; (d) Zhong, M.; Nowak, I.; Robins, M. J. *J. Org. Chem.* **2006**, *71*, 7773–7779.
21. An, T. Y.; Huang, R. Q.; Yang, Z. D.; Zhang, K.; Li, G. R.; Yao, Y. C.; Gao, J. *Phytochemistry* **2001**, *58*, 1267–1269.