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ARTICLE

Microwave promoted C-O coupling for synthesizing *O*-aryloxytriazole nucleoside analogues

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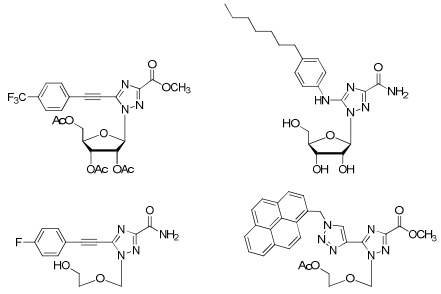
Synthetic nucleoside analogues with novel structural entities are of considerable importance in the search for new structural paradigms with biologically interesting activities. We report in this work that microwave promoted C-O coupling reaction is able to efficiently offer a large array of novel *O*-arylated triazole nucleosides with considerably improved yields and appreciably reduced reaction time. One of the synthesized compounds showed interesting anticancer activity against human prostate and pancreatic cancers. The synthetic method described in this work, not only highlights the importance of microwave irradiation in organic synthesis but also provides a promising access towards synthesizing novel nucleoside analogues which can offer molecular diversity in the quest for new and potential drug candidates.

Introduction

Nucleoside analogues remain one of the most fruitful drug classes for antiviral and anticancer therapeutics.[1] Many efforts have been directed towards the design and synthesis of nucleoside mimics *via* chemically modifying either the nucleobase or the sugar moiety, or both.[2] As part of our ongoing efforts to uncover new structural paradigms as drug candidates to combat cancer and viral infection with novel mechanisms of action, we have been working towards establishing original nucleoside analogues *via* appending aromatic moieties onto the triazole nucleobase.[3] The motivation behind us developing such triazole nucleosides is multifaceted. First of all, triazole is an unnatural heterocycle and nucleosides composed of triazole nucleobases are expected to be more resistant against nucleos(t)ide metabolizing enzymes and thus have better *in vivo* stability. Moreover, triazole is considered as a universal base capable of forming base-pairs with all five natural nucleobases,[4] and hence may more favorably interact with their biological targets. By conjugating aromatic systems to the triazole nucleobase, we may encompass the special characteristics of unnatural triazole heterocycles together with those of expanded and enlarged aromatic nucleobases;[5] this may offer stronger and more efficient binding to biological targets and thereby novel mechanisms of action.[6] It is also to note that triazole nucleosides can mimic guanosine and may possess potential immunoregulatory activity.[7] Among various triazole nucleoside analogues developed in our group, several ingenious triazole nucleosides bearing aromatic moiety at the nucleobase (Scheme 1) effectively demonstrated potent anticancer activity and antiviral activity.[6] Additionally, they exhibited entirely new mechanisms of action as well as dual immunomodulatory and anticancer activities.[6]

In our continuing efforts to develop triazole nucleosides as novel anticancer drug candidates, we have been interested in *O*-aryloxytriazol*e* nucleosides. This is because the frequent occurrence of the *O*-aryl motif in many natural products and clinical drugs[8] justifies the interest in their presence within nucleoside analogues with respect to establishing new structural paradigms with biological activities. Furthermore, we surmised that the relatively flexible *O*-linkage between the triazole ring and aryl moiety may confer an adaptive binding flexibility to the corresponding biological targets.[9] This would be an advantage over our previously identified lead compounds bearing arylethynyltriazolyl and bitriazolyl entities (Scheme 1), which carry a rather rigid bridge between the aryl moiety and triazole ring of the nucleobase. Finally, we wished to establish structural diverse analogues in order to gain better understanding of the relationships between structure and activity. Consequently, we embarked upon synthesizing *O*-aryloxytriazole nucleosides.



Scheme 1. Some representative triazole nucleoside analogues previously developed in our group.[6]

Aromatic nucleophilic substitution reaction (S_NAr) is the classical way for C-O coupling to introduce *O*-aryl moieties, and would appear to be the most convenient way to construct *O*-aryloxytriazole nucleosides. Nevertheless, this method often requires harsh conditions such as high temperature, long reaction time and unfriendly solvents like DMF or DMSO, making it neither economical nor ecological. Microwave

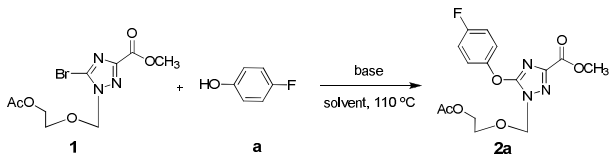
irradiation (MW) has been shown to promote organic reactions by shortening reaction time and lowering the temperature yet allowing higher product yield.[10] Indeed, microwave irradiation can heat molecules directly and bypass thermal conductivity, thereby leading to an enhanced reaction rate, improved selectivity and higher product yield.[10] We therefore explored the effect of submitting the aromatic nucleophilic substitution reaction S_NAr to microwave irradiation, in the synthesis of *O*-aryloxytriazole nucleosides. Indeed this combination has been proven to be very beneficial and rewarding, offering various *O*-aryloxytriazole nucleoside analogues with good to excellent yields within a very short reaction time. We present and discuss our results below.

Results and discussion

We first started with the screening of reaction conditions for direct aromatic nucleophilic substitution using bromotriazole nucleoside **1** and 4-fluorophenol as substrates (Table 1). 4-Fluorophenol is a challenging substrate for S_NAr reaction since it is electron-deficient, hence poorly nucleophilic. While we did manage to obtain the desired *O*-aryloxytriazole nucleoside **2a** using Cs_2CO_3 as base in CH_3CN under conventional oil bath heating (Table 1, entry 1), the yield of merely 41% was rather low. In addition, several by-products were also formed, possibly because of the decomposition of both the starting material and the product during the reaction process.

Microwave irradiation, introduced in order to promote the reaction, significantly improved the product yield from 41% to 78% and reduced the reaction time from 3 h to 30 minutes (Table 1, entry 2). Our results confirmed that the microwave-assisted reaction exhibited notable advantages over the conventional heating. It also suppressed the side reactions, considerably facilitating the purification process. Systematic screening of bases and solvents (Table 1) under MW using the optimised reagent ratio[11] revealed the best result was obtained with Cs_2CO_3 as base and CH_3CN as solvent (Table 1, entry 2).

Table 1. Optimization of S_NAr reaction using bromotriazole nucleoside **1** and 4-fluorophenol as substrates. [a]



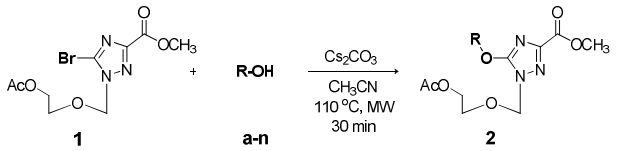
1 a 2a

entry	solvent	base	heating mode	reaction time	yields % [a]
1	CH_3CN	Cs_2CO_3	Oil bath	3 h	41 [b]
2	CH_3CN	Cs_2CO_3	MW	30 min	78 [b]
3	CH_3CN	K_2CO_3	MW	30 min	44
4	CH_3CN	Na_2CO_3	MW	30 min	4
5	CH_3CN	Li_2CO_3	MW	30 min	0
6	CH_3CN	TEA	MW	30 min	0
7	Toluene	Cs_2CO_3	MW	30 min	6
8	THF	Cs_2CO_3	MW	30 min	5
9	DMF	Cs_2CO_3	MW	30 min	45

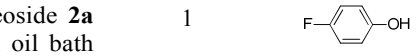
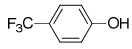
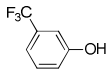
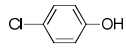
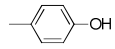
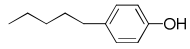
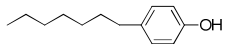
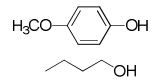
[a] compound **1** (0.10 mmol), phenol (0.30 mmol), base (0.10 mmol), solvent (0.75 mL), MW, 110 °C, calculated yield with ^{1}H NMR; [b] isolated yield.

Under these optimized conditions, we obtained the desired products successfully using electron-deficient phenols as nucleophiles (Table 2, entries 1-7). We expected the electron-neutral and electron-rich phenols to be stronger nucleophiles and hence better substrates for S_NAr reaction than the electron-deficient phenols.[12] Surprisingly however, the electron-neutral and electron-rich phenol substrates delivered the corresponding *O*-arylation products with markedly reduced yields (Table 2, entries 8-14).

Table 2. Synthesis of *O*-aryloxytriazole nucleosides **2** *via* microwave irradiation (MW) assisted S_NAr reaction with 5-bromotriazole nucleoside **1**.



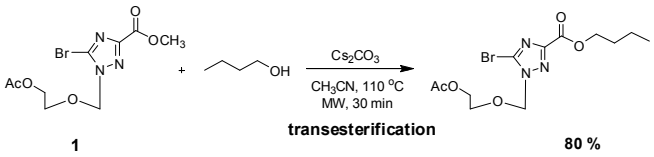
1 a-n 2

entry	R-OH	product	yields % [a]
1		**2a**	78
2		**2b**	74
3		**2c**	50
4		**2d**	62 [b]
5		**2e**	80
6		**2f**	35 [b]
7		**2g**	61
8		**2h**	44
9		**2i**	34
10		**2j**	37
11		**2k**	32
12		**2l**	44
13		**2m**	47
14		**2n**	40
15		**2o**	0

[a] compound **1** (0.10 mmol), phenol (0.30 mmol), Cs_2CO_3 (0.10 mmol), CH_3CN (0.75 mL), 110 °C, MW, 30 min, isolated yield; [b] Cs_2CO_3 (1.5 eq).

Further inspection of reaction products led us to isolate and identify a transesterification product (Scheme 2). This finding indicates that the electron-neutral and electron-rich phenols were also good substrates for the transesterification reaction on the methyl ester of the triazole ring, which competed with the

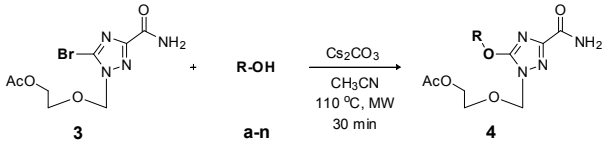
S_NAr reaction and led to significantly reduced yield for the corresponding *O*-arylation product.

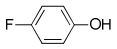
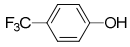
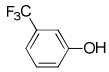
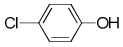
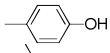
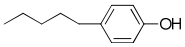
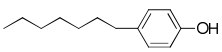
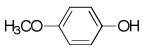


Scheme 2. Additional side product identified during the synthesis of *O*-oxytriazole nucleoside **2o.**

To avoid this transesterification, we then opted for triazole nucleoside **3** as substrate (Table 3), for which the methyl ester group in **1** was replaced by an amide group. Remarkably, both

Table 3. Synthesis of *O*-aryloxytriazole nucleosides **4** *via* microwave irradiation (MW) assisted S_NAr reaction with 5-bromotriazole nucleoside **3**.



entry	R-OH	product	yields % [a]
1		**4a**	86
2		**4b**	88 [b]
3		**4c**	84
4		**4d**	55
5		**4e**	81 [b]
6		**4f**	48 [b]
7		**4g**	84
8		**4h**	85
9		**4i**	88
10		**4j**	80
11		**4k**	74
12		**4l**	81
13		**4m**	84
14		**4n**	80
15		**4o**	0 [c]

[a] compound **3** (0.10 mmol), phenol (0.30 mmol), Cs_2CO_3 (0.10 mmol), CH_3CN (0.75 mL), 110 °C, MW, 30 min, isolated yield; [b] Cs_2CO_3 (1.5 eq). [c] The yield of recycled starting material is 90%.

electron-donating and electron-withdrawing substituted as well as sterically hindered phenols made all competent substrates (Table 3, entries 1-14). Moreover, there was little or no side-product formed during the reaction, which considerably facilitated the product isolation and purification, offering the desired *O*-arylation products with much improved yields. Noteworthy is the lower product yields afforded by the strongly electron-deficient substrates, *o*- and *p*-trifluoromethyl phenols. This can be ascribed to their unfavorable nucleophilicity for S_NAr reaction. It is also to note the poor deprotonation ability of the aliphatic alcohol rendering this substrate particularly challenging (entry 15).

With these newly synthesized *O*-aryloxytriazole nucleoside analogues in hand, we assessed their anticancer activity on human prostate cancer PC-3 and LNCaP cells, and human pancreatic cancer MiaPaCa-2 and Capan-2 cells. Compound **4m** showed interesting anticancer activity on both prostate and pancreatic cancer cells (Figure 1).

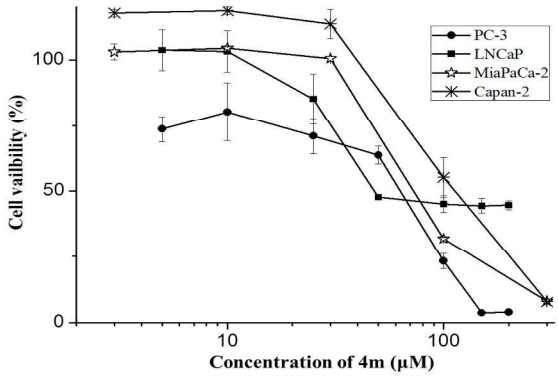
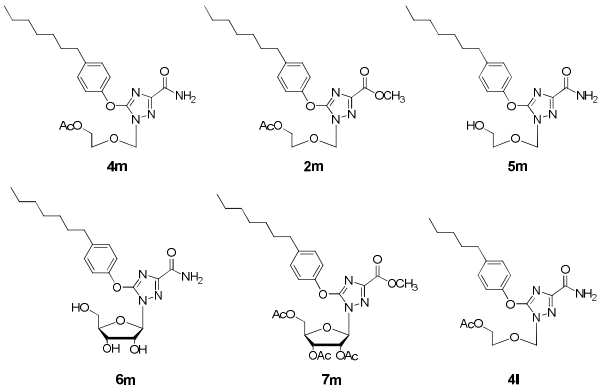


Figure 1. Inhibitory effect of compound **4m** on human prostate cancer PC-3 and LNCaP cells, and human pancreatic cancer, MiaPaCa-2 and Capan-2 cells.

Encouraged by this finding, we further synthesized and tested the ester analogue **2m** and its deprotected form **5m**, as well as the ribonucleoside derivatives **6m** and **7m,** and the derivative bearing shorter alkyl chain **4l** (Scheme 3). Unfortunately, none of these analogues exhibited any notable anticancer activity (data not shown). These seemingly negative results nevertheless provide important instrumental information for further structure/activity relationship studies of triazole nucleoside analogues, in the quest for better anticancer candidates.



Scheme 3. Structural analogues of **4m** studied in this work.

Conclusions

In summary, we have reported here an effective method for synthesizing *O*-aryloxytriazole nucleoside analogues using microwave promoted S_NAr reaction. Microwave irradiation significantly promoted the C-O coupling reaction, offering considerably improved product yield and appreciably reduced reaction time. Consequently, a large array of *O*-aryloxytriazole nucleosides was synthesized efficiently. The synthetic method described here, not only highlights the importance of microwave irradiation in organic synthesis but also provides a promising access towards synthesizing new triazole nucleoside analogues in the search for novel structural paradigms with biological activities. Indeed, compound **4m** exhibited interesting anticancer activity. The *O*-aryloxytriazole nucleosides newly synthesized in this study will also offer structural diversity on which to base further structure/activity relationship studies of nucleoside analogues in the quest for promising drug candidates. We are actively working in this direction.

Experimental section

General

Chemicals were purchased from Sigma Aldrich or Alfa Aesar and, except for all the solvents which were dried as described in methods and distilled before used, were employed directly without purification. The microwave equipment used was Explorer 12 Hybrid (Discover). The products were purified by flash chromatography on silica gel (Merck 200-300 mesh). ^{1}H NMR spectra were recorded at 250 or 400 MHz and ^{13}C NMR spectra recorded at 62.5 or 100 MHz, on Bruker Avance II 250, Bruker Avance III 400 spectrometers, and JEOL 400 spectrometers, respectively. The chemical shifts (δ) are expressed in parts per million (ppm) with the residual peak of $CHCl_3$ at 7.26 ppm. The high-resolution mass spectra (HRMS) were obtained with a MALDI/DHB or ESI-Positive mode on IonSpec 4.7 TESLA FTMS or Bruker Daltonics, Inc. APEXIII 7.0 TESLA FTMS. Thin Layer Chromatography (TLC) was performed on TLC plates of silica gel 60 F254, layer thickness 0.2 mm, Merck KGaA and the compounds were revealed by using UV light (254 and 364 nm). Human prostate cancer LNCaP and PC-3 cells as well as pancreatic cancer MiaPaCa-2 and Capan-2 cells were purchased from American Type Culture Collection. LNCaP cells were grown in Roswell Park Memorial Institute 1640 (RPMI 1640) medium supplemented with 10% fetal bovine serum (FBS). PC-3, MiaPaCa-2 and Capan-2 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS).

General Procedure of Synthesis

Compound **1** or **3** (0.10 mmol) and Cs_2CO_3 (0.10 mmol) were mixed in CH_3CN (0.75 mL) in an oven-dried 10 mL MW tube with a stirring bar under microwave irradiation at 110 °C for 30 min. The reaction mixture was concentrated under reduced pressure and the crude residue was purified by flash chromatography on silica gel using the mixture of cyclohexane and ethyl acetate as eluent. The purified material was dried in vacuum to afford the corresponding product **2a-2n** or **4a-4n.**

Anticancer activity evaluation

The relevant cancer cells were seeded into a 96-well plate and allowed to adhere overnight. Then the culture medium was removed and replaced with medium containing DMSO-vehicle control or different compounds at the indicated concentration. After 48 h treatment, the number of viable cells remaining was determined by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, MTT) colorimetric assay. All experiments were performed in triplicate and repeated three independent times.

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Notes and references

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

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11. The ratio of the reagents was also screened. The detail information was described in Table S1 in support information. The best reagent ratio turned out to be compound **1**/ phenol / Cs_2CO_3 = 1:3:1
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Graphical Abstract

A convenient and effective microwave-promoted synthesis of *O*-aryloxytriazole nucleosides was established, leading to an interesting candidate with anticancer activity.