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Synthesis and antiproliferative activity of RITA and its analogs

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ABSTRACT

The synthesis of RITA and a variety of five-membered heterocyclic triads by the cyclocondensation of 1,4-bis(5-substituted-2-thienyl or 2-furyl)-1,3-butadiynes with water or $Na_2S{\cdot}9H_2O$ in the presence of KOH in DMSO is described. The study on the antiproliferative activities against K562, MCF-7, A549, and HCT116 tumor cells has revealed that some of the heterocyclic triads show higher antiproliferative activities than RITA, depending on the structures of substituents, the property of heteroatoms as well as their numbers.

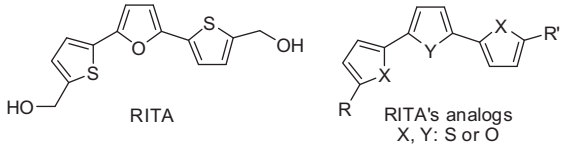
The tumor suppressor gene P53 plays a central transcription role in the regulation of DNA repair, cell cycle, apoptosis, and senescence.[1] MDM2 (murine double minute 2, also known as HDM2 in human) is a key negative regulator of P53 to inhibit the transcriptional activity.[2] RITA (Scheme 1), a five-membered heterocyclic triad: 2,5-bis(5-hydroxymethyl-2-thienyl)furan has been demonstrated to show high antitumor activity via its binding to p53 to block the interaction between p53 and HDM-2 (human double minute 2), so as to activate p53 function in antitumors.[3] Therefore, the investigation of antitumor activity of RITA and its analogs as well as their synthetic methods have become one of the interesting and important research topics in the filed of chemistry and biology.[4]

In continuation of our interest on the development of the synthetic routes with high-atom efficiency to construct five-membered heterocyclic compounds using 1,3-butadiyne derivatives as starting materials,[5] and evaluating and screening the antitumor activity of RITA and its analogs,[4d] in this Letter we would like to report not only an efficient and practical procedure for the synthesis of RITA and its analogs via the cyclocondensation reaction of 1,4-bis(5-substituted-2-thienyl or 2-furyl)-1,3-butadiynes with water or $Na_2S{\cdot}9H_2O$ in the presence of KOH in DMSO,[6] but also their antitumor activities against K562, MCF-7, A549, and HCT116 tumor cells.

Very recently, a general approach to arylated furans, pyrroles, and thiophenes via the cyclocondensation of 1,4-diaryl-1,3-butadiynes with water, primary amines, and $Na_2S{\cdot}9H_2O$ in the presence of superbase (KOH in DMSO) was reported by our group.[7] In order to develop an efficient procedure for the formation of RITA and its analogs via the cyclocondensation of 1,3-butadiynes bearing heteroaryl substituents, we re-optimized the reaction conditions, and found that in DMSO solvent, KOH could efficiently promote the formation of RITA and its analogs as concluded in Table 1.[8]

Since the synthesis of **2da** and **2ea** direct from the cyclocondensation of the corresponding 1,3-butadiynes was not successful under the present base conditions, they were then obtained by I_2-catalyzed deprotection of **2d** and **2e** in acetone (Scheme 2).

For comparison of antitumor activities of RITA's analogs, several other five-membered triheterocyclic compounds having the thienyl group as the central ring were also prepared via the cyclocondensation reactions of the corresponding 1,4-bis(5-substituted-2-thienyl or 2-furyl)-1,3-butadiynes with $Na_2S{\cdot}9H_2O$. As shown in Table 2, under the re-optimizing reaction conditions, the desired



Scheme 1. RITA and its analogs.

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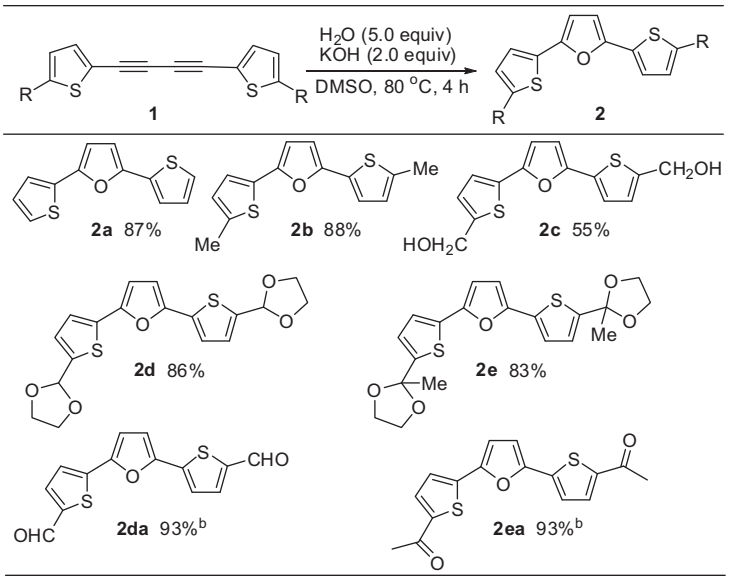
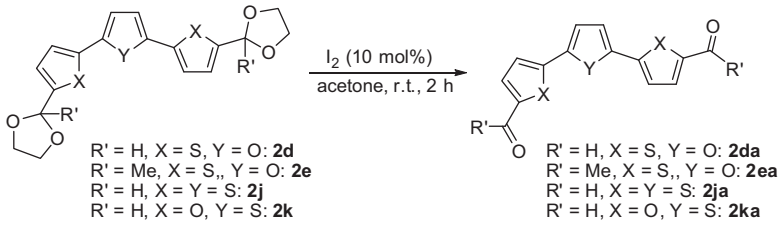
0040-4039/

Table 1
Synthesis of RITA and its analogs[a]



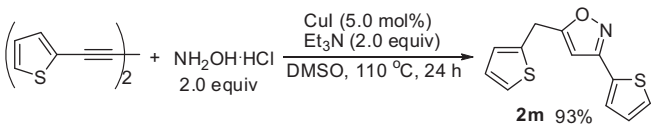
[b] **2da** and **2ea** were synthesized from **2d** and **2e**, respectively. See: Scheme 2.
[a] The reactions were carried out with 1.0 mmol of **1**, 5.0 mmol of H_2O, and 2.0 mmol of KOH in 2.5 mL of DMSO at 80 °C for 4 h.



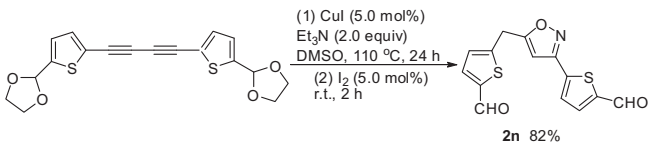
Scheme 2. Deprotection reactions.

2,5-bis(thienyl or furyl)thiophenes could be obtained in high yields in the presence of 0.5 equivalent of KOH at room temperature for 2 h.[8]

RITA is a derivative of furan bearing two thienyl groups, it should be also interesting to synthesize and evaluate the antitumor activity of other heterocyclic compounds bearing the thienyl group, which can be synthesized from the similar cyclocondensation of 1,4-bis(2-thienyl)-1,3-butadiyne. Therefore on the basis of the known process reported by Bao's group,[9] the synthesis of new thienyl-substituted isoxazoles via the cyclic hydroamination of thienyl-substituted 1,3-butadiynes with hydroxylamine hydrochloride was also performed (Eqs. (1) and (2)).



(1)



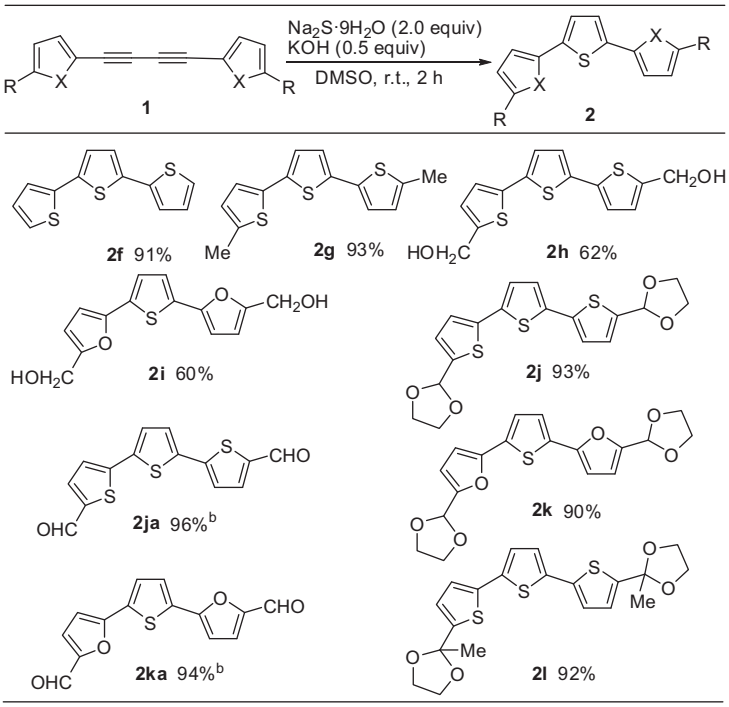
(2)

The antiproliferative activities of the synthesized heterocyclic compounds in K562, MCF-7, A549, and HCT116 cells were evaluated using MTT assay,[10] and the obtained results are summarized in Table 3. It was found that antiproliferative activities greatly depend on the substituents and heteroatoms, as well as the different tumor cells used, and some notable features can be concluded.

On the basis of the results reported in Table 3, the antiproliferative activities of RITA and its analogs are listed in Table 4. The IC_{50} of RITA (**2c**) on K562 cell was much lower, **2c** shows the best antiproliferative activity than its analogs, in which the CH_2OH group in **2c** is replaced by other groups. The order of the antiproliferative activities was found to be: **2c** (RITA) > **2e** (R = 2-methyl-1,3-dioxolanyl) > **2ea** (R = acetyl) > **2da** (R = formyl) > **2d** (R = 1,3-dioxolanyl) > **2a** (R = H) > **2b** (R = Me), indicating that the oxygen-containing groups are important to show the antiproliferative activity against K562. However, the antiproliferative activities of **2ea** and **2e** resulting in apoptosis in MCF-7 are higher than **2c**, and also in A549 and HCT116, **2e** shows the best activity. These results demonstrate that the use of the formyl group (–CHO) (**2e**) replacing of $–CH_2OH$ (**2c**) can expand and improve the antiproliferative activity against various tumor cells.

When oxygen atom in **2c** was replaced by sulfur atom (**2h**), or two sulfur atoms were replaced by oxygen atoms (**2i**), it was found that terthiophene (**2h**) shows the best antiproliferative activities in all the chosen tumor cells, and the trend of antiproliferative activity

Table 2
Synthesis of 2,5-disubstituted thiophenes[a]

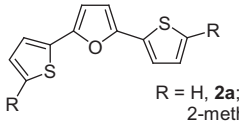


[b] **2ja** and **2ka** were synthesized from **2j** and **2k**, respectively. See: Scheme 2.
[a] The reactions were carried out with 1.0 mmol of **1**, 2.0 mmol of $Na_2S·9H_2O$, and 0.5 mmol of KOH in 4.0 mL of DMSO at rt for 2 h.

Table 3
Antiproliferative activity of K562, MCF-7, A549, and HCT116 cells

Entry	IC_{50} (μm)			
	K562	MCF-7	A549	HCT116
2a	6.745	25.611	14.474	4.645
2b	7.718	>50	24.781	3.657
2c (RITA)	1.133	33.160	28.750	5.778
2d	5.218	13.701	5.762	4.718
2da	4.093	19.246	10.915	10.812
2e	3.897	5.684	4.325	3.041
2ea	4.074	4.235	6.332	7.991
2f	>50	30.765	>50	18.336
2g	32.988	26.928	28.407	26.283
2h	0.523	25.541	26.644	2.790
2i	4.430	>50	>50	10.224
2j	4.703	>50	26.435	>50
2ja	2.432	8.542	5.221	5.430
2jb	6.057	18.568	18.196	6.719
2k	6.635	>50	28.710	17.915
2ka	7.306	>50	>50	>50
2l	3.035	5.573	3.493	2.750
2m	>50	>50	>50	>50
2n	>50	>50	>50	>50

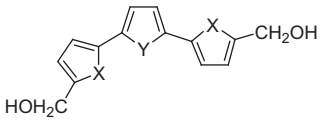
Table 4
Antiproliferative activity of RITA and its analogs



R = H, **2a**; Me, **2b**; CH_2OH, **2c** (RITA); 1,3-dioxolanyl, **2d**; 2-methyl-1,3-dioxolanyl, **2e**; formyl, **2da**; acetyl, **2ea**

K562	**2c** (RITA) > **2e** > **2ea** > **2da** > **2d** > **2a** > **2b**
MCF-7	**2ea** > **2e** > **2d** > **2da** > **2a** > **2c** (RITA) > **2b**
A549	**2e** > **2d** > **2ea** > **2da** > **2a** > **2b** > **2c** (RITA)
HCT116	**2e** > **2b** > **2a** > **2d** > **2c** (RITA) > **2ea** > **2da**

Table 5
Antiproliferative activity of triheterocyclic compounds



X = S, Y = O: **2c** (RITA)
X = Y = S: **2h**
X = O, Y = S: **2i**

K562	**2h** > **2c** > **2i**
MCF-7	**2h** > **2c** > **2i**
A549	**2h** > **2c** > **2i**
HCT116	**2h** > **2c** > **2i**

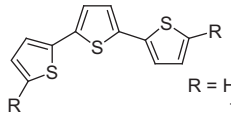
is similar to **2h** > **2c** > **2i** as shown in Table 5. These results indicate that the existence of sulfur atom greatly affects the antiproliferative activities, and with the increase in the number of sulfur atoms, it improves the apoptosis on tumor cells efficiently.[11]

Therefore, the relationship between the antiproliferative activity and substituents in terthiophene derivatives was further investigated as concluded in Table 6. It was revealed that when the CH_2OH group in **2h** was replaced by the 2-methyl-1,3-dioxolanyl group to derive **2l**, **2l** shows the best antiproliferative activity against MCF-7, A549, and HCT116 tumor cells.

As described in Table 5, **2i** has less number of sulfur atoms, which shows the lowest antiproliferative activity. In this study, it was also investigated whether the substituent effect could enhance the antiproliferative activity of **2i**'s analogs. As shown in Tables 5 and 7, **2k** bearing 1,3-dioxolanyl groups could induce the apoptosis of A549 cells with a higher cytotoxicity than **2i**, but **2ka** having the formyl group shows very low antiproliferative activity. Compared with **2e**, it can be concluded that the sulfur atoms are key factor to influence the antiproliferative activity.[11]

As shown in Table 3, it is unfortunate that **2m** and **2n** did not show efficient antiproliferative activities.

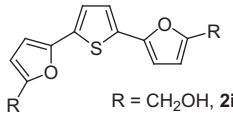
Table 6
Antiproliferative activity of terthiophene derivatives



R = H, **2f**; Me, **2g**; CH_2OH, **2h**; 1,3-dioxolanyl, **2j**; formyl, **2ja**; 2-methyl-1,3-dioxolanyl, **2l**

K562	**2h** > **2ja** > **2l** > **2j** > **2g** > **2f**
MCF-7	**2l** > **2ja** > **2h** > **2g** > **2f** > **2j**
A549	**2l** > **2ja** > **2j** > **2h** > **2g** > **2f**
HCT116	**2l** > **2h** > **2ja** > **2f** > **2g** > **2j**

Table 7
Antiproliferative activity of 2,5-bis(furyl) thiophene derivatives



R = CH_2OH, **2i**; 1,3-dioxolanyl, **2k**; formyl, **2ka**

K562	**2i** > **2k** > **2ka**
A549	**2k** > **2i** ~ **2ka**
HCT116	**2i** > **2k** > **2ka**

In conclusion, we have developed a practical method with high-atom efficiency for the synthesis of a variety of five-membered triheterocyclic compounds by the cyclocondensation of 1,4-bis(5-substituted-2-thienyl or 2-furyl)-1,3-butadiynes with water or $Na_2S{\cdot}9H_2O$ in the presence of KOH in DMSO, including RITA, which has been well known to have the high antitumor activity. The investigation on their antiproliferative activities against K562, MCF-7, A549, and HCT116 tumor cells disclosed that both structures of substituents and species as well as the number of heteroatoms greatly affect their antiproliferative activities. It was found that **2h** shows the higher antiproliferative activity than RITA against all the chosen tumor cells, and both **2e** and **2l** have higher activities in apoptosis of MCF-7, A549, and HCT116. The obtained results have revealed the relationship between the structures of RITA's analogs and the antiproliferative activity, providing the valuable and experimental knowledge for designing and synthesizing the new structures of RITA's analogs, which have good antitumor activity.

The general experimental procedure for the synthesis of RITA and its analogs (Table 1, **2a**–**e**): a mixture of 1,3-butadiyne derivatives (**1**) (1.0 mmol), H_2O (5.0 mmol), and KOH (2.0 mmol) in DMSO (2.5 mL) was heated at 80 °C with stirring for 4 h. After removal of the volatiles, the residue was then subjected to column chromatography isolation on silica gel using petroleum ether as eluent to afford the desired products.

The general experimental procedure for the synthesis of **2f**–**l** (Table 2): a mixture of 1,3-butadiyne derivatives (**1**) (1.0 mmol), $Na_2S{\cdot}9H_2O$ (2.0 mmol), and KOH (0.5 mmol) in DMSO (4.0 mL) was stirred at room temperature for 2 h. After removal of the volatiles, the residue was then subjected to column chromatography isolation on silica gel using petroleum ether as eluent to afford the desired products.

Acknowledgments

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Supplementary data

Supplementary data (general method, characterization data, charts of ^1H and ^{13}C NMR for all products, and the general method for measurement of the antiproliferative activity are concluded) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.10.074.

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