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Graphical Abstract:

Ultrasonic-assisted synthesis of 1,4-disubstituted 1,2,3-triazoles *via* various terminal acetylenes and azide and their quorum sensing inhibition

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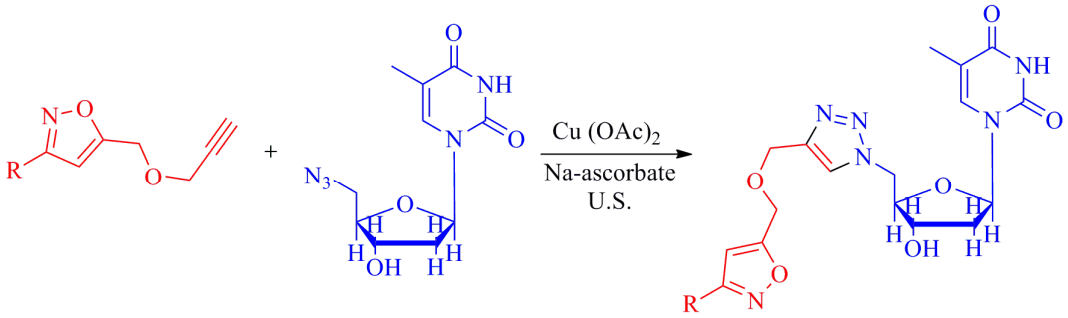

up to 96% yield

An efficient synthesis of 1,4-disubstituted 1,2,3-triazole derivatives was developed *via* 1,3-dispolar cycloaddition starting from various terminal isoxazole ether alkynes and *β*-thymidine azides under ultrasonic assisted conditions in satisfactory yields. The compounds **8a**, **8c** and **8f** exhibited considerable levels of inhibitory activity against violacein production, which highlighted the potential of these compounds as lead structures for further research towards the development of novel QS inhibitors.

Ultrasonic-assisted synthesis of 1,4-disubstituted 1,2,3-triazoles *via* various terminal acetylenes and azide and their quorum sensing inhibition

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Abstract

An efficient synthesis of 1,4-disubstituted 1,2,3-triazole derivatives was studied. 1,4-Disubstituted 1,2,3-triazoles containing isoxazole and thymidine structures were synthesized in 84–96% yields starting from various terminal isoxazole ether alkynes and *β*-thymidine azide derivatives *via* a 1,3-dispolar cycloaddition using copper acetate, sodium ascorbate as the catalyst under ultrasonic assisted condition. All the target compounds were characterized by HRMS, FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy. Furthermore, the quorum sensing inhibitory activities of synthesized compounds were evaluated with *Chromobacterium violaceum* (*C. Violaceum* CV026) based on their inhibition of violacein production, with compound C_{10}-HSL as a positive control. The compounds **8a**, **8c** and **8f** exhibited considerable levels of inhibitory activity against violacein production, and IC_{50} values were 217±19, 223±20 and 42.8±4.5 μM, respectively, which highlighted the potential of these compounds as lead structures for further research towards the development of novel QS inhibitors.

Keywords 1,2,3-Triazole; Isoxazole; Thymidine; 1,3-Dispolar cycloaddition; Ultrasonic heating; Quorum sensing

1. Introduction

Quorum sensing (QS) is one of cell communication mechanisms involving the generation, release and detection of small signaling molecules which can activate specific receptors associated with transcription signals that are responsible for controlling a variety of different biochemical processes.[1–3] QS can effectively regulate some important phenotypes that are mostly relevant to antibiotics resistance[4–6], such as the bioluminescence, virulence expression and formation of biofilm and so on. In recent years, much more bacterial strains showed resistance to antibiotics, and it is urgent to find an effective solution. QS inhibitors are new target agents, which can provide insights into bacterial signaling processes from fundamental and applied perspectives, conducive to the discovery of novel antibacterial strategy and antimicrobial agents.[7–9] It was reported that the amide structure of the pyrimidine ring has a potential quorum sensing inhibitory effect. *β*-Thymidine derivatives contain lactam structure are expected to possess good quorum sensing inhibitory effect to Gram negative bacteria.[10,11]

Thymidine derivatives are more widely used in the world of organic heterocyclic compounds,[12,13] in particular, the amide structure of the pyrimidine ring has a potential quorum sensing inhibitory effect. They are concerned by people because they exhibit good biological and pharmaceutical activity[14,15] *etc*.. Several 2',3'-dideoxynucleosides, including 3'-azido-3'-deoxythymidine (AZT, zidovudine), have been used as antiretroviral drugs for the treatment of acquired immunodeficiency syndrome (AIDS).[16,17] In addition, some nucleoside derivatives also have strong resistance to some tumour cells,[18,19] bacteria[20,21] and so on. Isoxazoles and 1,2,3-triazoles with better anti-bacterial,[22,23] anti-cancer,[24,25] antiviral,[26,27] analgesic and anti-inflammatory[28,29] are also important heterocyclic skeleton for synthesizing a drug molecule, and have been widely used in clinical practice.[30,31] Therefore, the study of the synthetical method and biological activity of heterocyclic compounds containing thymidine, isoxazole and 1,2,3-triazole has always been a search realm with vitality in the organic chemistry and medical chemistry. The structural features and physiochemical properties of their fragment are of great interest in drug design and discovery.

It is known that 1,3-dipolar cycloaddition reaction occurred between 1,3-dipole body and olefins, alkynes or corresponding derivatives. Nitrile oxides and azides as the most common

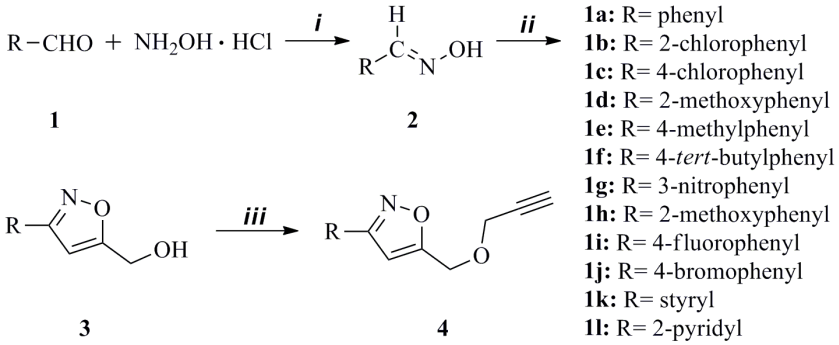
1,3-dipolar body were important precursor for synthesizing isoxazole and 1,2,3-triazole derivatives.[32–35] The most widely used method to build the 1,2,3-triazole system is the 1,3-dipolar cycloaddition of alkynes with azides, which is the famous Click reaction.[36] However, the major disadvantage of this method is the parallel formation of 1,4 and 1,5-regioisomers. Early as 2002, it was reported that Husigen-Click reaction, which copper(I)-catalyzed alkyne-azide independently conducted 1,3-dipolar cycloaddition reaction (CuAAC), was able to exclusively obtain 1,4-product with a good regioselectivity.[37] Successively, ruthenium-catalyzed method was reported for the regioselective synthesis of the 1,5-product.[38] However, the synthesis of isoxazole-thymidine derivatives has not been reported using copper(I) as a catalyst. With the development of technology, several improved methods including microwave and ultrasonic assisted method were reported to increase the yield of the cycloaddition.[39,40] Ultrasonic-assisted organic synthesis is a powerful technique that is widely being used in organic synthesis reaction. The notable features of the ultrasound approach are enhanced reaction rate, formation of pure product in high yield, and easier manipulation. In this work, we reported the cyclization between 3-aryl-5-((prop-2-yn-1-yloxy)methyl) isoxazoles and 1-(4-azido-5-(azidomethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione or 1-(4-azido-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione using the CuAAC reaction under conventional heating and ultrasonic-assisted condition. The structures of the synthesized compounds were characterized by HRMS, FT-IR, ^{1}H and ^{13}C NMR spectroscopy. Herein, we combined 1,2,3-trizole and isoxazole into thymidine derivative structure get novel target molecules, further evaluated their QS to Gram negative bacteria. It suggested that 4-substituted group of phenyl on isoxazole had important effect on antibacterial and antimicrobial agents.

2. Results and discussion

2.1 Synthesis

It is known that isoxazole is a versatile scaffold for the synthesis of varieties of complex natural products, and functionalized isoxazole derivatives are active pharmacophores in many pharmacologically important molecules.[41,42] In the present work, 3-substituted

phenyl-5-((prop-2-yn-1-yloxy)methyl)isoxazoles (**4**) were synthesized in three steps starting form substituted benzaldehyde (Scheme 1, Table 1) according to the literature.[43,44] In the process for synthesizing 3-substituted phenylisoxazol-5-yl)methanols (**3**), 1,3-dipolar cycloaddition reaction was catalyzed using $ZnCl_2$ as a catalyst by one-pot method under ultrasound-assisted with 58–88% yields, which resulted in the yield of the synthesized desired products enhancing 12–29% compared with conventional method. A series of compounds **4** were prepared by employing intermediate **3** and propargyl bromide under NaH with 68–96% yields at room temperature condition, and the influence of different substituents on the yield was investigated.



1a: R= phenyl
1b: R= 2-chlorophenyl
1c: R= 4-chlorophenyl
1d: R= 2-methoxyphenyl
1e: R= 4-methylphenyl
1f: R= 4-*tert*-butylphenyl
1g: R= 3-nitrophenyl
1h: R= 2-methoxyphenyl
1i: R= 4-fluorophenyl
1j: R= 4-bromophenyl
1k: R= styryl
1l: R= 2-pyridyl

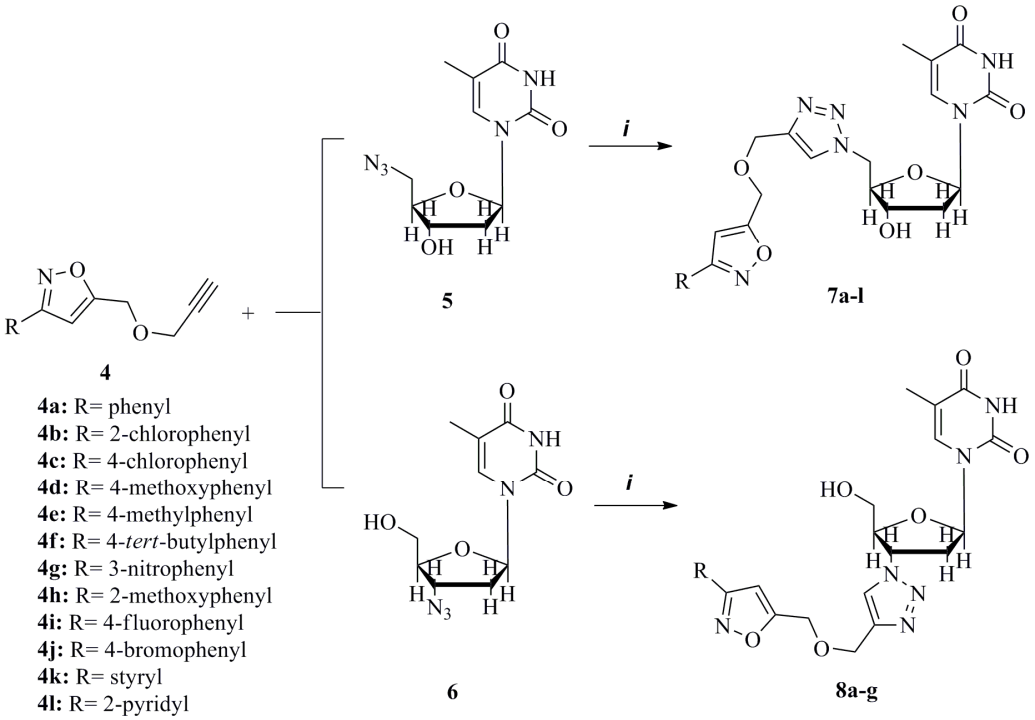
Scheme 1. The route for synthesizing 3-substituted phenyl-5-((prop-2-yn-1-yloxy)methyl)isoxazoles **4a-l** from aromatic aldehyde **1a-l**. Reagents and conditions: (i) NaOH aq. (6mol/L), EtOH, reflux. (ii) a) NCS, DMF. b) Propargyl alcohol, $ZnCl_2$, Et_3N, U.S. 0°C–r.t.. (iii) NaH, propargyl bromide, THF, 0°C–r.t..

Table 1. 3-substituted phenyl-5-((prop-2-yn-1-yloxy)methyl)isoxazoles yields under different substituent

Entry	Alcohol	R	Time / h	Product	Yield / % [a]
1	**3a**	C_6H_5	4	**4a**	80
2	**3b**	2-Cl-C_6H_4	5	**4b**	83
3	**3c**	4-Cl-C_6H_4	6	**4c**	90
4	**3d**	2-MeO-C_6H_4	5	**4e**	82
5	**3e**	4-MeO-C_6H_4	6	**4f**	96
6	**3f**	4-Me-C_6H_4	5	**4d**	83
7	**3g**	4-tBu-C_6H_4	8	**4g**	85
8	**3h**	4-F-C_6H_4	5	**4h**	97
9	**3i**	4-Br-C_6H_4	6	**4i**	86
10	**3j**	3-NO_2-C_6H_4	8	**4j**	93
11	**3k**	styryl	4	**4k**	98

12	**3l**	2-pyridyl	4	**4l**	97

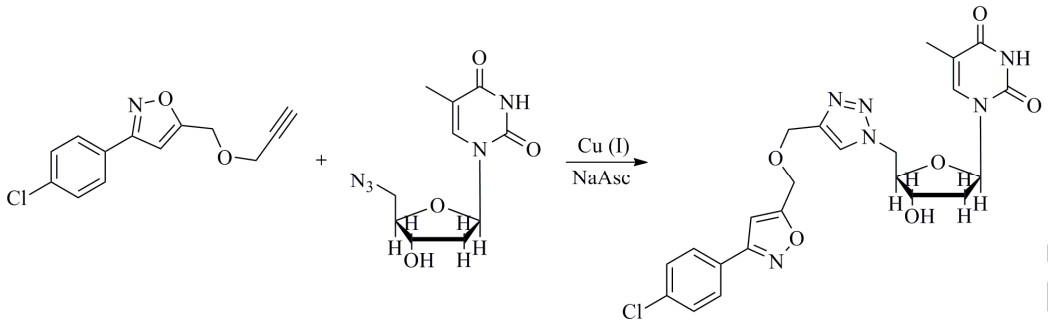
[a] Isolated product yields.



Scheme 2. Synthesis of 1,2,3-triazole derivatives **7a-l** and **8a-g** starting from various isoxazole ether alkynes and thymidine azide derivatives. Reagents and conditions: (i) $Cu(OAc)_2 \cdot H_2O$, sodium ascorbate(NaAsc), *i*-PrOH-H_2O (2:1, v/v), 45°C.

Sequentially, the synthesis of 5-((4-(((3-(4-chlorophenyl)isoxazol-5-yl)methoxy)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)-4-hydroxytetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (**7c**) were explored by employing 3-(4-chlorophenyl)-5-((prop-2-yn-1-yloxy)methyl)isoxazole (**4c**) and *β*-thymidine azide derivatives (**5**) in the presence of copper(I)(Scheme 2). In order to optimize the reaction conditions, we screened the different heating mode and catalyst, a variety of H_2O-organic solvents and examined their influence on reaction time and yield of the obtained target product. The results are summarized in Table 2.

Table 2. Effect of various solvents on the model reaction [a]



4c **5** **7c**

Entry	Cu source	Solvent	Temp. /°C	Traditional heating		Ultrasonic radiation	
				Time/h	Yield [b]/%	Time/min	Yield [b]/%
1	None	*i*-PrOH/H_2O (2:1)	45	8	N.D.	20	N.D.
2	$CuCl_2$	*i*-PrOH/H_2O (2:1)	45	10	73	15	82
3	$CuSO_4$	*i*-PrOH/H_2O (2:1)	45	8	77	10	88
4	$Cu(OAc)_2$	*i*-PrOH/H_2O (2:1)	45	8	78	10	92
5	CuI [c]	*i*-PrOH/H_2O (2:1)	45	8	74	10	85
6	$Cu(OAc)_2$	*i*-PrOH/H_2O (2:1)	35	8	58	10	70
7	$Cu(OAc)_2$	*i*-PrOH/H_2O (2:1)	r.t	8	35	10	56
8	$Cu(OAc)_2$	*i*-PrOH/H_2O (2:1)	55	7	78	10	90
9	$Cu(OAc)_2$	*i*-PrOH/H_2O (1:1)	45	8	68	10	84
10	$Cu(OAc)_2$	*i*-PrOH/H_2O (3:1)	45	8	72	10	87
11	$Cu(OAc)_2$	*i*-PrOH	45	24	46	20	58
12	$Cu(OAc)_2$	H_2O	45	24	trace	20	trace
13	$Cu(OAc)_2$	THF/H_2O (2:1)	45	8	76	10	81
14	$Cu(OAc)_2$	DMSO/H_2O (2:1)	45	4	85	10	91
15	$Cu(OAc)_2$	DMF/H_2O (2:1)	45	4	81	10	89
16	$Cu(OAc)_2$	MeCN/H_2O (2:1)	45	11	56	10	79

[a] The reaction was conducted with alkyne (1.2mmol), azide-thymidine (1mmol), Cu source (0.1mmol), Na-ascorbate (0.2mmol). N.D. (not detected).

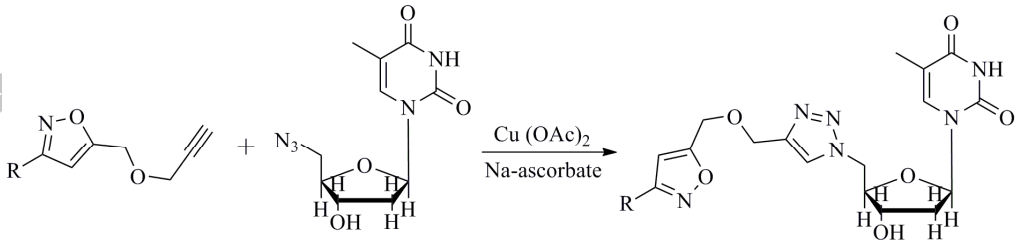
[b] Isolated product yields.

[c] No Na-ascorbate was added.

As shown in Table 2, the desired **7c** was successfully synthesized with 56–92% and 35–85% yields in the presence of organic solvents and catalyst under ultrasonic radiation and traditional

heating, respectively. Exhilaratingly, ultrasonic radiation as an efficient and convenient process, could shorten reaction time(20 h→20 min; 10 h→10 min and 4 h→10 min). Also, the yields of the obtained target product were enhanced 6–21% compared with traditional heating. Under the other identical reaction conditions, no desired product was obtained in the absence of catalyst. When copper acetate was used as in situ generation of copper(I) ion with sodium ascorbate as a catalyst, the yield of the obtained **7c** was higher than that of copper sulfate, copper chloride and directly loading copper(I) iodide (Table 2, entries 2–5). However, when the reaction temperature was decreased to room temperature and 35°C, respectively, the yield of the obtained **7c** respectively decreased to 35% and 70% (traditional heating) and 56% and 70% (ultrasonic radiation)(Table 2, entries 4, 6 and 7). Sequentially raising the reaction temperature to 55°C, the yield of the obtained target product was remained (78% and 90% under the different heating mode)(Table 2, entry 8). Besides, the solvent played a significant role in progress of reaction. Among the examined solvents, when *i*-PrOH and water were used alone, the yields of 46% (conventional heating), 58% (ultrasonic heating) and trace for the model reaction were obtained after 24h, respectively (Table 2, entries 11 and 12). The low yield obtained for the corresponding triazole derivatives using pure water as a solvent is attributed to lack of organic material solubility in water. The mixed solvents of *i*-PrOH–H_2O, DMF–H_2O and DMSO–H_2O (2:1, V/V) all afforded the better yield (Table 2, entries 4, 14 and 15). Considering economy and environment, the confirmed optimal synthesis condition was copper acetate, sodium ascorbate as the catalyst, *i*-PrOH–H_2O mixture (2:1, V/V), under ultrasound-assisted at 45°C for 10min.

Table 3 Synthesis and yields of 1,2,3-triazole derivatives **7a-l** under the traditional heating and ultrasonic radiation, respectively.



4 **5** **7**

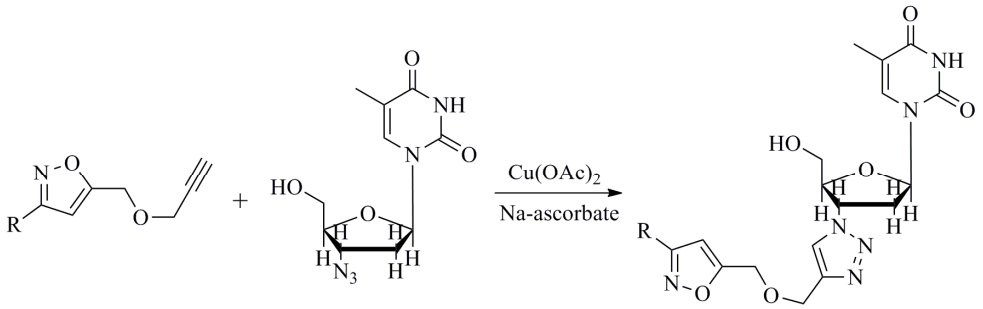
Entry	Alkyne	R	Product	Traditional heating		Ultrasonic radiation	
				Time/h	Yield[a]/%	Time/min	Yield[a]/%

1	**4a**	C_6H_5	**7a**	7	75	10	90
2	**4b**	2-Cl-C_6H_4	**7b**	8	78	10	93
3	**4c**	4-Cl-C_6H_4	**7c**	8	78	10	92
4	**4d**	4-MeO-C_6H_4	**7d**	10	70	10	95
5	**4e**	4-Me-C_6H_4	**7e**	8	84	10	95
6	**4f**	4-tBu-C_6H_4	**7f**	8	82	10	94
7	**4g**	3-NO_2-C_6H_4	**7g**	8	68	10	85
8	**4h**	2-MeO-C_6H_4	**7h**	10	71	10	96
9	**4i**	4-F-C_6H_4	**7i**	8	85	10	94
10	**4j**	4-Br-C_6H_4	**7j**	8	74	10	89
11	**4k**	styryl	**7k**	9	72	10	89
12	**4l**	2-pyridyl	**7l**	9	66	15	84

[a] Isolated product yields.

Having been optimized the reaction conditions for the model system (Table 2, entry 4) was chosen to explore the scope and limitations of this protocol (Table 3). As shown in Table 3, all scanned reactants afforded the corresponding 1,2,3-triazole derivatives under conventional heating and ultrasonic heating in 66–85% and 84–96% yields, respectively. The results indicated that the good yields were obtained when benzene ring of R on terminal alkynes was substituted by ortho and para directing group (Table 3, entries 1–6 and 8–11). However, when benzene ring of R on terminal alkynes was substituted by meta-directing group (–NO_2 and pyridyl *etc*), the yields of the synthesized compounds were lower (Table 3, entries 7 and 12). This is because the meta-directing group could lead to the electron cloud density of isoxazole terminal alkynes decreased.

Table 4 Synthesis and yields of 1,2,3-triazole derivatives **8a-g** under the traditional condition and ultrasonic radiation, respectively.



4 6 8

Entry	Alkyne	R	Product	Traditional heating		Ultrasonic radiation	
				Time /h	Yield [a]/%	Time /min	Yield [a]/%
1	**4a**	C_6H_5	**8a**	8	76	10	89
2	**4b**	2-Cl-C_6H_4	**8b**	9	82	10	92
3	**4c**	4-Cl-C_6H_4	**8c**	9	79	10	91
4	**4d**	4-MeO-C_6H_4	**8d**	13	71	10	96
5	**4e**	4-Me-C_6H_4	**8e**	9	83	10	94
6	**4f**	4-tBu-C_6H_4	**8f**	9	77	10	90
7	**4g**	3-NO_2-C_6H_4	**8g**	8	68	10	87

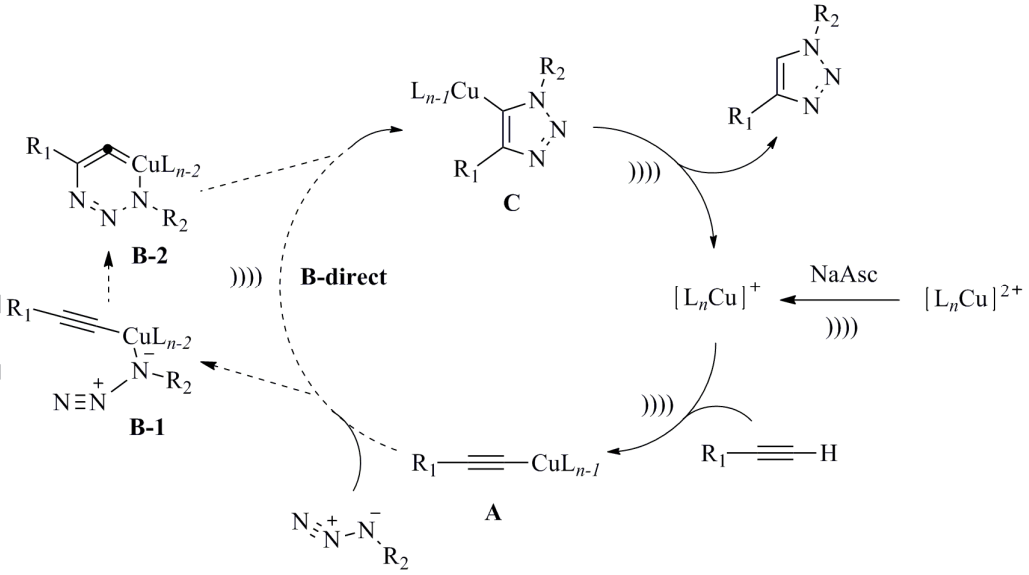
[a] Isolated product yields.

It is known that several 2',3'-dideoxynucleosides, including 3'-azido-3'-deoxythymidine (AZT, zidovudine) (**6**) (Scheme 2), have been used as antiretroviral drugs for the treatment of acquired immunodeficiency syndrome (AIDS).[45] In addition, there are some examples of application of AZT as an antitumor agent.[46,47] Triazole is versatile scaffold for the synthesis of a wide variety of complex natural products, and functionalized triazole derivatives are active pharmacophores in many pharmacologically important molecules.[48,49] Next, we successfully synthesized seven novel 1,2,3-triazole derivatives containing isoxazole ring and deoxythymidine in 68–83% and 87–96% yields starting from isoxazole terminal alkynes (**4a–g**) and AZT using the above obtained optimal reaction conditions under conventional heating and ultrasonic heating, respectively (Table 4). The effect of substituents on the yield was similar to that of compounds **7a–l**.

The scope of this protocol was examined by its application to between various isoxazole terminal alkynes and *β*-thymidine azide. The target compounds **7a**–**l** and **8a**–**g** were successfully

prepared in 66%–85% (traditional heating), 84%–96% (ultrasonic radiation) and 68%–83% (traditional heating), 87%–96% (ultrasonic radiation) yields, respectively (Table 3 and Table 4). The structures of compound **7a–l** and **8a**–**g** were confirmed by FT-IR, ^{1}H and ^{13}C NMR and HRMS spectra analysis. The $–CH_2–O–CH_2–$ protons and =CH– protons of 1,2,3-triazole ring exhibited resonances at δ 4.64–4.79 ppm and δ 7.80–8.67 ppm using DMSO as solvent, while the resonances for the corresponding $–CH_2–O–CH_2–$ and =CH– carbon atom were observed peaks at δ 61.78–64.18 ppm and δ 125.33–128.57 ppm, respectively.

A plausible mechanism for the preparation of 1,2,3-tiazole derivatives *via* 1,3-dispolar cycloaddition of various terminal isoxazole ether alkynes and β-thymidine azide derivatives using copper acetate, NaAsc as the catalyst under ultrasonic irradiation was suggested according to the reference[37,50,51] (Scheme 3). Firstly, copper(I) was in situ generated by redox reaction of copper(II) and NaAsc, and subsequently attacked terminal alkynes to form intermediate **A**. Then, the formation of intermediate **C** was achieved by the nucleophilic addition of azide and intermediate **A** with the formation of intermediate **B-1** and **B-2**. Finally, the target 1,2,3-triazole products were obtained by the removal of copper coordination compound from intermediate **C**. Herein, ultrasound accelerated the reaction rate and provided a certain amount of energy in the whole process of reaction.



Scheme 3. Proposed mechanism for the synthesis of 1,2,3-triazole derivatives under ultrasonic assisted

2.2 Quorum Sensing Inhibition

The QS inhibitory activities of the target compounds **7a–l** and **8a–g** were evaluated with *C.*

Violaceum CV026 based on their inhibition of violacein production, with compound C_{10}-HSL being used as a positive control. The IC_{50} value for each compound has been summarized in Table 5.

Table 5 IC_{50} of compounds for inhibiting violacein production

Entry	Compound	R	IC_{50} (μM) [a]
1	C_{10}-HSL		0.124±0.014
2	**7a**	C_6H_5	>256
3	**7b**	2-Cl-C_6H_4	>256
4	**7c**	4-Cl-C_6H_4	>256
5	**7d**	4-MeO-C_6H_4	>256
6	**7e**	4-Me-C_6H_4	>256
7	**7f**	4-tBu-C_6H_4	>256
8	**7g**	3-NO_2-C_6H_4	>256
9	**7h**	2-MeO-C_6H_4	>256
10	**7i**	4-F-C_6H_4	NA[b]
11	**7j**	4-Br-C_6H_4	NA[b]
12	**7k**	styryl	NA[b]
13	**7l**	2-pyridyl	NA[b]
14	**8a**	C_6H_5	217±19
15	**8b**	2-Cl-C_6H_4	>256
16	**8c**	4-Cl-C_6H_4	223±20
17	**8d**	4-MeO-C_6H_4	>256
18	**8e**	4-Me-C_6H_4	>256
19	**8f**	4-tBu-C_6H_4	42.8±4.5
20	**8g**	3-NO_2-C_6H_4	>256

[a] Half maximal inhibitory concentration.

[b] NA means no significant inhibition.

C. violaceum is a Gram-negative bacteria and the production of virulence factors, such as violacein production, is regulated in a cell density-dependent manner known as QS. The

autoinducer in QS of *C. violaceum* is *N*-decanoyl-*L*-Homoserine lactone (C10-HSL). *C. Violaceum* CV026 has been widely used as a model for screen novel QS inhibitors for rapid and easy detection method. To the best of our knowledge, C10-HSL and Chloro Lactone (CL) are the best inhibitors from violacein production. Thus, C10-HSL is used as positive control in this study.

As shown in Table 5, the synthesized compounds have certain inhibitory activity against violacein production except compound **7i**, **7j**, **7k**, **7l**. In particular, compounds **8a**, **8c** and **8f** exhibited considerable levels of inhibitory activity with IC_{50} values of 217±19, 223±20 and 42.8±4.5 μM, respectively. This maybe is because phenyl and substituted phenyl consisted in a specific interaction to the LuxR protein.[52] For the LuxR protein, the acknowledged model was that dimerization requested the preliminary binding of acyl-HSL.[53] It could deduce that the inhibitory activity of the synthesized compound might result from the interaction with one or several aromatic amino acid of the LuxR protein which contains the HSL binding site or in the dimerization domain or both. Besides, the introduction of an aryl group at the R position provided enhanced levels of inhibitory activity relative to the alkyl groups, as compound **8e** and **8f**. When the group at the R position was an alkyl group, the activity was dependent on the length of the chain, with a longer chain length providing the highest level of inhibitory activity among all these compounds.[54] Thus, compound **8f** showed good QS inhibition activity in this paper comparing with 8e. Methyl and chlorine are bioisostere, when they are substituted in the *para* of benzene ring, chlorine substituted benzene had inhibition activity. For chlorine is hydrophily group, which is important for inhibition activity.[52,54] In a word, the study suggested that the potential of these compounds could be used a lead structure for further research towards the development of novel QS inhibitors.

3. Conclusions

In conclusion, a convenient, efficient method for the synthesis of 1,4-disubstituted 1,2,3-triazoles containing isoxazole and thymidine structures using copper acetate, sodium ascorbate as the catalyst under ultrasonic assisted has been presented. Ultrasonic heating can

significantly shorten reaction time and increased yields of the products (9–25%) compared with conventional heating. Twelve novel 1-((2*R*,4*S*,5*R*)-4-hydroxy-5-((4-(((3-arylisoxazol-5-yl)methoxy)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione and seven 1-((2*R*,4*S*,5*S*)-5-(hydroxymethyl)-4-(4-(((3-arylisoxazol-5-yl)methoxy)methyl)-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione were obtained in 84–96% and 87–96% yields, respectively. The quorum sensing inhibitory activities of synthesized compounds were evaluated with CV026 based on their inhibition of violacein production, with compound C_{10}-HSL being used as a positive control. The compounds **8a**, **8c** and **8f** exhibited considerable levels of inhibitory activity against violacein production, and IC_{50} values were 217±19, 223±20 and 42.8±4.5 μM, respectively. These compounds have potential application prospect as lead structures towards the development of novel QS inhibitors.

4. Experimental

4.1 General Remarks

Various substituted benzaldehydes were of analytical-reagent grade from Aladdin reagent Co. (China) and used without further purification. The other solvents and reagents used were supplied by Tianjin Tiantai Chemical Co. Ltd (China) and Beijing Chemical Plant (China). *N*-decanoyl-*L*-Homoserine lactone (C10-HSL) was obtained from Cayman Chemical. All melting points were determined on an XT–4 melting point apparatus (China) and were uncorrected. ^{1}H and ^{13}C NMR spectra were measured using a Bruker AVANCE-500 NMR (Germany) spectrometer and with TMS as an internal standard. The chemical shift is given in δ relative to TMS. MS was collected using an Agilent 1290-micrOTOF Q II (USA) spectrometer, respectively. FT-IR spectra were obtained as KBr pellets using an IRAffinity-1 instrument (Shimadzu, Japan) in the range of 500–4000 cm^{-1}. An KQ2200E-type ultrasonic reactor (100 W) (China) with a thermometer for ultrasonic application was used in experiments.

*4.2 General synthesis process for 3-substituted phenyl-5-prop-2-ynyloxymethyl-isoxazoles (**4a-l**)*

In 100 mL three-necked flask, (3-substituted phenylisoxazol-5-yl)methanols (**3a-l**) (5 mmol)

was poured into a stirred mixture of sodium hydride (15 mmol) and anhydrous THF (10 mL) previously cooled in glacial bath. Propargyl bromide (6 mmol, 0.47 mL) was added, and the mixture was stirred at room temperature (20–25 ^{o}C) until the reaction was over by TLC monitoring. The slurry was filtrated by sabouraud funnel. Filtrate was evaporated under a vacuum to provide the crude product which was purified by column chromatography (silica gel, 200–300 mesh) using petroleum ether/ethyl acetate (φ_r = 4 : 1) to furnish the desired product **4a-l** in 68–96% yield.

*4.3 General synthesis process for 1,4-disubstituted 1,2,3-triazole derivatives (**7a-l** and **8a-g**)*

Traditional heating method: 3-Substituted phenyl-5-prop-2-ynyloxymethyl-isoxazoles (**4a-l**) (1.2 mmol), and β-thymidine azide derivatives (1 mmol) were dissolved into *i*-PrOH/H_2O=2/1 (6 mL) in 50 mL three-necked flask. After dissolving, CuOAc (10% mmol), H_2O (3 mL) and sodium ascorbate (20% mmol) was added dropwise, and the mixture was stirred at 45^{o}C until the reaction was over by TLC monitoring. After the reaction was over, the slurry was extracted with ethyl acetate. The organic layers were dried over anhydrous sodium sulfate, filtrated and then evaporated under a vacuum to provide the crude product which was purified by column chromatography (silica gel, 200-300 mesh) using ethyl acetate/ methanol (φ_r = 20 : 1) to furnish the product. The yields of obtained 1,4-disubstituted 1,2,3-triazole derivatives **7a-l** and **8a-g** were 66%–85% and 68%–83%, respectively.

Ultrasonic radiation method: 3-Substituted phenyl-5-prop-2-ynyloxymethyl-isoxazoles (**4a-l**) (1.2 mmol), and β-thymidine azide derivatives (1 mmol) were dissolved into *i*-PrOH/H_2O=2/1 (6 mL) in 50 mL three-necked flask. After dissolving, CuOAc (10% mmol), H_2O (3 mL) and sodium ascorbate (20% mmol) was added dropwise to the reaction mixture. The reaction mixture was submitted to ultrasound irradiation and stirring condition at 45^{o}C (the solution become clear), respectively, and the process was monitored by TLC. After the reaction was over, the slurry was extracted with ethyl acetate. The organic layers were dried over anhydrous sodium sulfate, filtrated and then evaporated under a vacuum to provide the crude product which was purified by column chromatography (silica gel, 200-300 mesh) using ethyl acetate/ methanol (φ_r = 20 : 1) to furnish the product. The yields of obtained 1,4-disubstituted 1,2,3-triazole derivatives **7a-l** and **8a-g** were 84%–96% and 87%–96%, respectively.

4.4. Representative spectral data

*4.4.1. 1-((2R,4S,5R)-4-hydroxy-5-((4-(((3-phenylisoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**7a**)*

Yield: 90%, orange solid, mp 200–201 °C. IR (KBr) (ν/cm^{-1}) 3746, 3391, 3178, 3117, 3067, 2947, 2924, 1721, 1655, 1577, 1507, 1474, 1439, 1366, 1273, 1204, 1096, 1040, 907, 860, 768, 691, 610; ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.31 (s, 1H), 8.17 (s, 1H), 7.89 – 7.86 (m, 2H), 7.54 – 7.50 (m, 3H), 7.34 (d, J = 1.2 Hz, 1H), 7.07 (s, 1H), 6.17 (t, J = 7.0 Hz, 1H), 5.50 (d, J = 4.4 Hz, 1H), 4.75 – 4.70 (m, 3H), 4.66 – 4.60 (m, 3H), 4.29 (td, J = 7.8, 4.0 Hz, 1H), 4.11 – 4.07 (m, 1H), 2.21 – 2.14 (m, 1H), 2.10 (ddd, J = 13.5, 6.5, 3.8 Hz, 1H), 1.79 (d, J = 1.0 Hz, 3H); ^{13}C NMR (126 MHz, DMSO) δ ppm: 170.14, 164.09, 162.29, 150.85, 143.74, 136.50, 130.71, 129.58, 128.91, 127.08, 125.49, 110.31, 102.27, 84.51, 84.40, 71.20, 63.71, 62.48, 51.64, 38.37, 12.50; HRMS (EI): calcd for $C_{23}H_{24}N_6O_6$ $[M+H]^+$ 481.1836, found 481.1863.

*4.4.2. 1-((2R,4S,5R)-5-((4-(((3-(2-chlorophenyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-4-hydroxytetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**7b**)*

Yield: 93%, light yellow solid, mp 226–228 °C. IR (KBr) (ν/cm^{-1}) 3750, 3391, 3178, 3110, 3059, 2947, 2924, 1717, 1655, 1512, 1474, 1436, 1369, 1273, 1204, 1099, 1045, 957, 764, 610; ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.31 (s, 1H), 8.17 (s, 1H), 7.70 (dd, J = 7.6, 1.8 Hz, 1H), 7.64 (dd, J = 8.0, 1.2 Hz, 1H), 7.55 (td, J = 7.8, 1.8 Hz, 1H), 7.49 (td, J = 7.5, 1.3 Hz, 1H), 7.33 (d, J = 1.2 Hz, 1H), 6.94 (s, 1H), 6.16 (t, J = 7.0 Hz, 1H), 5.50 (d, J = 4.4 Hz, 1H), 4.75 (s, 2H), 4.74 – 4.70 (m, 1H), 4.66 (s, 2H), 4.63 (dd, J = 14.3, 7.4 Hz, 1H), 4.28 (dt, J = 7.9, 3.9 Hz, 1H), 4.10 – 4.07 (m, 1H), 2.20 – 2.13 (m, 1H), 2.09 (ddd, J = 13.5, 6.5, 3.8 Hz, 1H), 1.78 (d, J = 1.0 Hz, 3H); ^{13}C NMR (126 MHz, DMSO) δ ppm: 169.53, 164.09, 160.93, 150.84, 143.72, 136.48, 132.29, 132.08, 131.53, 130.83, 128.15, 125.50, 110.31, 105.24, 84.50, 84.39, 71.19, 63.78, 62.36, 51.63, 38.38, 12.50; HRMS (EI): calcd for $C_{23}H_{23}ClN_6O_6$ $[M+H]^+$ 515.1446, found 515.1466.

*4.4.3. 1-((2R,4S,5R)-5-((4-(((3-(4-chlorophenyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-4-hydroxytetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**7c**)*

Yield: 92%, gray solid, mp 223–225 °C. IR (KBr) (ν/cm^{-1}) 3746, 3383, 3178, 3113, 3063, 2951, 2924, 1721, 1655, 1524, 1474, 1435, 1369, 1273, 1204, 1096, 1042, 980, 845, 652, 610; ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.31 (s, 1H), 8.16 (s, 1H), 7.91 (t, J = 2.1 Hz, 1H), 7.91 – 7.89 (m,

1H), 7.60 (d, *J* = 1.9 Hz, 1H), 7.58 (t, *J* = 2.1 Hz, 1H), 7.34 (d, *J* = 1.0 Hz, 1H), 7.10 (s, 1H), 6.16 (t, *J* = 7.0 Hz, 1H), 5.50 (d, *J* = 4.4 Hz, 1H), 4.74 – 4.70 (m, 3H), 4.66 – 4.60 (m, 3H), 4.28 (td, *J* = 7.8, 4.0 Hz, 1H), 4.10 – 4.06 (m, 1H), 2.17 (dt, *J* = 13.7, 6.9 Hz, 1H), 2.10 (ddd, *J* = 13.5, 6.4, 3.8 Hz, 1H), 1.78 (d, *J* = 0.8 Hz, 3H); ^{13}C NMR (126 MHz, DMSO) δ ppm: 170.44, 164.10, 161.38, 150.85, 143.73, 136.50, 135.40, 129.69, 128.88, 127.78, 125.50, 110.31, 102.30, 84.50, 84.39, 71.19, 63.71, 62.46, 51.63, 38.36, 12.51; HRMS (EI): calcd for $C_{23}H_{23}ClN_6O_6$ $[M+H]^+$ 515.1446, found 515.1462.

*4.4.4. 1-((2R,4S,5R)-4-hydroxy-5-((4-(((3-(4-methoxyphenyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**7d**)*

Yield: 95%, white solid, mp 138–139 °C. IR (KBr) (ν/cm^{-1}) 3750, 3379, 3178, 3113, 3059, 2952, 2928, 1717, 1655, 1524, 1474, 1431, 1366, 1273, 1204, 1096, 1042, 957, 837, 652, 606; ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.33 (s, 1H), 8.17 (s, 1H), 7.81 (d, *J* = 8.8 Hz, 2H), 7.35 (s, 1H), 7.06 (d, *J* = 8.8 Hz, 2H), 7.01 (s, 1H), 6.17 (t, *J* = 7.0 Hz, 1H), 4.75 – 4.69 (m, 3H), 4.66 – 4.60 (m, 3H), 4.31 – 4.25 (m, 1H), 4.11 – 4.06 (m, 1H), 3.82 (s, 3H), 2.18 (dt, *J* = 13.8, 6.9 Hz, 1H), 2.10 (ddd, *J* = 13.4, 6.3, 3.8 Hz, 1H), 1.79 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ ppm: 169.78, 164.12, 161.88, 161.20, 150.86, 143.75, 136.50, 128.57, 125.48, 121.30, 114.97, 110.31, 102.02, 84.52, 84.40, 71.20, 63.69, 62.50, 55.77, 51.64, 38.37, 12.51; HRMS (EI): calcd for $C_{24}H_{26}N_6O_7$ $[M+H]^+$ 511.1941, found 511.1958.

*4.4.5. 1-((2R,4S,5R)-4-hydroxy-5-((4-(((3-(p-tolyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**7e**)*

Yield: 95%, orange solid, mp 223–225 °C. IR (KBr) (ν/cm^{-1}) 3750, 3379, 3178, 3113, 3063, 2950, 2924, 1721, 1655, 1512, 1477, 1437, 1366, 1273, 1204, 1096, 1042, 980, 822, 652, 610; ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.31 (s, 1H), 8.16 (s, 1H), 7.78 – 7.75 (m, 2H), 7.33 (dd, *J* = 9.5, 4.5 Hz, 3H), 7.03 (s, 1H), 6.16 (t, *J* = 7.0 Hz, 1H), 5.50 (d, *J* = 4.4 Hz, 1H), 4.75 – 4.70 (m, 3H), 4.66 – 4.60 (m, 3H), 4.28 (td, *J* = 7.8, 3.9 Hz, 1H), 4.11 – 4.07 (m, 1H), 2.36 (s, 3H), 2.20 – 2.14 (m, 1H), 2.10 (ddd, *J* = 13.5, 6.4, 3.8 Hz, 1H), 1.79 (d, *J* = 1.0 Hz, 3H); ^{13}C NMR (126 MHz, DMSO) δ ppm: 169.94, 164.09, 162.18, 150.85, 143.75, 140.41, 136.50, 130.12, 126.98, 126.12, 125.48, 110.31, 102.16, 84.51, 84.40, 71.20, 63.69, 62.48, 51.63, 38.36, 21.42, 12.50; HRMS (EI): calcd for $C_{24}H_{26}N_6O_6$ $[M+H]^+$ 495.1992, found 495.2024.

*4.4.6. 1-((2R,4S,5R)-5-((4-(((3-(4-(tert-butyl)phenyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-4-hydroxytetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**7f**)*

Yield: 94%, light yellow solid, mp 195–197 °C. IR (KBr) (ν/cm^{-1}) 3746, 3383, 3173, 3110, 3059, 2952, 2924, 2855, 1717, 1659, 1512, 1474, 1435, 1366, 1273, 1204, 1099, 1045, 980, 907, 841, 606; ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.33 (s, 1H), 8.17 (s, 1H), 7.80 (d, J = 8.5 Hz, 2H), 7.53 (d, J = 8.6 Hz, 2H), 7.35 (d, J = 1.0 Hz, 1H), 7.03 (s, 1H), 6.17 (t, J = 7.0 Hz, 1H), 5.52 (d, J = 4.4 Hz, 1H), 4.76 – 4.69 (m, 3H), 4.67 – 4.60 (m, 3H), 4.28 (td, J = 7.5, 3.6 Hz, 1H), 4.11 – 4.06 (m, 1H), 2.18 (dt, J = 13.8, 6.8 Hz, 1H), 2.09 (ddd, J = 13.5, 6.4, 3.8 Hz, 1H), 1.79 (d, J = 0.8 Hz, 3H), 1.31 (s, 9H); ^{13}C NMR (126 MHz, DMSO) δ ppm: 169.93, 164.09, 162.11, 153.38, 150.84, 143.74, 136.50, 126.86, 126.34, 126.15, 125.48, 110.30, 102.20, 84.51, 84.40, 71.20, 63.72, 62.50, 51.63, 38.37, 35.04, 31.41, 12.50; HRMS (EI): calcd for $C_{27}H_{32}N_6O_6$ $[M+H]^+$ 537.2462, found 537.2483.

*4.4.7. 1-((2R,4S,5R)-4-hydroxy-5-((4-(((3-(3-nitrophenyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**7g**)*

Yield: 85%, light yellow solid, mp 191–193 °C. IR (KBr) (ν/cm^{-1}) 3750, 3383, 3179, 3113, 3063, 2954, 2928, 1721, 1655, 1512, 1477, 1435, 1350, 1273, 1204, 1096, 1042, 910, 864, 806, 745, 696, 610; ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.32 (s, 1H), 8.68 – 8.64 (m, 1H), 8.36 (td, J = 8.2, 4.7 Hz, 2H), 8.17 (s, 1H), 7.84 (t, J = 8.0 Hz, 1H), 7.35 (s, 1H), 7.31 (s, 1H), 6.16 (t, J = 7.0 Hz, 1H), 5.52 (d, J = 4.3 Hz, 1H), 4.77 – 4.70 (m, 3H), 4.69 – 4.60 (m, 3H), 4.28 (dt, J = 7.7, 3.9 Hz, 1H), 4.09 (dt, J = 7.8, 4.1 Hz, 1H), 2.18 (dt, J = 13.7, 6.9 Hz, 1H), 2.10 (ddd, J = 13.4, 6.4, 3.8 Hz, 1H), 1.79 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ ppm: 170.99, 164.09, 160.86, 150.84, 148.82, 143.72, 136.50, 133.41, 131.36, 130.47, 125.50, 125.33, 121.53, 110.31, 102.59, 84.52, 84.40, 71.20, 63.77, 62.47, 51.65, 38.37, 12.50; HRMS (EI): calcd for $C_{23}H_{23}N_7O_8$ $[M+H]^+$ 526.1686, found 526.1655.

*4.4.8. 1-((2R,4S,5R)-4-hydroxy-5-((4-(((3-(2-methoxyphenyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**7h**)*

Yield: 96%, white solid, mp 138–139 °C. IR (KBr) (ν/cm^{-1}) 3750, 3383, 3178, 3110, 3059, 2954, 2928, 1721, 1655, 1511, 1474, 1435, 1366, 1273, 1204, 1096, 1052, 1018, 957, 860, 752, 610; ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.33 (s, 1H), 8.17 (s, 1H), 7.74 (dd, J = 7.6, 1.5 Hz, 1H), 7.51 – 7.46 (m, 1H), 7.34 (d, J = 0.8 Hz, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.08 – 7.04 (m, 1H),

6.88 (s, 1H), 6.16 (t, *J* = 7.0 Hz, 1H), 5.51 (d, *J* = 4.3 Hz, 1H), 4.75 – 4.70 (m, 3H), 4.66 – 4.60 (m, 3H), 4.28 (dt, *J* = 7.6, 3.8 Hz, 1H), 4.08 (dt, *J* = 7.6, 4.0 Hz, 1H), 3.87 (s, 3H), 2.17 (dt, *J* = 13.7, 6.9 Hz, 1H), 2.09 (ddd, *J* = 13.5, 6.4, 3.8 Hz, 1H), 1.79 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ ppm: 168.84, 164.09, 159.88, 157.41, 150.85, 143.78, 136.48, 132.08, 129.31, 125.47, 121.19, 117.54, 112.70, 110.31, 105.35, 84.50, 84.40, 71.19, 63.71, 62.42, 56.17, 51.62, 38.37, 12.50; HRMS (EI): calcd for $C_{24}H_{26}N_6O_7$ $[M+H]^+$ 511.1941, found 511.1933.

*4.4.9. 1-((2R,4S,5R)-5-((4-(((3-(4-fluorophenyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-4-hydroxytetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**7i**)*

Yield: 94%, orange solid, mp 212–214 ^{o}C. IR (KBr) (ν/cm^{-1}) 3746, 3383, 3173, 3113, 3063, 2955, 2924, 1721, 1655, 1524, 1474, 1435, 1369, 1273, 1227, 1096, 1042, 980, 845, 652, 610; ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.32 (s, 1H), 8.17 (s, 1H), 7.94 (dd, *J* = 8.8, 5.5 Hz, 2H), 7.40 – 7.32 (m, 3H), 7.08 (s, 1H), 6.16 (t, *J* = 7.0 Hz, 1H), 5.51 (d, *J* = 4.4 Hz, 1H), 4.72 (q, *J* = 4.2 Hz, 3H), 4.67 – 4.59 (m, 3H), 4.28 (td, *J* = 7.5, 3.7 Hz, 1H), 4.11 – 4.06 (m, 1H), 2.18 (dt, *J* = 13.7, 6.9 Hz, 1H), 2.10 (ddd, *J* = 13.5, 6.4, 3.8 Hz, 1H), 1.78 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ ppm: 170.28, 164.65, 164.09, 162.68, 161.42, 150.85, 143.74, 136.50, 129.47, 129.40, 125.49, 116.72, 116.54, 110.31, 102.26, 84.52, 84.40, 71.20, 63.72, 62.48, 51.64, 38.37, 12.50; HRMS (EI): calcd for $C_{23}H_{23}FN_6O_6$ $[M+H]^+$ 499.1741, found 499.1721.

4.4.10. 1-((2R,4S,5R)-5-((4-(((3-(4-bromophenyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-4-hydroxytetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (7j)

Yield: 89%, yellow solid, mp 232–234 ^{o}C. IR (KBr) (ν/cm^{-1}) 3750, 3379, 3173, 3113, 3063, 2950, 2924, 1721, 1655, 1512, 1477, 1427, 1369, 1273, 1204, 1096, 1041, 977, 822, 652, 610; ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.33 (s, 1H), 8.17 (s, 1H), 7.84 (d, *J* = 8.5 Hz, 2H), 7.79 – 7.69 (m, 2H), 7.35 (s, 1H), 7.11 (s, 1H), 6.17 (t, *J* = 7.0 Hz, 1H), 5.54 (s, 1H), 4.73 (q, *J* = 4.7 Hz, 3H), 4.68 – 4.57 (m, 3H), 4.28 (d, *J* = 2.1 Hz, 1H), 4.13 – 4.04 (m, 1H), 2.18 (dt, *J* = 13.7, 6.8 Hz, 1H), 2.10 (ddd, *J* = 13.4, 6.4, 3.9 Hz, 1H), 1.77 (d, *J* = 16.4 Hz, 3H); ^{13}C NMR (126 MHz, DMSO) δ ppm: 170.47, 164.11, 161.47, 150.86, 143.71, 136.51, 132.61, 129.10, 128.13, 125.50, 124.14, 110.31, 102.27, 84.51, 84.40, 71.19, 63.72, 62.46, 51.64, 38.37, 12.51; HRMS (EI): calcd for $C_{23}H_{23}BrN_6O_6$ $[M+H]^+$ 559.0941, found 561.0887.

*4.4.11. 1-((2R,4S,5R)-4-hydroxy-5-((4-(((3-((E)-styryl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**7k**)*

Yield: 89%, orange solid, mp 206–208 °C. IR (KBr) (ν/cm^{-1}) 3746, 3507, 3416, 3173, 3135, 3065, 3021, 2957, 2924, 1713, 1639, 1466, 1436, 1369, 1281, 1240, 1142, 1065, 964, 895, 810, 756, 694, 640, 579; ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.32 (s, 1H), 8.17 (s, 1H), 7.67 (d, J = 7.3 Hz, 2H), 7.48 – 7.34 (m, 5H), 7.24 (d, J = 16.5 Hz, 1H), 6.96 (s, 1H), 6.17 (t, J = 7.0 Hz, 1H), 5.52 (d, J = 4.3 Hz, 1H), 4.76 – 4.67 (m, 3H), 4.67 – 4.60 (m, 3H), 4.29 (td, J = 7.6, 3.8 Hz, 1H), 4.12 – 4.06 (m, 1H), 2.18 (dt, J = 13.8, 6.9 Hz, 1H), 2.10 (ddd, J = 13.5, 6.4, 3.8 Hz, 1H), 1.79 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ ppm: 169.22, 164.10, 162.05, 150.85, 143.77, 137.00, 136.51, 136.09, 129.42, 129.31, 127.59, 125.47, 116.02, 110.32, 101.35, 84.52, 84.40, 71.21, 63.68, 62.49, 51.64, 38.36, 12.51; HRMS (EI): calcd for $C_{25}H_{26}N_6O_6$ $[M+H]^+$ 507.1992, found 507.1974.

*4.4.12. 1-((2R,4S,5R)-4-hydroxy-5-((4-(((3-(pyridin-2-yl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**7l**)*

Yield: 84%, white solid, mp 178–179 °C. IR (KBr) (ν/cm^{-1}) 3750, 3391, 3173, 3113, 3055, 2950, 2924, 1721, 1655, 1512, 1477, 1403, 1371, 1273, 1204, 1096, 918, 864, 787, 610; ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.32 (s, 1H), 8.72 (d, J = 4.8 Hz, 1H), 8.18 (s, 1H), 8.03 (d, J = 7.8 Hz, 1H), 7.97 (td, J = 7.8, 1.7 Hz, 1H), 7.55 – 7.51 (m, 1H), 7.34 (s, 1H), 7.05 (s, 1H), 6.17 (t, J = 7.0 Hz, 1H), 5.51 (d, J = 4.3 Hz, 1H), 4.76 – 4.70 (m, 3H), 4.67 – 4.60 (m, 3H), 4.28 (dt, J = 7.6, 3.9 Hz, 1H), 4.12 – 4.06 (m, 1H), 2.17 (dt, J = 13.8, 6.9 Hz, 1H), 2.09 (ddd, J = 13.4, 6.3, 3.8 Hz, 1H), 1.78 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ ppm: 170.41, 164.09, 163.23, 150.84, 150.43, 147.98, 143.75, 137.99, 136.48, 125.58, 125.49, 121.85, 110.31, 102.82, 84.51, 84.40, 71.20, 63.69, 62.32, 51.63, 38.38, 12.50; HRMS (EI): calcd for $C_{22}H_{23}N_7O_6$ $[M+H]^+$ 482.1788, found 482.1783.

*4.4.13. 1-((2R,4S,5S)-5-(hydroxymethyl)-4-(4-(((3-phenylisoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**8a**)*

Yield: 89%, white solid, mp 132–134 °C. IR (KBr) (ν/cm^{-1}) 3746, 3447, 3229, 3116, 3071, 3008, 2957, 2928, 1694, 1607, 1466, 1404, 1365, 1277, 1206, 1076, 976, 930, 791, 768, 691, 610; ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.35 (s, 1H), 8.37 (s, 1H), 7.90 – 7.87 (m, 2H), 7.82 (d, J = 1.2 Hz, 1H), 7.53 – 7.51 (m, 2H), 7.08 (s, 1H), 6.42 (t, J = 6.6 Hz, 1H), 5.38 (dt, J = 8.7, 5.4 Hz, 1H), 5.28 (t, J = 5.2 Hz, 1H), 4.74 (s, 2H), 4.69 (s, 2H), 4.28 (q, J = 7.1 Hz, 1H), 4.24 – 4.20 (m, 1H), 3.70 (ddd, J = 11.9, 5.2, 3.5 Hz, 1H), 3.62 (ddd, J = 12.1, 5.1, 3.9 Hz, 1H), 2.74 (ddd, J =

13.8, 6.7, 5.5 Hz, 1H), 2.68 – 2.61 (m, 1H), 1.81 (d, *J* = 1.0 Hz, 3H); ^{13}C NMR (126 MHz, DMSO) δ ppm: 170.16, 164.18, 162.28, 150.89, 143.96, 136.69, 131.95, 130.72, 129.58, 129.10, 128.90, 127.08, 124.41, 110.08, 102.29, 84.91, 84.34, 63.78, 62.53, 61.78, 61.18, 59.67, 37.61, 12.72; HRMS (EI): calcd for $C_{23}H_{24}N_6O_6$ $[M+H]^+$ 481.1836, found 481.1814.

*4.4.14. 1-((2R,4S,5S)-4-(4-(((3-(2-chlorophenyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**8b**)*

Yield: 92%, yellow solid, mp 120–122 °C. IR (KBr) (ν/cm^{-1}) 3746, 3429, 3240, 3174, 3116, 3067, 2957, 2924, 1732, 1686, 1607, 1454, 1409, 1362, 1265, 1096, 1045, 972, 756, 621, 571; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 9.13 (s, 1H), 7.80 (s, 1H), 7.70 (dd, *J*= 7.5, 1.7 Hz, 1H), 7.50 – 7.46 (m, 1H), 7.43 (s, 1H), 7.38 (tdd, *J*= 15.0, 10.6, 4.6 Hz, 2H), 6.77 (s, 1H), 6.22 (t, *J*= 6.5 Hz, 1H), 5.47 (dd, *J*= 13.1, 5.4 Hz, 1H), 4.79 (s, 2H), 4.77 (s, 2H), 4.46 – 4.42 (m, 1H), 4.00 (d, *J*= 10.8 Hz, 1H), 3.79 (d, *J*= 10.9 Hz, 1H), 2.96 (qd, *J*= 14.1, 6.7 Hz, 2H), 1.91 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm: 168.32, 163.62, 161.07, 150.41, 144.52, 137.89, 132.86, 131.02, 130.94, 130.45, 128.00, 127.17, 123.19, 111.32, 104.93, 88.90, 85.23, 64.18, 63.09, 61.64, 59.51, 37.45, 12.42; HRMS (EI): calcd for $C_{23}H_{23}ClN_6O_6$ $[M+H]^+$ 515.1446, found 515.1424.

*4.4.15. 1-((2R,4S,5S)-4-(4-(((3-(4-chlorophenyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**8c**)*

Yield: 91%, yellow solid, mp 78–80 °C. IR (KBr) (ν/cm^{-1}) 3746, 3397, 3132, 3063, 2924, 2867, 1697, 1607, 1512, 1474, 1427, 1366, 1273, 1092, 1045, 949, 907, 833, 610; ^{1}H NMR (600 MHz, DMSO) δ ppm: 11.36 (s, 1H), 8.36 (s, 1H), 7.92 – 7.91 (m, 1H), 7.91 – 7.90 (m, 1H), 7.82 (d, *J* = 1.2 Hz, 1H), 7.61 – 7.60 (m, 1H), 7.59 – 7.58 (m, 1H), 7.12 (s, 1H), 6.42 (t, *J* = 6.6 Hz, 1H), 5.39 – 5.35 (m, 1H), 5.28 (t, *J* = 5.2 Hz, 1H), 4.74 (s, 2H), 4.68 (s, 2H), 4.23 – 4.20 (m, 1H), 3.69 (ddd, *J* = 12.0, 5.2, 3.5 Hz, 1H), 3.64 – 3.60 (m, 1H), 2.75 – 2.70 (m, 1H), 2.67 – 2.61 (m, 1H), 1.81 (d, *J* = 1.0 Hz, 3H); ^{13}C NMR (151 MHz, DMSO) δ ppm: 170.47, 164.19, 161.38, 150.90, 143.95, 136.69, 135.42, 129.70, 128.88, 127.77, 110.10, 102.33, 84.90, 84.34, 63.79, 62.51, 61.18, 59.69, 37.61, 12.73; HRMS (EI): calcd for $C_{23}H_{23}ClN_6O_6$ $[M+H]^+$ 515.1446, found 515.1410.

*4.4.16. 1-((2R,4S,5S)-5-(hydroxymethyl)-4-(4-(((3-(4-methoxyphenyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**8d**)*

Yield: 96%, white solid, mp 126–128 °C. IR (KBr) (ν/cm^{-1}) 3746, 3447, 3225, 3129, 3075, 3008, 2928, 2874, 1701, 1612, 1528, 1460, 1435, 1365, 1254, 1177, 1080, 1022, 930, 814, 747,

652, 601; ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.38 (s, 1H), 8.37 (s, 1H), 7.82 (d, *J*= 2.0 Hz, 2H), 7.82 – 7.80 (m, 1H), 7.08 – 7.06 (m, 1H), 7.06 – 7.05 (m, 1H), 7.02 (s, 1H), 6.42 (t, *J*= 6.6 Hz, 1H), 5.37 (dt, *J*= 8.7, 5.4 Hz, 1H), 5.30 (t, *J*= 5.2 Hz, 1H), 4.72 (s, 2H), 4.68 (s, 2H), 4.23 – 4.19 (m, 1H), 3.82 (s, 3H), 3.69 (ddd, *J*= 11.9, 5.2, 3.5 Hz, 1H), 3.64 – 3.59 (m, 1H), 2.76 – 2.70 (m, 1H), 2.67 – 2.60 (m, 1H), 1.81 (d, *J*= 0.7 Hz, 3H); ^{13}C NMR (126 MHz, DMSO) δ ppm: 169.80, 164.18, 161.87, 161.20, 150.92, 143.98, 136.69, 128.57, 124.40, 121.27, 114.97, 110.08, 102.03, 84.90, 84.34, 63.77, 62.55, 61.18, 59.67, 55.77, 37.61, 12.72; HRMS (EI): calcd for $C_{24}H_{26}N_6O_7$ $[M+H]^+$ 511.1941, found 511.1918.

*4.4.17. 1-((2R,4S,5S)-5-(hydroxymethyl)-4-(4-(((3-(p-tolyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**8e**)*

Yield: 94%, white solid, mp 149–151 ^{o}C. IR (KBr) (ν/cm^{-1}) 3746, 3435, 3225, 3129, 3071, 3008, 2928, 2874, 1701, 1612, 1531, 1458, 1429, 1371, 1277, 1080, 1052, 934, 814, 664, 608; ^{1}H NMR (600 MHz, DMSO) δ ppm: 11.36 (s, 1H), 8.37 (s, 1H), 7.82 (d, *J* = 1.1 Hz, 1H), 7.78 – 7.75 (m, 2H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.05 – 7.02 (m, 1H), 6.42 (t, *J* = 6.6 Hz, 1H), 5.36 (ddd, *J* = 41.9, 22.5, 8.5 Hz, 2H), 4.73 (s, 2H), 4.68 (s, 2H), 4.21 (dt, *J* = 5.5, 3.7 Hz, 1H), 3.69 (dd, *J* = 12.1, 3.4 Hz, 1H), 3.62 (dd, *J* = 12.1, 3.7 Hz, 1H), 2.73 (ddd, *J* = 15.1, 6.7, 5.6 Hz, 1H), 2.67 – 2.61 (m, 1H), 2.36 (s, 3H), 1.81 (d, *J* = 0.9 Hz, 3H); ^{13}C NMR (151 MHz, DMSO) δ ppm: 169.97, 164.20, 162.18, 150.90, 143.97, 140.44, 136.70, 130.13, 126.98, 126.10, 110.09, 102.18, 84.91, 84.33, 63.77, 62.53, 61.17, 59.67, 37.61, 21.43, 12.73; HRMS (EI): calcd for $C_{24}H_{26}N_6O_6$ $[M+H]^+$ 495.1992, found 495.1975.

*4.4.18. 1-((2R,4S,5S)-4-(4-(((3-(4-(tert-butyl)phenyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**8f**)*

Yield: 90%, light yellow solid, mp 107–109 ^{o}C. IR (KBr) (ν/cm^{-1}) 3734, 3435, 3180, 3135, 3063, 2959, 2924, 2868, 1697, 1563, 1470, 1429, 1362, 1269, 1096, 1045, 903, 836, 774, 691, 614; ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.38 (s, 1H), 8.38 (s, 1H), 7.82 (d, *J*= 1.1 Hz, 1H), 7.81 (d, *J*= 1.8 Hz, 1H), 7.80 – 7.78 (m, 1H), 7.55 – 7.53 (m, 1H), 7.53 – 7.52 (m, 1H), 7.04 (s, 1H), 6.43 (t, *J*= 6.6 Hz, 1H), 5.38 (dt, *J*= 8.6, 5.4 Hz, 1H), 5.30 (t, *J*= 5.2 Hz, 1H), 4.73 (s, 2H), 4.68 (s, 2H), 4.21 (dd, *J*= 8.9, 3.6 Hz, 1H), 3.69 (ddd, *J*= 11.9, 5.1, 3.5 Hz, 1H), 3.64 – 3.59 (m, 1H), 2.76 – 2.69 (m, 1H), 2.67 – 2.60 (m, 1H), 1.81 (d, *J*= 0.9 Hz, 3H), 1.31 (s, 9H); ^{13}C NMR (126 MHz, DMSO) δ ppm: 169.96, 164.18, 162.11, 153.40, 150.89, 143.97, 136.69, 126.86,

126.35, 126.14, 124.41, 110.09, 102.23, 84.91, 84.34, 63.81, 62.56, 61.19, 59.68, 37.61, 35.04, 31.40, 12.72; HRMS (EI): calcd for $C_{27}H_{32}N_6O_6$ $[M+H]^+$ 537.2462, found 537.2429.

*4.4.19. 1-((2R,4S,5S)-5-(hydroxymethyl)-4-(4-(((3-(3-nitrophenyl)isoxazol-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**8g**)*

Yield: 87%, light yellow solid, mp 96–98 °C. IR (KBr) (ν/cm^{-1}) 3750, 3653, 3266, 3152, 3074, 2928, 2874, 1697, 1531, 1458, 1346, 1269, 1048, 999, 945, 903, 810, 745, 694, 594; ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.35 (s, 1H), 8.67 – 8.65 (m, 1H), 8.38 – 8.34 (m, 3H), 7.86 – 7.81 (m, 2H), 7.32 (s, 1H), 6.42 (t, J = 6.6 Hz, 1H), 5.38 (dt, J = 8.6, 5.4 Hz, 1H), 5.28 (t, J = 5.2 Hz, 1H), 4.78 (s, 2H), 4.71 (s, 2H), 4.23 – 4.20 (m, 1H), 3.70 (ddd, J = 12.0, 5.2, 3.5 Hz, 1H), 3.65 – 3.60 (m, 1H), 2.77 – 2.71 (m, 1H), 2.68 – 2.61 (m, 1H), 1.81 (d, J = 1.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ ppm: 171.02, 164.17, 160.86, 150.89, 148.82, 143.94, 136.68, 133.41, 131.38, 130.46, 125.34, 124.43, 121.54, 110.09, 102.62, 84.91, 84.36, 63.84, 62.53, 61.19, 59.70, 37.61, 12.71; HRMS (EI): calcd for $C_{23}H_{23}N_7O_8$ $[M+H]^+$ 526.1686, found 526.1670.

4.5 Evaluation of the biological activity

In preliminary color screens for QS activity, we used the biomonitor CV026 for short-chain AHL inhibitor detection. CV026 was cultured in LB broth with shaking at 170 rpm overnight. C6-HSL (15 μL, 0.125 mM) was then added and the mixture was gently mixed with molten semi-solid LB agar (5 mL). Then, the mixture was overlaid on the solid LB agar. After solidification of the top layer, the synthesized compounds were spotted on the plate and the plates were incubated overnight at 30 °C. Finally, the inhibiting activities were determined by the presence of white colonies/holes in a purple background of the plates.[55,56]

For the secondary screening of the compounds inhibited activities, CV026 were cultured in LB broth and subsequently diluted and added into 12-well plates. The PBS solution of C6-HSL (15 μL, 0.125 mM) and different concentrations of synthesized compounds were added to the wells. After incubation for 16–18 h, 1 mL of the culture was taken off each mixture and centrifuged at 12,000 g for 10 min. The supernatant was then discarded and DMSO (500 μL) was added to each tube. Then the mixtures were centrifuged at 12,000 g for 10 min. Finally, 200 μL of the upper layer was put into a 96-well plate, and the absorbance was read at 585 nm.[57] This is because the maximum absorbance wavelength of violacein solution appears on 585 nm. Also, the absorbance change showed the concentration changes of violacein. Thus, the QS inhibition effect of the

synthesized compound to Gram-negative bacteria was evaluated by detecting the absorbance change at 585 nm. The expriments were carried out three times. The IC50 (half maximum inhibitory concentration) of each compounds were calculated using Graphpad 6.0 software and were defined to be the average of three determinations.

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Highlights

- An efficient and eco-friendly ultrasonic-assisted Click chemistry method for synthesizing 1,2,3-tiazoles containing isoxazole and β-thymidine structures was developed.
- The reaction conditions were optimized. Nineteen new target products were obtained in 84–96% yields.
- The synthesized compounds have a significant quorum sensing inhibitory activities.