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Asymmetric Synthesis and Antitumor Activity of Spiro-Oxadiazole Derivatives from 1,4:3,6-Dianhydro-*D*-fructose

Wenke Xu,[a,b] Yongxun Ge,[c] Yu Hou,[d] Yingju Liu,[a,b] Yingchun Hua,[a,b] Weiwei Han,[a,b] Zhiyan Qin,[a] and Fengwu Liu*[,a,b]

[a] *School of Pharmaceutical Sciences, Zhengzhou University, Zhengzhou, Henan 450001, China*
[b] *Collaborative Innovation Center of Henan New Drug Research and Safety Evaluation, Zhengzhou, Henan 450001, China*
[c] *Zhengzhou Central Hospital Affiliated to Zhengzhou University, Zhengzhou, Henan 450007, China*
[d] *Department of Medicine, Kaifeng University, Kaifeng, Henan 475000, China*

A series of spiro-oxadiazoles were synthesized from 1,4:3,6-dianhydro-*D*-fructose and hydrazides via a stereoselective two-step reaction sequence. The structures of newly synthesized compounds were established by spectral analysis. The absolute configuration of compound **2a** was further confirmed by single crystal X-ray analysis. All the synthesized compounds were screened for their *in vitro* antitumor activity, showing that these compounds have poor inhibitory activities against A549, MGC-803 tumor cells.

Keywords 1,4:3,6-dianhydro-*D*-froctose, spiro-oxadiazole, single crystal X-ray analysis, antitumor activity

Introduction

1,3,4-Oxadiazoles, containing an oxygen atom and two nitrogen atoms in a five-membered ring, have attracted significant attention in the field of drug discovery for decades due to their broad spectra of biological activities such as anti-fungal, antibacterial, analgesic, anti-inflammatory, anti-hypertension, antitumor and muscle relaxing activities.[1] The synthesis and biological evaluation of novel 1,3,4-oxadiazole derivatives has accelerated in the last two decades.[2] A number of therapeutic agents, such as HIV-integrase inhibitor Raltegravir[3] and anticancer agent Zibotentan,[4] consist of 1,3,4-oxadiazole moiety. 3-Acetyl-2,3-dihydro-1,3,4-oxadiazoles, an important class of 1,3,4-oxadiazole derivatives, have been well documented with biological activities.[5] Substituted 3-acetyl-2,3-dihydro-1,3,4-oxadiazoles are generally obtained from hydrazones in acetic anhydride or acetyl chloride under heating.[6] On the other hand, introduction of chirality into bioactive molecules may improve their biological activity or endow molecules new bioactivity. Carbohydrates are an important class of natural chiral source. Therefore, carbohydrate-based 1,3,4-oxadiazoles have been synthesized in the past years.[7-9]

In our previous paper, we reported a V-shaped chiral precursor 1,4:3,6-dianhydro-*D*-fructose (DAF), which consists of a highly reactive carbonyl group and allows good control in diastereoselectivity of such reactions as nucleophilic addition to the carbonyl group. Because of the potent bioactivity and pharmaceutical applications of 1,4:3,6-dianhydrohexitol derivatives and the wide utilization of the characteristic V-shaped skeleton in asymmetric synthesis,[10-14] we have made great efforts for years to produce novel chiral compounds from the V-shaped precursors and investigate their stereochemistry and bioactivities.[15] As the continuation of our program aimed at the discovery and development of chiral bioactive product, encouraged by the above-mentioned interesting bioactivity of 1,3,4-oxadiazole derivatives, we designed and synthesized a series of 1,4:3,6-dianhydro-*D*-fructose based 1,3,4-oxadiazole derivatives (Figure 1). To our knowledge, this class of compounds have not been reported in previous literatures. Herein, we describe the synthesis and antitumor activity of these 1,3,4-oxadiazole derivatives.

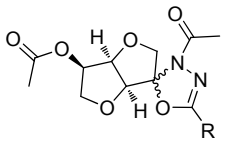

Figure 1 Novel chiral 1,3,4-oxadiazole derivatives.

Experimental

General methods

Unless otherwise specified, all reactants and reagents

* E-mail: fwliu@zzu.edu.cn
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were purchased commercially and used without further purification. Solvents were purified by standard procedures. Melting points were determined on an XT5B melting-point apparatus and are uncorrected. Optical rotations were obtained on a polAAr 3001 automatic polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVANCE III 400 MHz spectrometer. The ^{1}H and ^{13}C NMR chemical shifts (δ) were referenced to internal tetramethylsilane (Me_4Si). HRMS (high-resolution mass spectra) were taken with a Q-Tof Micromass spectrometer. Thin-layer chromatography (TLC) was performed on glass plates precoated with silica gel GF 254 (5－40 μm) and detected by placing under the UV lamp or by spraying the chromatograms with 5% ethanolic sulfuric acid and charring them with a heating gun.

X-ray diffraction experiment

X-ray diffraction analysis was carried out on a Rigaku RAXIS-IV imaging plate with graphite monochromated Mo Kα radiation (λ＝0.71073 Å). An monoclinic crystal was selected and mounted on a glass fiber. All data were collected at a temperature of 291(2) K and corrected for Lorentz-polarization effects. The structure was solved via direct methods and expanded using the Fourier technique. The non-hydrogen atoms were refined with anisotropic thermal parameters. Hydroxyl hydrogen atoms were refined with isotropic thermal parameters. Other hydrogen atoms were included but not refined. All calculations were performed using the SHELX-97 crystallographic software package.[16]

General procedures for the preparation of acylhydrazine

Hydrazine hydrate (80%, 2 mL) and ethyl carboxylate (10 mL) were added to 20 mL of ethanol.The mixture was refluxed and monitored by TLC detection until the disappearance of hydrazine hydrate. The resulting mixture was concentrated under reduced pressure and coevaporated with toluene twice to give crude acylhydrazine. The crude product was recrystallized with anhydrous alcohol to afford acylhydrazine as a white solid.

General procedures for the preparation of hydrazones

The solution of 1,4:3,6-dianhydro-*D*-fructose (288 mg, 2 mmol) and acylhydrazide (2.4 mmol) in EtOH (40.0 mL) was heated to 80 ℃. The reaction was monitored by TLC until the reaction was completed in about 12 h, the resulting mixture was concentrated under vacuum to give a foam which was directly used in the next step without further purification.

General procedures for the preparation of 2

The solution of hydrazones (4 mmol) and acetic anhydride (12 mmol) in pyridine (10.0 mL) under stirring was heated to 100 ℃ and kept the temperature until the disappearance of hydrazones by TLC detection. The solution was evaporated and coevaporated twice under reduced pressure. The residual syrup was purified by column chromatography (Silica Gel, 200－300 mesh) with gradient elution (dichloromethane/petroleum ether/acetone, 0－25∶25∶1, *V*∶*V*∶*V*) to afford **2**.

(3*R*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-methyl-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate (**2a**): White solid; m.p. 82－84 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.09 (s, 3H, CH_3), 2.14 (s, 3H, $COCH_3$), 2.22 (s, 3H, $COCH_3$), 3.78 (t, J＝8.9 Hz, 1H, H-6b), 3.95 (d, J＝10.3 Hz, 1H, H-1b), 4.15－4.18 (m, 1H, H-6a), 4.42 (d, J＝10.4 Hz, 1H, H-1a), 4.91 (t, J＝5.0 Hz, 1H, H-4), 4.97－5.05 (m, 1H, H-5), 5.09 (d, J＝5.0 Hz, 1H, H-3); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 11.3 (CH_3), 20.6 ($COCH_3$), 22.0 ($COCH_3$), 68.6 (C-6), 72.7 (C-5), 73.0 (C-1), 80.1 (C-4), 83.7 (C-3), 104.0 (C-2), 155.23 (C-7), 167.1 (C＝O), 170.3 (C＝O); HRMS (ESI) calcd for $C_{12}H_{17}N_2O_6$ [M＋H$^+$] 285.1081, found 285.1084.

(3*R*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-ethyl-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate (**2b**): White solid; m.p. 61－63 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.22 (t, J＝7.7 Hz, 3H, CH_3), 2.11 (s, 3H, $COCH_3$), 2.20 (s, 3H, $COCH_3$), 2.39 (q, J＝7.5 Hz, 2H, CH_2), 3.75 (t, J＝8.9 Hz, 1H, H-6b), 3.91 (d, J＝10.3 Hz, 1H, H-1b), 4.14 (t, J＝7.6 Hz, 1H, H-6a), 4.40 (d, J＝10.3 Hz, 1H, H-1a), 4.87 (s, 1H, H-4), 4.93－5.04 (m, 1H, H-5), 5.08 (d, J＝4.5 Hz, 1H, H-3); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 9.6 (CH_3), 19.2 (CH_2), 20.6 ($COCH_3$), 22.0 ($COCH_3$), 68.5 (C-6), 72.6 (C-5), 72.8 (C-1), 79.9 (C-4), 83.7 (C-3), 103.8 (C-2), 159.2 (C-7), 167.2 (C＝O), 170.3 (C＝O); HRMS (ESI) calcd for $C_{13}H_{19}N_2O_6$ [M＋H$^+$] 299.1238, found 299.1243.

(3*R*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-propyl-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate (**2c**): White solid; m.p. 80－81 ℃; $[\alpha]_D^{20}$ ＋167.9 (*c* 1.00, EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.02 (t, J＝7.4 Hz, 3H, CH_3), 1.68－1.73 (m, 2H, CH_2), 2.15 (s, 3H, $COCH_3$), 2.23 (s, 3H, $COCH_3$), 2.37 (t, J＝7.4 Hz, 2H, CH_2), 3.78 (t, J＝8.9 Hz, 1H, H-6b), 3.93 (d, J＝10.3 Hz, 1H, H-1b), 4.17 (t, J＝7.6 Hz, 1H, H-6a), 4.44 (d, J＝10.3 Hz, 1H, H-1a), 4.90 (t, J＝5.1 Hz, 1H, H-4), 5.04－4.96 (m, 1H, H-5), 5.12 (d, J＝5.1 Hz, 1H, H-3); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 13.5 (CH_3) , 19.0 (CH_2), 20.7 ($COCH_3$), 22.1 ($COCH_3$), 27.3 (CH_2), 68.5 (C-6), 72.7 (C-5), 72.8 (C-1), 79.9 (C-4), 83.7 (C-3), 103.7 (C-2), 158.2 (C-7), 167.2 (C＝O), 170.4 (C＝O); HRMS (ESI) calcd for $C_{14}H_{21}N_2O_6$ [M＋H$^+$] 313.1394, found 313.1398.

(3*R*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-pentyl-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate(**2d**): White foam; m.p. 53－54 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.90 (t, J＝6.8 Hz, 3H, CH_3), 1.34 (dd, J＝8.7, 5.2 Hz, 4H, CH_2CH_2), 1.70－1.60 (m, 2H, CH_2), 2.12 (s, 3H, $COCH_3$), 2.21 (s, 3H, $COCH_3$), 2.36 (t, J＝7.5 Hz, 2H, CH_2), 3.75 (t, J＝8.9 Hz, 1H, H-6b), 3.90 (d, J＝10.3 Hz, 1H, H-1b), 4.14 (t, J＝7.6 Hz, 1H, H-6a), 4.41 (d, J＝10.3 Hz, 1H, H-1a), 4.87 (t, J＝5.1 Hz, 1H, H-4), 4.94－5.02 (m, 1H, H-5),

5.09 (d, J=5.1 Hz, 1H, H-3); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 13.8 (CH_3), 20.6 ($COCH_3$), 22.0 ($COCH_3$), 22.2 (CH_2), 25.0 (CH_2), 25.4 (CH_2), 31.0 (CH_2), 68.5 (C-6), 72.7 (C-5), 72.8 (C-1), 79.9 (C-4), 83.7 (C-3), 103.7 (C-2), 158.4 (C-7), 167.2 (C=O), 170.3 (C=O); HRMS (ESI) calcd for $C_{16}H_{25}N_2O_6$ [M+H$^+$] 341.1707, found 341.1719.

(3*R*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-benzyl-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate (**2e**): White solid; m.p. 81−82 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.14 (s, 3H, $COCH_3$), 2.25 (s, 3H, $COCH_3$), 3.63 (t, J=8.3 Hz, 1H, H-6b), 3.73 (s, 2H, CH_2), 3.89 (d, J=10.3 Hz, 1H, H-1b), 4.04 (dd, J=8.3, 7.1 Hz, 1H, H-6a), 4.43 (d, J=10.3 Hz, 1H, H-1a), 4.87 (t, J=5.2 Hz, 1H, H-4), 4.96 (ddd, J=9.6, 7.0, 5.2 Hz, 1H, H-5), 5.12 (d, J=5.2 Hz, 1H, H-3), 7.31−7.39 (m, 5H, Ar-H); ^{13}C NMR (101 MHz,$CDCl_3$) δ: 20.6 ($COCH_3$), 22.0 ($COCH_3$), 32.0 (CH_2), 68.4 (C-6), 72.6 (C-5), 72.6 (C-1), 79.8 (C-4), 83.6 (C-3), 104.3 (C-2), 127.5, 128.7, 128.8, 133.3 (Ar-C), 156.8 (C-7), 167.5 (C=O), 170.3 (C=O); HRMS (ESI) calcd for $C_{18}H_{21}N_2O_6$ [M+H$^+$] 361.1394, found 361.1397.

(3*R*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-(2-chlorobenzyl)-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate (**2f**): Off-white solid; m.p. 83−85 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.07 (s, 3H, $COCH_3$), 2.21 (s, 3H, $COCH_3$), 3.57−3.61 (m 1H, H-6b), 3.77−3.95 (m, 3H, H-1b CH_2), 3.98−4.04 (m, 1H, H-6a), 4.42 (d, J=10.3 Hz, 1H, H-1a), 4.84 (d, J=4.3 Hz, 1H, H-4), 4.87−4.93 (m, 1H, H-5), 5.11 (d, J=4.4 Hz, 1H, H-3), 7.20−7.29 (m, 2H, Ar-H), 7.34−7.40 (m, 2H, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.6 ($COCH_3$), 22.0 ($COCH_3$), 29.7 (CH_2), 68.4 (C-6), 72.6 (C-1, C-5), 79.7 (C-3), 83.5 (C-4), 104.4 (C-2), 127.0, 129.0, 129.6, 130.7, 131.4, 134.2 (Ar-C), 155.8 (C-7), 167.5 (C=O), 170.3 (C=O); HRMS (ESI) calcd for $C_{18}H_{20}ClN_2O_6$ [M+H$^+$] 395.1004, found 395.1010.

(3*R*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-(furan-2-yl)-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]-oxadiazol]-6-yl acetate (**2g**): White foam; m.p. 127−128 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.18 (s, 3H, $COCH_3$), 2.36 (s, 3H, $COCH_3$), 3.88 (t, J=8.7 Hz, 1H, H-6b), 4.07 (d, J=10.5 Hz, 1H, 1b), 4.23 (t, J=7.6 Hz, 1H, H-6a), 4.51 (d, J=10.5 Hz, 1H, H-1a), 4.96 (t, J=5.1 Hz, 1H, H-4), 5.01−5.08 (m, 1H, H-5), 5.19 (d, J=5.1 Hz, 1H, H-3), 6.57 (dd, J=3.4, 1.7 Hz, 1H, C_4H_3O), 7.00 (d, J=3.2 Hz, 1H, C_4H_3O), 7.62 (s, 1H, C_4H_3O); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.7 ($COCH_3$), 22.2 ($COCH_3$), 68.7 (C-6), 72.67 (C-5), 72.9 (C-1), 80.0 (C-4), 83.7 (C-3), 104.7 (C-2), 111.9 (C_4H_3O), 115.0 (C_4H_3O), 139.3 (C_4H_3O), 146.0 (C_4H_3O), 148.0 (C-7), 167.8 (C=O), 170.3 (C=O); HRMS (ESI) calcd for $C_{15}H_{17}N_2O_7$ [M+H$^+$] 337.1030, found 337.1033.

(3*R*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-phenyl-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate (**2h**): White solid; m.p. 122−124 ℃; $[\alpha]_D^{20}$ +155.3 (*c* 0.50, EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.18 (s, 3H, $COCH_3$), 2.36 (s, 3H, $COCH_3$), 3.92 (t, J=8.7 Hz, 1H, H-6b), 4.06 (d, J=10.4 Hz, 1H, H-1b), 4.23 (t, J=7.6 Hz, 1H, H-6a), 4.53 (d, J=10.4 Hz, 1H, H-1a), 4.96 (t, J=5.0 Hz, 1H, H-4), 5.01−5.09 (m, 1H, H-5), 5.21 (d, J=5.1 Hz, 1H, H-3), 7.46 (t, J=7.4 Hz, 2H, Ar-H), 7.52 (d, J=7.0 Hz, 1H, Ar-H), 7.87 (d, J=7.5 Hz, 2H, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.7 ($COCH_3$), 22.1 ($COCH_3$), 68.7 (C-6), 72.7 (C-5), 72.9 (C-1), 80.0 (C-4), 83.8 (C-3), 104.6 (C-2), 124.1 (Ar-C), 126.9 (Ar-C), 128.7 (Ar-C), 131.8 (Ar-C), 154.8 (C-7), 167.7 (C=O), 170.3 (C=O); HRMS (ESI) calcd for $C_{17}H_{19}N_2O_6$ [M+H$^+$] 346.1165, found 347.1239.

(3*R*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-(*p*-tolyl)-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate (**2i**): White foam; m.p. 114−115 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.18 (s, 3H, $COCH_3$), 2.34 (s, 3H, $COCH_3$), 2.42 (s, 3H, CH_3), 3.91 (t, J=8.7 Hz, 1H, H-6b), 4.05 (d, J=10.3 Hz, 1H, H-1b), 4.23 (t, J=7.3 Hz, 1H, H-6a), 4.52 (d, J=10.3 Hz, 1H, H-1a), 4.95 (t, J=4.3 Hz, 1H, H-4), 5.00−5.08 (m, 1H, H-5), 5.21 (d, J=4.5 Hz, 1H, H-3), 7.75 (d, J=7.2 Hz, 2H, Ar-H), 7.26 (d, J=7.5 Hz, 2H, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.7 ($COCH_3$), 21.7 (CH_3), 22.2 ($COCH_3$), 68.6 (C-6), 72.7 (C-5), 72.9 (C-1), 79.9 (C-4), 83.7 (C-3), 104.4 (C-2), 121.2, 126.9, 129.4, 142.4 (Ar-C), 155.0 (C-7), 167.6 (C=O), 170.3 (C=O); HRMS (ESI) calcd for $C_{18}H_{21}N_2O_6$ [M+H$^+$] 361.1394, found 361.1390.

(3*R*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-(4-methoxyphenyl)-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate (**2j**): White foam; m.p. 146−147 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.18 (s, 3H, $COCH_3$), 2.34 (s, 3H, $COCH_3$), 3.87 (s, 3H), 3.91 (d, J=8.9 Hz, 1H, H-6b), 4.05 (d, J=10.3 Hz, 1H, H-1b), 4.23 (t, J=7.6 Hz, 1H, H-6a), 4.52 (d, J=10.3 Hz, 1H, H-1a), 4.95 (t, J=4.9 Hz, 1H, H-4), 5.04 (dd, J=15.0, 6.4 Hz, 1H, H-5), 5.20 (d, J=5.0 Hz, 1H, H-3), 6.95 (d, J=8.5 Hz, 2H, Ar-H), 7.80 (d, J=8.5 Hz, 2H, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.7 ($COCH_3$), 22.1 ($COCH_3$), 55.4 (OCH_3), 68.6 (C-6), 72.7 (C-5), 72.9 (C-1), 80.0 (C-4), 83.7 (C-3), 104.2 (C-2), 114.2, 116.3, 128.7, 154.8, 162.4 (Ar-C), 167.6 (C=O), 170.4 (C=O); HRMS (ESI) calcd for $C_{18}H_{21}N_2O_7$ [M+H$^+$]: 377.1343, found 377.1349.

(3*R*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-(2-methoxyphenyl)-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate (**2k**): White solid; m.p. 149−150 ℃; $[\alpha]_D^{20}$ +145.7 (*c* 0.50, EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.17 (s, 3H, $COCH_3$), 2.37 (s, 3H, $COCH_3$), 3.86−3.97 (m, 4H, H-6b, OCH_3), 4.05 (d, J=10.2 Hz, 1H, H-1b), 4.23 (t, J=7.3 Hz, 1H, H-6a), 4.52 (d, J=10.2 Hz, 1H, H-1a), 4.96 (s, 1H, H-4), 5.00−5.08 (m, 1H, H-5), 5.21 (d, J=4.2 Hz, 1H, H-3), 7.00−7.08 (m, 2H, Ar-H), 7.49 (t, J=7.7 Hz, 1H, Ar-H), 7.78 (d, J=7.4 Hz, 1H, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.7 ($COCH_3$), 22.2 ($COCH_3$), 56.0 (OCH_3), 68.5 (C-6), 72.8 (C-5), 73.0 (C-1), 80.0 (C-4), 83.8 (C-3), 103.2 (C-2), 111.8, 112.9, 120.6, 130.2,

133.0, 153.3 (Ar-C), 158.4 (C-7), 167.8 (C=O), 170.4 (C=O); HRMS (ESI) calcd for $C_{18}H_{21}N_2O_7$ [M+H$^+$] 377.1343, found 377.1344.

(3*R*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-(4-fluorophenyl)-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate (**2l**): White foam; m.p. 136−138 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.19 (s, 3H $COCH_3$), 2.35 (s, 3H, $COCH_3$), 3.90 (t, J=8.9 Hz, 1H, H-6b), 4.06 (d, J=10.4 Hz, 1H, H-1b), 4.24 (t, J=7.6 Hz, 1H, H-6a), 4.53 (d, J=10.4 Hz, 1H, H-1a), 4.97 (t, J=5.1 Hz, 1H, H-4), 5.01−5.10 (m, 1H, H-5), 5.21 (d, J=5.1 Hz, 1H, H-3), 7.16 (t, J=8.6 Hz, 2H, Ar-H), 7.88 (dd, J=8.6, 5.4 Hz, 2H, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.7 ($COCH_3$), 22.1 ($COCH_3$), 68.7 (C-6), 72.7 (C-5), 72.9 (C-1), 80.0 (C-4), 83.8 (C-3), 104.8 (C-2), 154.0 (C-7), 116.0, 116.2, 120.3, 120.4, 129.2, 129.3, 163.6, 166.1 (Ar-C), 167.7 (C=O), 170.3 (C=O); HRMS (ESI) calcd for $C_{17}H_{18}FN_2O_6$ [M+H$^+$] 365.1143, found 365.1151.

(3*R*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-(4-nitrophenyl)-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate (**2m**): Light yellow foam; m.p. 134−136 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.19 (s, 3H, $COCH_3$), 2.39 (s, 3H, $COCH_3$), 3.92 (t, J=7.4 Hz, 1H, H-6b), 4.10 (d, J=8.5 Hz, 1H, H-1b), 4.27 (t, J=7.6 Hz, 1H, H-6a), 4.54 (d, J=6.6 Hz, 1H, H-1a), 4.99 (d, J=4.5 Hz, 1H, H-4), 5.01−5.03 (m, 1H, H-5), 5.20 (s, 1H, H-3), 8.05 (d, J=6.0 Hz, 2H, Ar-H), 8.33 (d, J=6.0 Hz, 2H, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.6 ($COCH_3$), 22.1 ($COCH_3$), 68.8 (C-6), 72.6 (C-5), 73.0 (C-1), 80.1 (C-4), 83.8 (C-3), 105.6 (C-2), 153.0 (C-7), 124.0, 127.8, 130.0, 149.5 (Ar-C), 168.0 (C=O), 170.3 (C=O); HRMS (ESI) calcd for $C_{17}H_{18}N_3O_8$ [M+H$^+$] 392.1088, found 392.1089.

(3*R*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-(3-nitrophenyl)-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate (**2n**): Light yellow solid; m.p. 168−169 ℃; $[\alpha]_D^{20}$ +132.2 (*c* 1.00, EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.20 (s, 3H, $COCH_3$), 2.39 (s, 3H, $COCH_3$), 3.93 (t, J=8.9 Hz, 1H, H-6b), 4.10 (d, J=10.5 Hz, 1H, H-1b), 4.25 (t, J=7.6 Hz, 1H, H-6a), 4.55 (d, J=10.6 Hz, 1H, H-1a), 4.98 (t, J=5.1 Hz, 1H, H-4), 5.03−5.10 (m, 1H, H-5), 5.21 (d, J=5.1 Hz, 1H, H-3), 7.69 (t, J=8.0 Hz, 1H, Ar-H), 8.19 (d, J=7.8 Hz, 1H, Ar-H), 8.38 (d, J=8.2 Hz, 1H, Ar-H), 8.69 (s, 1H, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.7 ($COCH_3$), 22.2 ($COCH_3$), 68.9 (C-6), 72.6 (C-5), 72.9 (C-1), 80.1 (C-4), 83.8 (C-3), 105.6 (C-2), 121.9, 126.0, 126.1, 130.0, 132.3, 148.4 (Ar-C), 152.8 (C-7), 168.0 (C=O), 170.3 (C=O); HRMS (ESI) calcd for $C_{17}H_{18}N_3O_8$ [M+H$^+$] 392.1088, found 392.1091.

(3*R*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-(4-acetoxyphenyl)-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate (**2o**): White solid; m.p. 131−132 ℃; $[\alpha]_D^{20}$ +151.5 (*c* 0.50, EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.18 (s, 3H, $COCH_3$), 2.34 (s, 3H, CH_3), 2.35 (s, 3H, $COCH_3$), 3.90 (t, J=8.9 Hz, 1H, H-6b), 4.02−4.10 (m, 1H, H-1b), 4.23 (t, J=7.6 Hz, 1H, H-6a), 4.53 (d, J=10.4 Hz, 1H, H-1a), 4.97 (dd, J=11.3, 6.2 Hz, 1H, H-4), 5.05 (dt, J=9.5, 9.0 Hz, 1H, H-5), 5.20 (d, J=4.9 Hz, 1H, H-3), 7.21 (d, J=8.6 Hz, 2H, Ar-H), 7.90 (d, J=8.6 Hz, 2H, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.7 ($COCH_3$), 21.1 (CH_3), 22.1 ($COCH_3$), 68.7 (C-6), 72.7 (C-5), 72.9 (C-1), 80.0 (C-4), 83.8 (C-3), 104.7 (C-2), 122.1, 128.3, 153.3, 154.2 (Ar-C), 167.8 (C-7), 168.9 (C=O), 170.3 (C=O); HRMS (ESI) calcd for $C_{17}H_{19}N_2O_7$ [M+H$^+$] 363.1187, found 363.1187.

General procedures for the preparation of 3

The reaction temperature was changed to reflux, others were the same as the method descripted in general procedures for the preparation of **2**. The residual syrup was purified by chromatography (Silica gel, 200−300 mesh) with gradient elution (dichloromethane/petroleum ether/acetone, 0−25∶25∶1, V∶V∶V) to afford **3** and then **2**, successively.

(3*S*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-phenyl-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate (**3h**): White solid; m.p. 108−110 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.18 (s, 3H, $COCH_3$), 2.41 (s, 3H, $COCH_3$), 3.96−4.17 (m, 3H, H-1b, H-6), 4.47 (s, 1H, H-3), 4.99 (s, 1H, H-4), 5.05 (d, J=7.4 Hz, 1H, H-5), 5.22 (d, J=10.6 Hz, 1H, H-1a), 7.47 (d, J=7.5 Hz, 2H, Ar-H), 7.52 (d, J=7.1 Hz, 1H, Ar-H), 7.85 (d, J=7.1 Hz, 2H, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.7 ($COCH_3$), 23.0 ($COCH_3$), 69.2 (C-6), 70.2 (C-1), 73.7 (C-5), 79.6 (C-4), 85.7 (C-3), 105.4 (C-2), 124.1 (Ar-C), 126.8 (Ar-C), 128.7 (Ar-C), 131.8 (Ar-C), 153.6 (C-7), 168.7 (C=O), 170.5 (C=O); HRMS (ESI) calcd for $C_{17}H_{19}N_2O_6$ [M+H$^+$] 347.1238, found 347.1249.

(3*S*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-(2-methoxyphenyl)-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate (**3k**): White solid; m.p. 155−156 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.18 (s, 3H, $COCH_3$), 2.42 (s, 3H, $COCH_3$), 3.95 (s, 3H, OCH_3), 4.05−4.13 (m, 3H, H-1b, H-6), 4.52 (d, J=4.8 Hz, 1H, H-3), 4.97 (t, J=5.1 Hz, 1H, H-4), 5.07 (ddd, J=9.1, 7.5, 5.3 Hz, 1H, H-5), 5.23 (d, J=10.6 Hz, 1H, H-1a), 6.97−7.09 (m, 2H, Ar-H), 7.45−7.54 (m, 1H, Ar-H), 7.70 (dd, J=7.7, 1.7 Hz, 1H, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.7 ($COCH_3$), 22.9 ($COCH_3$), 56.0 (OCH_3), 69.1 (C-6), 70.2 (C-1), 73.8 (C-5), 79.6 (C-4), 85.5 (C-3), 104.2 (C-2), 111.8, 113.0, 120.6, 130.1, 133.1, 152.4 (Ar-C), 158.4 (C-7), 168.8 (C=O), 170.6 (C=O); HRMS (ESI) calcd for $C_{18}H_{21}N_2O_7$ [M+H$^+$] 377.1343, found 377.1347.

(3*S*,3a*S*,6*R*,6a*R*)-3'-Acetyl-5'-(4-nitrophenyl)-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-6-yl acetate (**3m**): Light yellow foam; m.p. 149−150 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.18 (s, 2H, $COCH_3$), 2.42 (s, 3H, OCH_3), 4.48 (d, J=4.8 Hz, 1H, H-3), 4.03−4.16 (m, 3H, H-1b, H-6), 5.00 (t, J=5.1 Hz, 1H, H-4), 5.04−5.14 (m, 1H, H-5), 5.20 (d, J=10.8 Hz, 1H, H-1a), 8.02 (d, J=8.6 Hz, 2H, Ar-H), 8.31

(d, J=8.6 Hz, 2H, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.6 ($COCH_3$), 23.0 ($COCH_3$), 69.3 (C-6), 70.2 (C-1), 73.5 (C-5), 79.7 (C-4), 85.7 (C-3), 106.4 (C-2), 124.0, 127.7, 129.9, 149.5 (Ar-C), 151.8 (C-7), 168.8 (C=O), 170.4 (C=O); HRMS (ESI) calcd for $C_{17}H_{18}N_3O_8$ [M+H^+] 392.1088, found 392.1088.

General procedures for the preparation of 4 and 5

Potassium carbonate (30 mg) was added to the stirred solution of compound **2** or **3** (100 mg) in methanol (5.0 mL) at room temperature until TLC showed that the reactant had disappeared. The solution was concentrated under reduced pressure. The residual syrup was purified by chromatography (ethyl acetate/petroleum ether 1∶1, V∶V) to afford **4** or **5**.

1-((3*R*,3a*S*,6*R*,6a*R*)-6-Hydroxy-5'-phenyl-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]-oxadiazol]-3'-yl)ethan-1-one (**4h**): White foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.37 (s, 3H, $COCH_3$), 2.62 (d, J=8.9 Hz, 1H, OH), 3.71 (t, J=8.7 Hz, 1H, H-6b), 4.03−4.18 (m, 2H, H-1b, H-6a), 4.33 (s, 1H, H-5), 4.61 (d, J=10.4 Hz, 1H, H-1a), 4.78 (d, J=5.0 Hz, 1H, H-3), 5.19 (d, J=4.7 Hz, 1H, H-4), 7.48−7.53 (m, 3H, Ar-H), 7.88 (d, J=6.8 Hz, 2H, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 22.2 ($COCH_3$), 71.9 (C-6), 72.4 (C-5), 73.0 (C-1), 81.1 (C-3), 83.7 (C-4), 104.9 (C-2), 124.1, 127.0, 128.7, 131.8 (Ar-C), 154.8 (C-7), 167.8 (C=O); HRMS (ESI) calcd for $C_{15}H_{16}N_2O_5$ [M+H^+] 305.1132, found 305.1139.

1-((3*R*,3a*S*,6*R*,6a*R*)-6-Hydroxy-5'-(2-methoxyphenyl)-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]oxadiazol]-3'-yl)ethan-1-one (**4k**): White solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.37 (s, 3H, $COCH_3$), 2.71 (d, J=10.0 Hz, 1H, OH), 3.70 (t, J=8.8 Hz, 1H, H-6b), 3.95 (s, 3H, OCH_3), 4.05 (d, J=10.3 Hz, 1H, H-1b), 4.10 (dd, J=8.4, 6.9 Hz, 1H, H-6a), 4.22−4.38 (m, 1H, H-5), 4.58 (d, J=10.3 Hz, 1H, H-1a), 4.76 (t, J=5.5 Hz, 1H, H-4), 5.19 (d, J=5.5 Hz, 1H, H-3), 6.98−7.08 (m, 2H, Ar-H), 7.43−7.54 (m, 1H, Ar-H), 7.78 (dd, J=7.7, 1.4 Hz, 1H, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 22.2 ($COCH_3$), 56.0 (OCH_3), 71.9 (C-6), 72.3 (C-5), 73.0 (C-1), 81.0 (C-4), 83.6 (C-3), 103.5 (C-2), 153.4 (C-7), 111.8, 112.8, 120.6, 130.2, 133.1, 158.3 (Ar-C), 167.8 (C=O); HRMS (ESI) calcd for $C_{16}H_{19}N_2O_6$ [M+H^+] 335.1238, found 335.1233.

1-((3*S*,3a*S*,6*R*,6a*R*)-6-Hydroxy-5'-phenyl-3a,5,6,6a-tetrahydro-2*H*,3'*H*-spiro[furo[3,2-*b*]furan-3,2'-[1,3,4]-oxadiazol]-3'-yl)ethan-1-one (**5h**): White solid; m.p. 108−109 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.42 (s, 3H, $COCH_3$), 3.56 (d, J=11.0 Hz, 1H, OH), 3.88 (dd, J=9.3, 6.7 Hz, 1H, H-6b), 3.97 (dd, J=9.3, 6.0 Hz, 1H, H-6a), 4.16 (d, J=10.8 Hz, 1H, H-1b), 4.33 (td, J=11.7, 6.0 Hz, 1H, H-5), 4.46 (d, J=5.6 Hz, 1H, H-3), 4.84 (t, J=5.5 Hz, 1H, H-4), 5.24 (d, J=10.8 Hz, 1H, H-a), 7.47 (t, J=7.5 Hz, 2H, Ar-H), 7.54 (t, J=7.4 Hz, 1H, Ar-H), 7.85−7.93 (m, 2H, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 22.9 ($COCH_3$), 71.5 (C-6), 72.8 (C-1), 73.7 (C-5), 82.6 (C-4), 85.9 (C-3), 106.0 (C-2), 124.0, 126.8, 128.8, 131.9 (Ar-C), 153.8 (C-7), 168.9 (C=O); HRMS (ESI) calcd for $C_{15}H_{17}N_2O_5$ [M+H^+] 305.1032, found 305.1024.

SRB assays

Cancer cells were seeded in 96-well plates with 5×10^3 cells per well in 200 μL RPMI 1640 media supplemented with 10% FCS (fetal calf serum). After 24 h incubation, the medium was replaced with fresh RPMI 1640 medium containing the tested compounds **2**−**5** at concentration of 20 μmol•L^{-1}, respectively. After being incubated at 37 ℃ for 72 h, the supernatant medium was discarded from the 96-well plates, and the cells were fixed with 10% trichloroacetic acid (TCA). For a thorough fixation, the plates were allowed to rest at 4 ℃ for 1 h. After fixation, the cells were washed five times with deionized water and allowed to air-dry overnight. The plates were dyed with 50 μL of 0.4% SRB for about 20 min. After dying, the plates were washed with 1% acetic acid to remove the excess of the dye and repeated four times and allowed to air-dry overnight. Tris base solution (150 μL, 10 mmol•L^{-1}) was added to each well. After complete dissolution, the absorbance was measured at 515 nm using microplate reader of multi-wavelength measurement system. Growth inhibition rate was calculated as follows:

growth inhibition rate=(1–absorbance value of the drug group/control group)×100%.

Results and Discussion

Chemistry

1,4:3,6-Dianhydro-*D*-fructose (**1**) was used as carbonyl precursor to construct 3-acetyl-2,3-dihydro-1,3,4-oxadiazoles. Compound **1** was firstly treated with acetyl hydrazide in ethanol by heating without catalyst. After disappearance of starting precursor, the mixture was concentrated and directly dissolved in acetic anhydride, followed by heating at 100 ℃ for 8 h to afford compound **2a** as a white solid with 20% yield. Compound **2a** was elucidated as an oxadiazole by the HRMS, ^{1}H NMR, ^{13}C NMR, and 2D NMR spectral analysis. The absolute configuration of **2a** was further established by X-ray crystallographic analysis of a suitable crystal of **2a** after recrystallization from ethanol, which has an *R*-configuration at newly formed spiro C-2 (as shown in Figure 2).

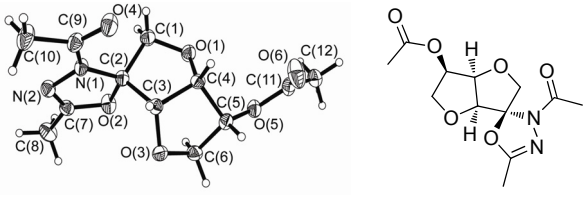


Figure 2 ORTEP plot of **2a** and the structure of **2a**.

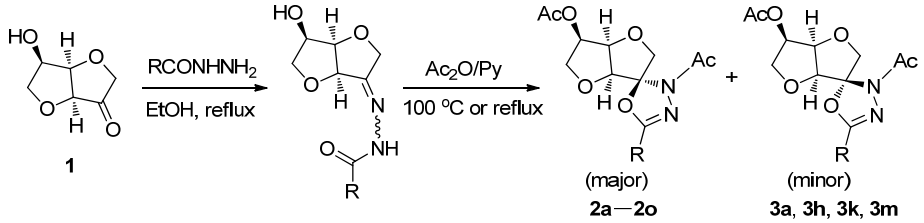
The low yield of the oxadiazole resulted from the

easily-charring features of carbohydrate at high temperature under strong acidic condition. Thus, the conditions for heterocyclization were optimized by using several solvents such as toluene, pyridine and acetonitrile to substitute part of the acetic anhydride to reduce the acidity of the reaction mixture. The result showed that pyridine is the most suitable one among the tested solvents. Then we used pyridine as solvent at 100 ℃ in the heterocyclization later to give oxadiazole in a 45% yield.

A series of hydrazides were obtained by refluxing solution of hydrazine hydrate and various esters in ethanol until the disappearance of hydrazine. Subsequently, the obtained hydrazides were used in the above reaction to replace acetyl hydrazide to afford a series of oxadiazoles with *R*-configuration at C-2 (**2a**—**2o**) as shown in Scheme 1 and Table 1 (Entries 1—15). The result showed that the aromatic hydrazides are slightly more favorable than aliphatic hydrazides in the synthesis of oxadiazoles.

When the reaction temperature of the heterocyclization was raised to reflux by accident in synthesis of **2a**, a minor product **3a** with a lower-polarity appeared together with the major **2a**. Due to their very similar polarity, a mixture of **2a** and **3a** was obtained by chromatography on silica gel. ^{1}H NMR analysis indicated that both compounds have similar chemical shifts and their mole ratio is about 6∶1 (**2a**∶**3a**). In order to further identify the new product, acetyl hydrazine was substituted by benzoyl hydrazine in the reactions, a new minor compound **3h** was obtained. HRMS spectral analysis showed that compound **3h** has the same formula as **2h**, which suggested that **3h** is an isomer of **2h** at C-2. That is, the new minor compounds are (2*S*)-oxadiazoles. Subsequently compounds **3k** and **3m** were also synthesized by following the methodology as described above (shown in Table 2, Entries 16—19). However, because of the more serious charring resulting from higher temperature, total yield of both 2*R* and 2*S* isomers is

Scheme 1 Synthesis of 1,3,4-oxadiazole derivatives **2** and **3**



a R = CH_3 **f** R = *o*-Cl-$C_6H_4CH_2$ **k** R = *o*-CH_3O-C_6H_4
b R = CH_3CH_2 **g** R = C_4H_3O **l** R = *p*-F-C_6H_4
c R = $CH_3CH_2CH_2$ **h** R = C_6H_5 **m** R = *p*-NO_2-C_6H_4
d R = $CH_3(CH_2)_3CH_2$ **i** R = *p*-CH_3-C_6H_4 **n** R = *m*-NO_2-C_6H_4
e R = $C_6H_5CH_2$ **j** R = *p*-CH_3O-C_6H_4 **o** R = *p*-AcO-C_6H_4

Table 1 Reactions of various hydrazides with DAF

Entry	R	*T*/℃	Time/h	Product	Configuration	Yield/%
1	Me	100	8	**2a**	*R*	45
2	Et	100	8	**2b**	*R*	42
3	Pr	100	8	**2c**	*R*	44
4	Pentyl	100	8	**2d**	*R*	43
5	$PhCH_2$	100	8	**2e**	*R*	48
6	*o*-Cl-$C_6H_4CH_2$	100	8	**2f**	*R*	47
7	2-Furyl	100	8	**2g**	*R*	46
8	Ph	100	8	**2h**	*R*	54
9	*p*-Me-C_6H_4	100	8	**2i**	*R*	46
10	*p*-MeO-C_6H_4	100	8	**2j**	*R*	53
11	*o*-MeO-C_6H_4	100	8	**2k**	*R*	55
12	*p*-F-C_6H_4	100	8	**2l**	*R*	52
13	*p*-NO_2-C_6H_4	100	8	**2m**	*R*	51
14	*m*-NO_2-C_6H_4	100	8	**2n**	*R*	53
15	*p*-AcO-C_6H_4	100	8	**2o**	*R*	52
16	Me	Reflux	8	**2a**/**3a**	*R*/*S*	40 (34/6)[a]
17	Ph	Reflux	8	**2h**/**3h**	*R*/*S*	42 (36/7)
18	*o*-MeO-C_6H_4	Reflux	8	**2k**/**3k**	*R*/*S*	45 (37/8)
19	*p*-NO_2-C_6H_4	Reflux	8	**2m**/**3m**	*R*/*S*	41 (35/6)

[a] Mole ratio is based on ^{1}H NMR analysis.

CHINESE JOURNAL OF CHEMISTRY

lower than the yield of sole 2*R*-isomer at lower temperature.

The change of compound solubility often affects its biological activity. Therefore, we prepared some free hydroxyl 1,3,4-oxadiazole derivatives (**4h**, **4k**, and **5h**), which were obtained in 90%—93% yields by selective removal of the acetyl group at fused furan ring via treatment of corresponding protected compounds (**2h**, **2k** and **3h**) with potassium carbonate in methanol.

The stereoselectivity in the reaction could be explained as follows. The hydrazide condensed with the carbonyl group of the DAF to give a mixture of *Z* and *E* hydrazones which are interchangable. Then, the stereochemistry of the heterocyclization should be influenced by the steric hindrance of the V-shaped skeleton of DAF. The acetic anhydride group attacked the nitrogen of imine to increase the electropositivity of imine carbon (C-2). Subsequently, the oxygen of acylhydrazone attacked C-2 to generate an oxazolidine ring. Due to the larger steric hindrance from the *endo* face than the *exo* face with respect to the V-shaped molecule, the acetic anhydride group preferred to attack from the less hindered *exo*-face, which resulted in the major (*R*)-isomer of oxazolidine. The result indicated that acetic anhydride attacking nitrogen atom of imine from the less hindered *exo* face of the V-shaped molecule should be crucial to the highly stereoselective heterocyclization (Scheme 2).

Preliminary evaluation of *in vitro* anti-tumor activity

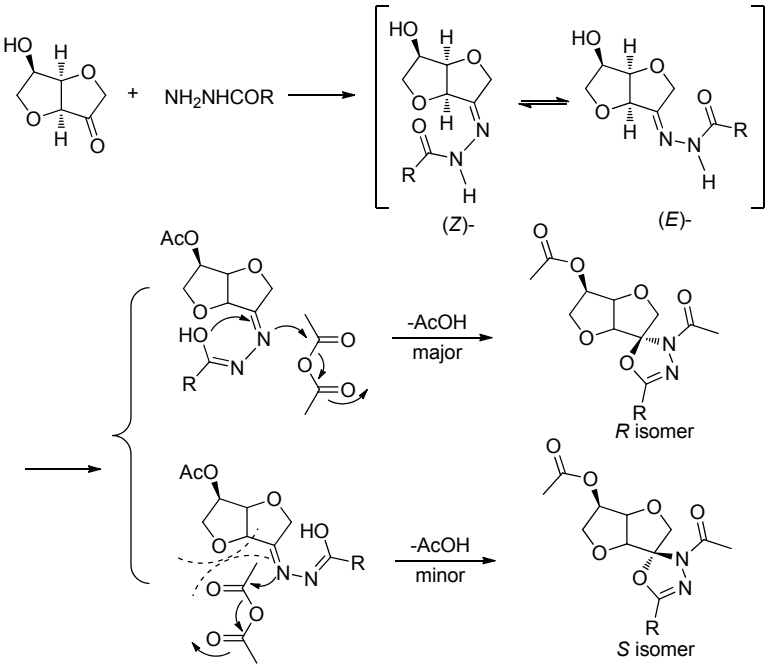
The preliminary *in vitro* inhibitory activities of the prepared compounds **2**—**5** against A549, MGC-803 tumor cells were evaluated by SRB assays.[17] The inhibitory rates of the tested compounds in 20 μmol•L^{-1} were listed in Table 2. The results indicated that all the tested compounds with both *R*- and *S*-configuration have not

Table 2 Inhibition rates (%) for tumor cell proliferation by **2**—**5**

Compd.	C^2-config.	Inhibition rate/%	
		A549[a]	MGC803[b]
2a	*R*	14	19
2b	*R*	12	7
2c	*R*	15	17
2d	*R*	21	12
2e	*R*	18	25
2f	*R*	12	17
2g	*R*	9	10
2h	*R*	15	20
3h	*S*	8	17
2i	*R*	13	21
2j	*R*	24	9
2k	*R*	16	26
3k	*S*	14	19
2l	*R*	19	11
2m	*R*	21	18
3m	*S*	12	23
2n	*R*	9	17
2o	*R*	10	9
4h	*R*	23	19
5h	*S*	20	21
4k	*R*	24	13

[a] Human lung carcinoma cell, 72 h. [b] Gastric carcinoma cell, 72 h.

Scheme 2 Proposed mechanism for the stereo-selective formation of oxadiazoles



obvious antitumor activity in general.

Conclusions

In summary, we have synthesized a series of chiral spiro oxadiazole derivatives based on the stereocontrol of 1,4:3,6-dianhydro-*D*-fructose. The configuration of the newly generated chiral center of oxadiazole was confirmed by X-ray crystallographic analysis of **2a**. The mechanism for steroselective cyclization of oxadiazole was proposed. *In vitro* evaluation of inhibitory activities against two human tumor cell lines showed poor antitumor activity of the obtained compounds.

Supplementary Data

Complete crystallographic data for the structural analysis in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC-1526929. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0) 1223 336033; e-mail: deposit@ccdc.cam.ac.uk; web: http://www.ccdc.cam.ac.uk/products/csd/request/].

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(Zhao, C.)

