

Synthesis and Antitumor Activity of 5-Trifluoromethyl-2,4-dihydropyrazol-3-one Nucleosides

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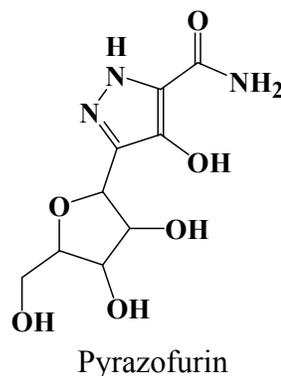
Abstract: 2,4-Dihydropyrazole glucosides **3a-3c** were prepared and tested for their antitumor activity. The structures of these compounds were established by ¹H and ¹³C-NMR spectroscopy. Glucoside **3b** shows an *in vitro* IC₅₀ value of 16.4 μM against proliferation of the human promyelotic leukemia (HL60) cell line.

Keywords: Trifluoromethylpyrazole; glucopyranose; antitumor agents.

Introduction

Fluorinated compounds in general and fluorinated heterocycles in particular are the focus of much interest in modern medicinal chemistry. A variety of pharmacological agents bearing a trifluoromethyl group are of special interest as they exhibit wide ranging biological properties [1-4]. Many efficient methods for the introduction of a trifluoromethyl group into organic molecules have been developed. Trifluoromethyl-1,3-dicarbonyl compounds have been proven to be versatile building blocks for a wide variety of trifluoromethyl-substituted heterocycles. These include pyrazoles [5-7], isoxazoles [8,9],

thiazoles [10], pyridines [11,12], pyrimidines [13,14], and pyrones [15]. The trifluoromethyl-substituted compounds have been reported to possess biological activities as herbicides [16], fungicides [17], analgesic agents [18], antipyretic agents [19], and inhibitors for platelet aggregation [20]. Recently, much attention has been focused on pyrazoles as antiviral and anticancer agents after the discovery of the pyrazole C-glycoside pyrazofurin [21, 22]. This report describes the synthesis and anticancer evaluation of several trifluoromethyl-substituted pyrazole N-nucleosides.



Results and Discussion

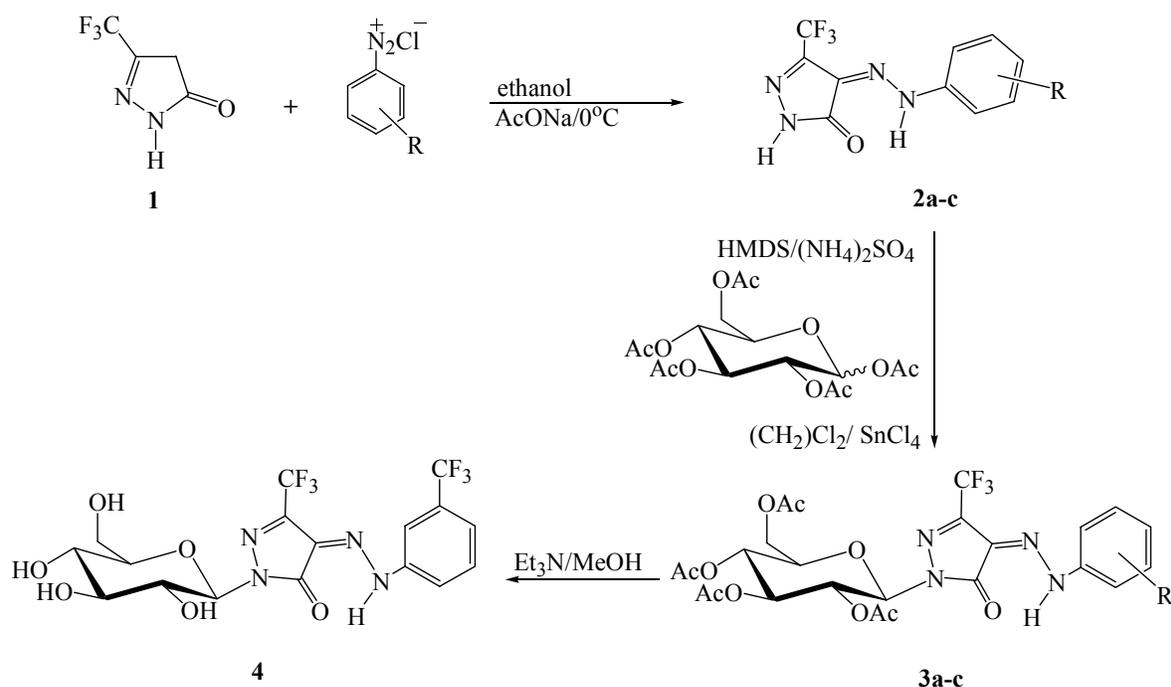
Chemistry

5-Trifluoromethyl-2,4-dihydropyrazol-3-one (**1**) was the chosen starting material for the synthesis of the new trifluoromethyl-substituted pyrazol-3-ones **2a-c** and their corresponding glucosides **3a-c**, **4** (Scheme 1). Compound **1** is readily available by the reaction of ethyl 4,4,4-trifluoro-3-oxobutanoate with hydrazine in ethanol in the presence of sulfuric acid [5,23]. Coupling reactions of **1** with aryldiazonium reagents were conducted by using a well-developed methodology [23-25] and the desired hydrazones **2a-c** were obtained in high yields.

In order to obtain glucosides **3a-c** as single nucleosides, compounds **2a-c** were treated with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) in the presence of a catalytic amount of ammonium sulfate, and the resultant trimethylsilyl derivatives, without isolation, were allowed to react with β -D-glucose pentaacetate in 1,2-dichloroethane in the presence of tin(IV) chloride. Nucleosides **3a-c** were thus obtained in good yield. Selected nucleoside **3a** was then subjected to deacetylation at room temperature in a solution of triethylamine in anhydrous methanol to give 2-(β -D-glucopyranosyl)-4-(3'-trifluoro-methylphenylhydrazono)-5-trifluoromethyl-2,4-dihydropyrazol-3-one (**4**) in high yield.

The structures of all new compounds are fully consistent with the NMR data and elemental analysis. In particular, the β -configuration of the pyranosyl moieties was established by $^1\text{H-NMR}$ from a large coupling constant ($J > 5$ Hz) for a diaxial interaction between H-1'' and H-2'' [26,27].

Scheme 1



Compound	R
2a, 3a	<i>m</i> -CF ₃
2b, 3b	<i>p</i> -F
2c, 3c	<i>m</i> -F

Anticancer activity.

The effects of nucleosides **3a-c** on proliferation of human promyelotic leukemia (HL60) and mouse T lymphocytic leukemia (EL4) cell lines were evaluated. The IC₅₀ values for compounds **3a-c** indicate that HL60 cells are more sensitive than EL4 cells.

Table 1. Evaluation of pyrazole nucleosides

Compound	R	IC ₅₀ (μM)	
		HL60 proliferation (human)	EL4 proliferation (mouse)
3a	<i>m</i> -CF ₃	20.2±1.7	25.3±0.9
3b	<i>p</i> -F	16.4±1.8	19.4±2.0
3c	<i>m</i> -F	27.2±1.3	28.7±2.0

The results presented in Table 1 indicate that these newly synthesized compounds exhibit promising anticancer activities. The most potent compound **3b** contains a fluorine atom in the *para* position of the

aromatic ring. The less active analogues **3a** and **3c** are substituted with a *meta*-trifluoromethyl group and *meta*-fluoro atom, respectively.

Conclusions

New 2,4-dihydropyrazol-3-one nucleosides **3a-c**, **4** were synthesized. Of these, the nucleosides **3a-c** were tested for their anticancer activities. The collective results clearly show that nucleoside 2-(2'',3'',4'',6''-tetra-*O*-acetyl- β -D-glucopyranosyl)-4-(3'-fluorophenylhydrazono)-5-trifluoromethyl-2,4-dihydropyrazol-3-one (**3b**), exhibits better biological activities than its structurally related analogues **3a** and **3c**.

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Experimental

General

All reagents were purchased from commercial sources and used without further purification. Melting points were determined on Gallenkamp apparatus (Pyrex capillary) and are not corrected. All reactions were carried out under an inert atmosphere of nitrogen. Thin-layer chromatography (TLC) was performed on aluminum sheets coated with Kieselgel (silica gel, type 60 F₂₅₄, Merck). Column chromatography was performed on Kieselgel 60. Elemental analyses were performed by the Central Laboratory Unit (CLU), UAE University. The IR spectra were recorded on a Shimadzu 940 spectrophotometer. ¹H-NMR and selected ¹³C-NMR spectra were recorded on Varian XL200 and Bruker AM 300 MHz instruments by using solutions in CDCl₃ and TMS as an internal reference. Specific optical rotations (*c* = 0.1 g/100 mL in all cases, sodium D line, 25 °C) were obtained for solutions in CDCl₃ (**3a-c**) and MeOH (**4**), respectively.

5-Trifluoromethyl-2,4-dihydropyrazol-3-one (**1**).

This compound was obtained by the reaction of ethyl 4,4,4-trifluoro-3-oxobutanoate with hydrazine hydrate in ethanol in the presence of sulfuric acid by using a general literature procedure [5, 23]: yield 85%; m.p. 218-220 °C; ¹H-NMR δ 2.41 (s, 2H), 8.72 (bs, exchangeable with D₂O); ¹³C-NMR δ 14.2 (C-4), 114.0 (q, CF₃), 143.0 (C-5), 159.7 (C-2); IR, 3300 (N-H), 1600 (C=O), 1500 cm⁻¹(C=N).

General Method for the Preparation of 4-Arylhydrazono-5-trifluoromethyl-2,4-dihydropyrazol-3-ones (2a-c).

To a cold solution of 5-trifluoromethyl-2,4-dihydropyrazol-3-one (**1**, 0.76 g, 5 mmol) in ethanol (25 mL) containing sodium acetate (0.82 g, 10 mmol), a solution of an appropriate aryldiazonium chloride (5 mmol/2 mL H₂O) was added dropwise with stirring at 0-5 °C. The reaction mixture was stirred at room temperature for 3 h and then the precipitated crude product was filtered, washed with cold H₂O (2 x 10 mL), dried and crystallized from 95% ethanol.

4-(3'-Trifluoromethylphenylhydrazono)-5-trifluoromethyl-2,4-dihydropyrazol-3-one (2a): yield 80%; yellow crystals; m.p. 206-208 °C; R_f = 0.65 (EtOAc/hexanes, 1:4); ¹H-NMR δ 7.51-7.68 (m, 4H), 8.26 (s, exchangeable with D₂O), 13.68 (bs, exchangeable with D₂O); ¹³C-NMR δ 113.5, 113.6, 116.6, 123.5, 123.6, 130.6, 140.9, 159.4; IR, 3410 (NH), 3060 (aromatic C-H), 1680 (C=O), 1600 (C=C), 1559 (C=N), 1523 cm⁻¹(N-N); Analysis. Calculated for C₁₁H₆F₆N₄O (324.04): C, 40.75; H, 1.87; N, 17.28. Found: C, 40.75; H, 1.86; N, 17.27.

4-(4'-Fluorophenylhydrazono)-5-trifluoromethyl-2,4-dihydropyrazol-3-one (2b): yield 75%; yellow crystals; m.p. 212-214 °C; R_f = 0.7 (EtOAc/hexanes, 1:4); ¹H-NMR δ 2.48 (s, 1H, H-4), 7.10-7.20 (m, 2H), 7.33-7.50 (m, 2H), 8.60 (s, exchangeable with D₂O), 12.96 (bs, exchangeable with D₂O); ¹³C-NMR δ 116.8, 117.1, 118.3, 118.4, 160.0, 161.0, 164.0, 179.0; IR, 3450 (NH), 3070 (aromatic C-H), 2985 (aliphatic C-H), 1675 (C=O), 1600 (C=C), 1550 (C=N), 1522 cm⁻¹(N-N); Analysis. Calculated for C₁₀H₆F₄N₄O (274.05): C, 43.81; H, 2.21; N, 20.43. Found: C, 43.82; H, 2.19; N, 20.42.

4-(3'-Fluorophenylhydrazono)-5-trifluoromethyl-2,4-dihydropyrazol-3-one (2c): yield 78%; brown crystals; m.p. 201-203 °C; R_f = 0.72 (EtOAc/hexanes, 1:4); ¹H-NMR δ 6.90 (t, 1H), 7.17-7.29 (m, 2H), 7.36-7.40 (m, 1H), 7.80 (s, exchangeable with D₂O), 13.56 (bs, exchangeable with D₂O); ¹³C-NMR δ 103.8, 104.1, 112.6, 112.7, 114.1, 114.3, 131.2, 131.3, 141.0, 162.0; IR: 3450 (NH), 3060 (aromatic C-H), 2995 (aliphatic C-H), 1670 (C=O), 1560 (C=C), 1550 (C=N), 1528 cm⁻¹(N-N); Analysis. Calculated for C₁₀H₆F₄N₄O (274.05): C, 43.81; H, 2.21; N, 20.43. Found: C, 43.81; H, 2.20; N, 20.42.

General Procedure for the Synthesis of 2-(2'',3'',4'',6''-Tetra-O-acetyl-β-D-glucopyranosyl)-4-arylhydrazono-5-trifluoromethyl-2,4-dihydropyrazol-3-ones (3a-c).

A mixture of a 4-arylhydrazono-5-trifluoromethyl-2,4-dihydropyrazol-3-one (**2a-c**, 10 mmol), HMDS (60 mL), and (NH₄)₂SO₄ (125 mg) was heated under reflux for 6 h. Excess HMDS was removed by distillation and then residual HMDS was removed under a reduced pressure. A solution of the resultant silyl intermediate in anhydrous 1,2-dichloroethane (20 mL) was stirred and treated with a solution of β-D-glucose pentaacetate (9 mmol) in anhydrous 1,2-dichloroethane (20 mL). Following cooling to 5 °C and then addition of anhydrous SnCl₄ (1.6 mL), the mixture was stirred at 23 °C until

the reaction was completely as judged by TLC (EtOAc/hexanes, 1:4). The mixture was diluted with CHCl_3 (150 mL), washed with a saturated solution of NaHCO_3 (50 mL) and water (2 x 25 mL), and then dried (Na_2SO_4). Removal of solvent under a reduced pressure followed by column chromatography and then crystallization from 95% EtOH gave the analytically pure compounds **3a-c**.

2-(2'',3'',4'',6''-Tetra-O-acetyl- β -D-glucopyranosyl)-4-(3'-trifluoromethylphenylhydrazono)-5-trifluoromethyl-2,4-dihydropyrazol-3-one (3a): Yield 78% after silica gel chromatography (EtOAc/hexanes, 1:9); m.p. 235-238 °C; $R_f = 0.3$ (EtOAc/hexanes,1:4); $[\alpha]_D^{25} = 38.3$; $^1\text{H-NMR}$ δ 1.98-2.06 (4s, 12H), 3.90 (m, 2H), 4.09 (m, 1H), 5.12 (m, 1H), 5.36 (m, 1H), 5.50 (m, 1H), 6.26 (d, $J = 6.0$ Hz, 1H, H1'), 7.49-7.63 (m, 4H), $^{13}\text{C-NMR}$ δ 20.3, 20.4, 20.5, 20.7, 61.6, 67.6, 68.3, 73.4, 74.3, 80.2, 113.5, 113.6, 119.6, 123.4, 123.7, 130.6, 132.1, 132.8, 140.6, 158.0, 168.6, 168.7, 169.2, 170.2, 170.5; IR, 3090 (aromatic C-H), 2998 (aliphatic C-H), 1755 (acetyl C=O), 1689 (C=O), 1620 (C=C), 1560 (C=N), 1528 (N-N), 1040 cm^{-1} (C-O-C); Analysis: Calculated for $\text{C}_{25}\text{H}_{24}\text{F}_6\text{N}_4\text{O}_{10} \bullet \text{H}_2\text{O}$ (672.14): C, 44.63; H, 3.86; N, 8.33. Found: C, 44.60; H, 3.88; N, 8.35.

2-(2'',3'',4'',6''-Tetra-O-acetyl- β -D-glucopyranosyl)-4-(4'-fluorophenylhydrazono)-5-trifluoromethyl-2,4-dihydropyrazol-3-one (3b): Yield 65% after silica gel chromatography (EtOAc/hexanes, 1:9); m.p. 159 °C; $R_f = 0.3$ (EtOAc/hexanes, 1:4); $[\alpha]_D^{25} = +52.0$; $^1\text{H-NMR}$ δ 1.90-2.08 (4s, 12H), 3.82 (m, 2H), 4.09 (s, 1H, H-4), 4.22 (m, 1H), 5.15 (m, 2H), 5.53 (m, 1H), 5.96 (d, $J = 5.4$ Hz, 1H, H1'), 7.01-7.17 (m, 2H), 7.33-7.40 (m, 2H); $^{13}\text{C-NMR}$ δ 20.3, 20.5, 20.7, 62.0, 68.0, 68.5, 73.8, 73.9, 80.8, 115.6, 121.3, 123.2, 129.7, 150.4, 159.0, 168.7, 169.3, 170.2, 170.5; IR, 3080 (aromatic C-H), 2995 (aliphatic C-H), 1750 (acetyl C=O), 1680 (C=O), 1610 (C=C), 1560 (C=N), 1526 (N-N), 1028 cm^{-1} (C-O-C); Analysis. Calculated for $\text{C}_{24}\text{H}_{24}\text{F}_4\text{N}_4\text{O}_{10} \bullet \text{H}_2\text{O}$ (622.14): C, 46.29; H, 4.17; N, 9.00. Found: C, 46.25; H, 4.22; N, 9.02.

2-(2'',3'',4'',6''-Tetra-O-acetyl- β -D-glucopyranosyl)-4-(3'-fluoro-phenylhydrazono)-5-trifluoromethyl-2,4-dihydropyrazol-3-one (3c): yield 68% after silica gel chromatography (EtOAc/hexanes, 9:1); m.p. 123-125 °C; $R_f = 0.3$ (EtOAc/hexanes,1:4); $[\alpha]_D^{25} = +36.8$; $^1\text{H-NMR}$ δ 1.90-198 (4s, 12H), 3.81 (m, 2H), 4.12 (m, 1H), 5.02 (m, 1H), 5.36 (m, 1H), 5.65 (m, 1H), 5.70 (d, $J = 6.2$ Hz, 1H, H1'), 7.15-7.40 (m, 3H); $^{13}\text{C-NMR}$ δ 20.4, 20.5, 20.6, 20.8, 76.6, 68.3, 69.7, 73.5, 74.3, 80.3, 103.7, 104.2, 112.7, 114.2, 114.6, 122.9, 128.3, 131.2, 141.6, 158.1, 168.6, 169.3, 170.2, 170.6; IR, 3070 (aromatic C-H), 2996 (C-H), 1750 (acetyl C=O), 1682 (C=O), 1604 (C=C), 1558 (C=N), 1528 (N-N), 1026 cm^{-1} (C-O-C); Analysis. Calculated for $\text{C}_{24}\text{H}_{24}\text{F}_4\text{N}_4\text{O}_{10} \bullet \text{H}_2\text{O}$ (622.14): C, 46.29; H, 4.17; N, 9.00. Found: C, 46.27; H, 4.20; N, 9.10.

2-(β -D-glucopyranosyl)-4-(3'-trifluoromethyl-phenylhydrazono)-5-trifluoromethyl-2,4-dihydropyrazol-3-one (4): yield 88% after silica gel chromatography (EtOAc/MeOH, 9:1); m.p. 196-199 °C (decomp.); $[\alpha]_D^{25} = +25.3$; $^1\text{H-NMR}$ δ 3.83-4.58 (m, 6H), 4.17 (m, exchangeable with D_2O , 4H), 5.22 (m, 1H), 5.53 (d, $J = 8.4$ Hz, 1H, H-1'), 7.44-7.71 (m, 4H), IR, 3070 (aromatic C-H), 2995 (aliphatic C-H), 1680

(C=O), 1612 (C=C), 1560 (C=N), 1528 (N-N), 1023 cm^{-1} (C-O-C); Analysis. Calculated for $\text{C}_{17}\text{H}_{16}\text{F}_6\text{N}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$ (522.10): C, 39.07; H, 3.83; N, 10.72. Found: C, 38.96; H, 3.87; N, 10.70.

MTT Cell Proliferation

All compounds tested were dissolved in DMSO (100 mM solution) and subsequently diluted in the culture medium before treatment of the cultured cells. Tested cells were plated in 96-well plates at a density 4×10^3 cells/well/100 μL of the proper culture medium and treated with the compounds at concentration 1.25 to 100 μM for 48h. In parallel, the cells were treated with 0.1% of DMSO as control.

A MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] Assay (Roche Molecular Biochemicals, 1 465 007) was performed 30 h later according to the instructions provided by Roche. This assay is based on the cellular cleavage of the tetrazolium salt, MTT, into a formazan that is soluble in cell culture medium and is measured at 550 nm directly in 96-well assay plates. Absorbance is directly proportional to the number of living cells in culture. Two types of cells were used in these studies, human promyelocytic leukemia HL60 and mouse T lymphocytic leukemia cell lines, provided by ATCC and cultivated in MEM (for HL60) or RPMI 1640 (for EL4) supplemented with 10% fetal bovine serum and 2 mM of L-glutamine. Tissue culture reagents were obtained from Gibco BRL.

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