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A facile synthesis, structure, and antimicrobial evaluation of novel 4-arylhydrazono-5-trifluoromethyl-2,4-dihydropyrazol-3-ones, their *N*- and *N*,*O*-bis-β-D-glucosides

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ABSTRACT

Synthesis of some novel 4-arylhydrazono-5-trifluoromethyl-2,4-dihydropyrazol-3-ones, their *N*- and *N*,*O*-bis- β-D-glucosides is described. Antimicrobial evaluation of eight selected compounds against *Aspergillus fumigatus* RCMB 002008 (1), *Penicillium italicum* RCMB 001018 (1), *Syncephalastrum racemosum* RCMB 016001, *Candida albicans* RCMB 005003, *Staphylococcus aureus* RCMB 106-001 (1), *Pseudomonas aeruginosa* RCMB 102-002, *Bacillus subtilis* RCMB 101-001, and *Escherichia coli* RCMB 103-001 has been achieved. The screening results indicated that all the tested compounds exhibited different inhibitory effects against five to seven different organisms of the eight test organisms.

1. Introduction

Fluorinated analogues of natural substances are mainly of interest in bioorganic chemistry[1] since single fluorine atoms or trifluoromethyl groups introduced in place of hydrogen atoms often increase the biological activity of the parent compounds. The trifluoromethyl-substituted compounds were found to possess biological activities as herbicides,[2] fungicides,[3] analgesic agents,[4] antipyretic agents,[5] and inhibitors for platelet aggregation.[6]

The chemistry of pyrazolone derivatives has attracted much attention because of their interesting structural properties and applications in diverse areas.[7] Pyrazolone derivatives find potential application in medicinal chemistry as analgesic,[8] anti-inflammatory,[9] and therapeutic agents.[10] As an example, edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) has been recently shown to produce marked attenuation of brain damage caused by ischemia-reperfusion,[11] and its pharmacological actions were attributed to its antioxidant activity, as a potent hydroxyl radical scavenger.[12] Many pyrazolone derivatives are useful reagents for the extraction and separation of various metal ions.[13–17] They can also be used in laser materials, as ^{1}H NMR shift reagents, in chromatographic study and in the petrochemical industry.[18–22] Recently, photochromism of pyrazolone derivatives has also been reported.[23] Many of these ligands exhibit tautomerism, and because of this they show interesting structural and spectroscopic properties which have been the subject of many reports.[24,25] Among these ligands, acyl pyrazolones have been studied extensively owing to their effective properties with respect to extracting metal ions.[26,27] On the other hand, arylhydrazonopyrazolone chemistry is less extensive. It has been found that some pyrazole glycosides show anticancer and/or antiviral activity, alone, or in combination with other drugs.[28]

Following the reported modified Hilbert-Johnson reaction,[29] only few *N*-glucosyl derivatives of 4-arylhydrazono-5-trifluoromethyl-2,4-dihydropyrazol-3-ones[30] have been synthesized via coupling of the silylated pyrazole derivatives with the acylated glucosyl halide in the presence of Friedel–Crafts catalyst. Recently, we reported simple convenient base-induced glycosylation of different heterocyclic systems, as a part of an ongoing program, directed for preparation of bio-active molecules.[31–44] Thus, the current work describes simple synthetic approach as well as antimicrobial evaluation of some novel 4-arylhydrazono-5-trifluoromethyl-2,4-dihydropyrazol-3-ones and their glucosides.

2. Results and discussion

2.1. Synthesis

Scheme 1 illustrates the synthetic route towards some novel biologically active fluorinated pyrazoles. With this aim, 5-trifluoromethyl-2,4-dihydropyrazol-3-one (**1**) was coupled with

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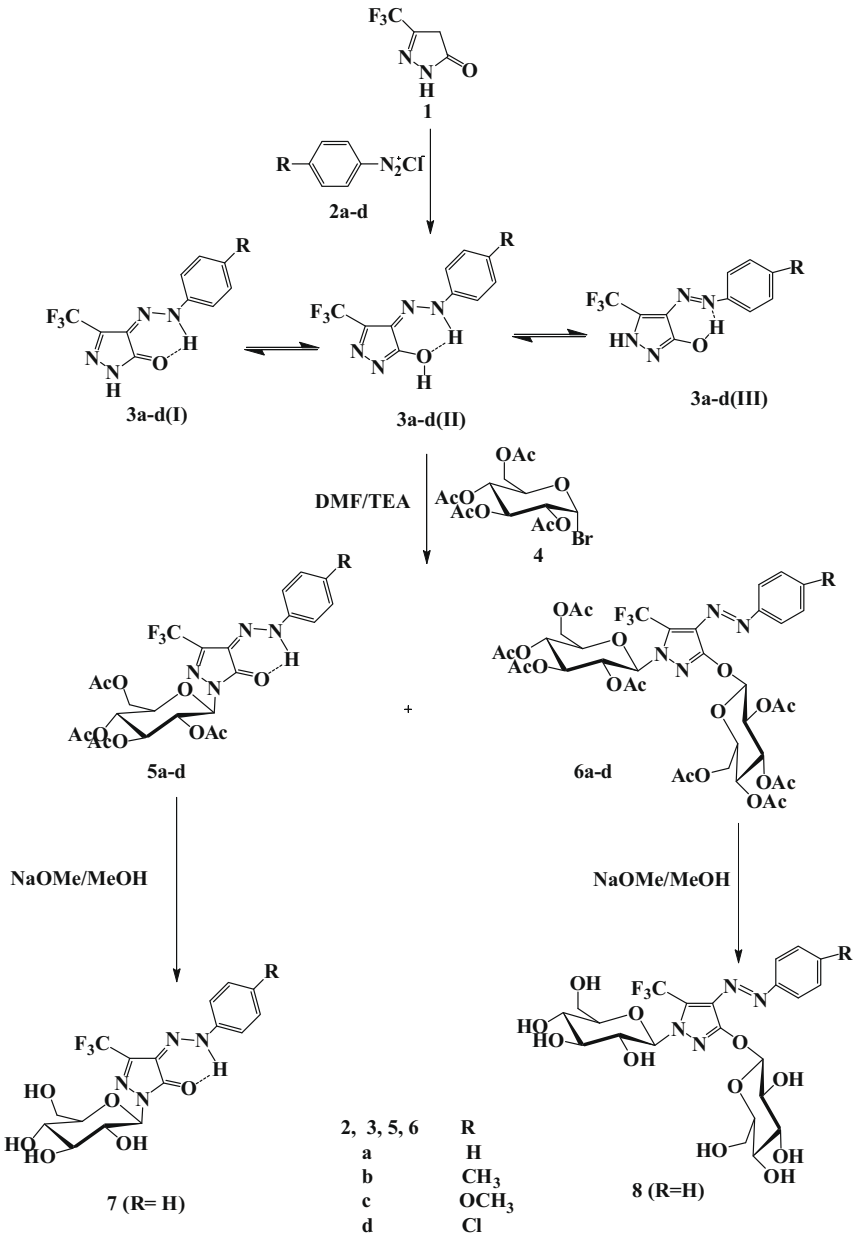

Scheme 1.

aryldiazonium chlorides (**2a–d**), to give the new 4-arylhydrazono-5-trifluoromethyl-2,4-dihydropyrazol-3-ones (**3a–d**). The molecular structures of these compounds are such that they can exist in three possible tautomeric forms, namely, the hydrazo-keto form **3a–d (I)**, the hydrazo-enol form **3a–d (II)**, and the azo-enol form **3a–d (III).** Detailed spectral and chemical studies were carried out to prove their existence. Thus, the IR spectra of compounds **3a–d** showed two characteristic bands in the ranges 3171–3279 and 1662–1670 cm^{-1}, which are assigned to NH and C=O functions, respectively, for the tautomeric hydrazo-keto form **3a–d (I)**. In solution, the ^1H NMR spectra of compounds **3a–d** were acquired in $CDCl_3$ and compared to those acquired for compounds **3a,b** in DMSO-d_6 under the same conditions. In $CDCl_3$, compounds **3a–d** revealed two signals at δ 9.10–9.96 (s, 1H, D_2O exchangeable) and 13.70–13.94 (br, 1H, D_2O exchangeable), which can be assigned to either the pyrazole NH and hydrazo NH functions, respectively, for the tautomeric hydrazo-keto form **3a–d (I)** or the pyrazole NH and OH functions, respectively for the tautomeric hydrazo-enol form **3a–d (II)**. In either case the hydrazo NH function of the tautomeric hydrazo-keto form **3a–d (I)** and the OH function of hydrazo-enol form **3a–d (II)** are strongly deshielded because of the possible hydrogen bonding. Thus, in $CDCl_3$ and comparing with the solid state study (IR) together with glucosidation study (discussed later), it is preferred to assign the signal at δ 13.70–13.94 (br) to hydrazo NH of the hydrazo-keto form **3a–d (I)**. In DMSO-d_6, the ^1H NMR spectra of compounds **3a–d** showed two signals at 5.17 (br, D_2O exchangeable) and 12.55–12.58 (s, D_2O exchangeable) which are assigned to the NH and OH functions, respectively, for the tautomeric azo-enol form **3a–d (III)**. In this case, the OH function is strongly deshielded because of the possible hydrogen bonding. The formation of the bis(glucosides) (**6a–d**) during glucosidation of compounds **3a–d** (discussed later) gave further evidence and supported the possible existence of the tautomeric azo-enol form **3a–d (III)** in solution.

Compounds **3a–d** were used as starting materials to prepare new functionalized pyrazole *N*- and *N*,*O*-bis-β-D-glucosides. Thus,

glucosidation of compounds **3a–d** with equimolar amount of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**4**) afforded a chromatographically separable mixture (65–79% overall yield) of two products, namely, 2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-4-arylhydrazono-5-trifluoromethyl-2,4-dihydropyrazol-3-ones (**5a–d**) (59–72%) and 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-4-arylhydrazono-5-trifluoromethyl-1*H*-pyrazoles (**6a–d**) (6–7%). Increasing the molar ratio (2:1) of compound **4,** when it was reacted with compounds **3a**,**b**, gave the same mixture but with enhanced yield of the bis(glucosides) **6a,b** (68–71%) and lower yield of the mono glucosides **5a,b** (5–8%).

The structures assigned for compounds **5a–d** and **6a–d** were based on spectroscopic and chemical data. Thus, the *N*-β-D-configuration of compounds **5a–d** is supported by their ^{1}H NMR data which revealed the anomeric proton signal at δ 5.73–5.80 with a coupling constant value 6.1–6.6 Hz consistent with reported data for *N*-β-D-glycosides.[31,35,36,40,42,44] The β-configuration was confirmed from the large coupling constant ($J_{H\text{-}1'\text{-}H\text{-}2'} > 5$ Hz) for a diaxial interaction.[45,46] The appearance of an amide carbonyl function at 1651–1678 cm^{-1} in the IR spectra of compounds **5a,b** is further support of the proposed structures for compounds **5a–d**. For compounds **6a–d**, the ^{1}H NMR spectra revealed signals due to acetyl protons (24 H) which appear as overlapped singlets in the region 1.80–2.12, indicating the presence of two glucosyl moieties. Also, ^{1}H NMR spectra of these compounds revealed two characteristic signals at δ 5.07–5.18 (d, 1H, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 9.2–9.3 Hz) and 5.51–5.59 (d, 1H, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 9.0–9.3 Hz), which can be assigned to two anomeric protons of *O*- and *N*-types, respectively. The large coupling constant ($J_{H\text{-}1'\text{-}H\text{-}2'} > 5$ Hz) of these anomeric protons confirms a diaxial interaction[45,46] and thus supports their β-configuration. The positions and coupling constants of these anomeric protons at δ 5.07–5.18 ($J_{H\text{-}1'\text{-}H\text{-}2'}$ = 9.2–9.3 Hz) and 5.51–5.59 ($J_{H\text{-}1'\text{-}H\text{-}2'}$ = 9.0–9.3 Hz) are consistent with those reported for *O*-β-D-glucosides[47] and *N*-β-D-glucosides,[31,35,36,40,42,44] respectively. Moreover, the IR spectrum of compound **6b**, as a typical example, revealed the absence of amide carbonyl function at 1651–1678 and thus gave a further confirmation of the proposed structures for compounds **6a–d**. Furthermore, the appearance of the azo group (N=N) at 1435 cm^{-1} in compound **6b**, as a typical example, together with the steric hinderance considerations, exclude any other possible isomeric bis(glucosides).

Deacetylation of compounds **5a** and **6a** using sodium methoxide in methanol gave the corresponding 2-β-D-glucopyranosyl-4-phenylhydrazono-5-trifluoromethyl-2,4-dihydropyrazol-3-one **(7)** and 1-β-D-glucopyranosyl-3-β-D-glucopyranosyloxy-4-phenylhydrazono-5-trifluoromethyl-1*H*-pyrazole **(8)**. The ^{1}H NMR data of these compounds revealed the absence of the acetyl protons at δ 1.80–2.12 and the appearance of the D_2O exchangeable OH protons at δ 4.55–5.52.

2.2. Antimicrobial activity

Compounds **1, 3a–d, 5a,b**, and **6a** were evaluated for their antibacterial and antifungal activities against two Gram-positive bacteria (*Bacillus subtilis* RCMB 101-001 and *Staphylococcus aureus* RCMB 106-001 (1)), two Gram-negative bacteria (*Pseudomonas aeruginosa* RCMB 102-002 and *Escherichia coli* RCMB 103-001), one yeast (*Candida albicans* RCMB 005003), and three fungal strains (*Aspergillus fumigatus* RCMB 002008 (1), *Penicillium italicum* RCMB 001018 (1) and *Syncephalastrum racemosum* RCMB 016001). The screening results (Table 1) indicated that all the tested compounds exhibited different inhibitory effects against five to seven different organisms of eight test organisms. As representative examples, the minimum inhibitory concentrations (MICs) of compounds **3a** and **5a** were determined and found to be 400 μg/mL.

3. Experimental

3.1. Synthesis

3.1.1. General

All melting points are uncorrected. IR spectra were recorded on a Perkin-Elmer 1430 spectrometer. NMR spectra were measured with a Varian Mercury 300 spectrometer (300 MHz ^{1}H NMR, 75 MHz ^{13}C NMR). Mass spectra were recorded on a GCMS-QP 1000 EX (70 EV) spectrometer. Elemental analyses were carried out at the Micro Analytical Center, Cairo University, Giza, Egypt. The starting 5-trifluoromethyl-2,4-dihydropyrazol-3-one (**1**)[48] and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**4**)[49] were prepared as reported. TLC was performed on Fluka silica gel 60 F_{254} aluminum sheets, and products were detected using 254 nm light. Fluka silica gel 60 (70–230 mesh) was used for column chromatography.

3.1.1.1. General procedure for the synthesis of 4-arylhydrazono-5-trifluoromethyl-2,4-dihydropyrazol-3-ones (3a–d). To a cold solution of 5-trifluoromethyl-2,4-dihydropyrazol-3-one (**1**) (1.52 g, 10 mmol) in EtOH (50 mL) containing NaOAc (1.64 g, 20 mmol), an aqueous solution of the appropriate aryldiazonium salt **2a–d** (10 mmol/4 mL H_2O) was added dropwise with stirring at 0–5 °C. The reaction mixture was stirred at room temperature for 3 h and the formed precipitate was collected by filtration, washed several times with cold water, dried, and recrystallized from EtOH.

*3.1.1.1.1. 4-Phenylhydrazono-5-trifluoromethyl-2,4-dihydropyrazol-3-one (**3a**).* Yield 2.29 g (89%); orange yellow crystals, mp 182–184 °C. IR: 3271, 3063, 1670, 1597, 1554, 1527, 1493, 1462, 1431, 1373, 1265, 1192, 1142, 1061, 976, 876, 764, 721, 667, 640, 594, 478; ^{1}H NMR ($CDCl_3$) δ 7.30 (tt, 1H, J = 1.5, 7.5 Hz, ArH), 7.46 (tt, 2H, J = 1.5, 7.5 Hz, ArH), 7.51 (m, 2H, ArH), 9.84 (s, 1H, D_2O exchangeable NH), 13.70 (br, 1H, D_2O exchangeable NH); ^{1}H NMR (DMSO-d_6) δ 5.17 (br, 1H, D_2O exchangeable NH), 7.28 (tt, 1H, J = 1.6, 7.9 Hz, ArH), 7.47 (tt, 2H, J = 1.6, 7.9 Hz, ArH), 7.60 (dd, 2H, J = 1.6, 7.9 Hz, ArH), 12.58 (s, 1H, D_2O exchangeable OH). Anal. Calcd for $C_{10}H_7F_3N_4O$ (256.1): C, 46.88; H, 2.75; N, 21.87. Found: C, 46.75; H, 2.66; N, 22.00.

*3.1.1.1.2. 4-(4-Methylphenylhydrazono)-5-trifluoromethyl-2,4-dihydropyrazol-3-one (**3b**).* Yield 2.51 g (93%); orange yellow crystals, mp 187 °C. IR: 3279, 3043, 2933, 2862, 2739, 1670, 1597, 1551, 1524, 1493, 1443, 1416, 1373, 1300, 1269, 1211, 1188, 1138, 1061, 976, 879, 818, 779, 760, 714, 663, 509, 482, 420; ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3H, CH_3), 7.25 (d, 2H, J = 8.7 Hz, ArH), 7.40 (d, 2H, J = 8.5 Hz, ArH), 9.96 (s, 1H, D_2O exchangeable NH), 13.76 (br, 1H, D_2O exchangeable NH); ^{1}H NMR (DMSO-d_6) δ 2.32 (s, 3H, CH_3), 5.17 (br, 1H, D_2O exchangeable NH), 7.27 (d, 2H, J = 8.5 Hz, ArH), 7.48 (d, 2H, J = 8.5 Hz, ArH), 12.55 (s, 1H, D_2O exchangeable OH). Anal. Calcd for $C_{11}H_9F_3N_4O$ (270.1): C, 48.89; H, 3.36; N, 20.73. Found: C, 48.67; H, 3.44; N, 20.85.

*3.1.1.1.3. 4-(4-Methoxyphenylhydrazono)-5-trifluoromethyl-2,4-dihydropyrazol-3-one (**3c**).* Yield 2.28 g (80%); reddish brown crystals, mp 192–194 °C. IR: 3259, 3019, 2938, 2816, 2756, 1662, 1601, 1551, 1527, 1493, 1446, 1419, 1373, 1308, 1250, 1180, 1142, 1065, 1026, 976, 876, 833, 779, 752, 710, 667, 521, 478, 428; ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3H, OCH_3), 6.98 (dd, 2H, J = 2.2, 6.9 Hz, ArH), 7.46 (dd, 2H, J = 2.2, 6.9 Hz, ArH), 9.31 (s, 1H, D_2O exchangeable NH), 13.94 (br, 1H, D_2O exchangeable NH). Anal. Calcd for $C_{11}H_9F_3N_4O_2$ (286.1): C, 46.16; H, 3.17; N, 19.58. Found: C, 46.01; H, 3.21; N, 19.35.

*3.1.1.1.4. 4-(4-Chlorophenylhydrazono)-5-trifluoromethyl-2,4-dihydropyrazol-3-one (**3d**).* Yield 2.72 g (94%); yellow crystals, mp 220–2 °C. IR: 3171, 3101, 3028, 1666, 1593, 1554, 1531, 1485,

Table 1
Antimicrobial activity of compounds **1**, **3a–d**, **5a**,**b**, and **6a** compared to standard antimicrobial agents

Test organisms	Compound								
	Concentration (mg/mL)								
	1	2.5	5	1	2.5	5	1	2.5	5
	1[a]			**3a**[a]			**3b**[a]		
Aspergillus fumigatus	0	0	+	+	+	+ (400)[c]	+	+	+
Penicillium italicum	+	+	+	++	++	++ (400)[c]	+	+	+
Syncephalastrum racemosum	0	0	+	0	+	+ (400)[c]	+	+	+
Candida albicans	0	0	0	0	0	0	0	0	0
Staphylococcus aureus	0	0	0	+	+	++ (400)[c]	+	+	+
Pseudomonas aeruginosa	0	0	0	0	0	0	0	0	0
Bacillus subtilis	0	0	+	++	++	++ (400)[c]	+	+	+
Escherichia coli	+	+	++	+	+	+ (400)[c]	+	+	+
	3c[a]			**3d**[a]			**5a**[a]		
Aspergillus fumigatus	0	0	+	0	0	+	0	0	+ (400)[c]
Penicillium italicum	+	+	+	+	+	++	+	+	+ (400)[c]
Syncephalastrum racemosum	0	0	0	+	+	+	0	0	+ (400)[c]
Candida albicans	0	0	0	0	0	0	0	0	+ (400)[c]
Staphylococcus aureus	0	+	+	+	++	++	++	++	++ (400)[c]
Pseudomonas aeruginosa	0	0	0	0	0	0	0	0	0
Bacillus subtilis	++	++	++	+	+	++	0	+	+ (400)[c]
Escherichia coli	+	+	+	+	+	+	0	+	+ (400)[c]
	5b[a]			**6a**[a]			**St.**[a,b]		
Aspergillus fumigatus	0	+	+	0	+	+	++	+++	+++
Penicillium italicum	+	+	+	+	+	+	++	+++	+++
Syncephalastrum racemosum	0	0	+	0	0	+	+++	+++	+++
Candida albicans	0	0	0	0	0	+	++	++	++
Staphylococcus aureus	+	+	+	0	0	+	++	++	++
Pseudomonas aeruginosa	0	0	0	0	0	0	++	+++	+++
Bacillus subtilis	0	0	+	0	0	0	++	+++	+++
Escherichia coli	+	+	+	+	+	+	++	++	++

Note: The test was done using the diffusion agar technique. Inhibition values = 0.1–0.5 cm beyond control = +; inhibition values = 0.6–1.0 cm beyond control = ++; inhibition values = 1.0–1.5 cm beyond control = +++; 0 = not detected.

[a] 100 μL of each concentration was tested (5, 2.5, 1.0 mg/mL); well diameter = 0.6 cm.

[b] St. = reference standard; chloramphenicol was used as a standard antibacterial agent and terbinafin was used as a standard antifungal agent.

[c] MIC values are given in brackets; MIC (μg/mL) = minimum inhibitory concentration, that is, lowest concentration to completely inhibit microbial growth.

1443, 1377, 1296, 1254, 1211, 1173, 1142, 1088, 1053, 1011, 976, 876, 833, 771, 725, 667, 644, 509, 471, 420; ^{1}H NMR ($CDCl_3$) δ 7.43 (s, 4H, ArH), 9.10 (s, 1H, D_2O exchangeable NH), 13.70 (br, 1H, D_2O exchangeable NH). Anal. Calcd for $C_{10}H_6ClF_3N_4O$ (290.0): C, 41.33; H, 2.08; N, 19.28. Found: C, 41.27; H, 1.97; N, 19.33.

3.1.1.2. General procedure for the synthesis of compounds 5a–d and 6a–d. (A) To a solution of each of compounds **3a–d** (2.1 mmol) in DMF (2.5 mL) and TEA (0.36 mL, 2.6 mmol) was added 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **(4)** (1.03 g, 2.5 mmol) and the reaction mixture was stirred overnight. The next day, the reaction mixture was diluted with ice-water mixture and the formed precipitate was collected by filtration, washed several times with water, and dried at room temperature. The precipitate was extracted with DCM, the solution was concentrated, and the residue was subjected to silica gel (70–230 mesh) column chromatography. Compounds **5a–d** were eluted first with 30–40% EtOAc/petroleum ether (bp 40–60 °C), followed by compounds **6a–d** with 40–70% EtOAc/petroleum ether (bp 40–60 °C). The chromatographically separated crude products were recrystallized from DCM/petroleum ether (bp 40–60 °C). R_f values of the latter compounds were determined on TLC aluminum sheets using EtOAc/petroleum ether (bp 40–60 °C) [60:40, v/v] as a developing system.

(B) The same previous method using compound **3a,b** (2.1 mmol), DMF (5 mL), TEA (0.72 mL, 5.2 mmol), and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **(4)** (1.73 g, 4.2 mmol).

*3.1.1.2.1. 2-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-4-phenylhydra zono-5-trifluoromethyl-2,4-dihydropyrazol-3-one (**5a**).* Yield 763 mg (62%, A), 98 mg (8%, B); yellow crystals, mp 130–2 °C (R_f = 0.21). IR: 3271, 3105, 3067, 2959, 2773, 1755, 1678, 1558, 1500, 1462, 1439, 1377, 1288, 1223, 1146, 1072, 1038, 976, 906, 806, 768, 729, 690, 598, 571, 536, 501, 459, 417; ^{1}H NMR ($CDCl_3$) δ 1.88, 1.90, 1.95, 1.96 (4s, 12H, CH_3CO), 3.80 (ddd, 1H, $J_{H\text{-}5'\text{-}H\text{-}6'}$ = 2.1 Hz, $J_{H\text{-}5'\text{-}H\text{-}6''}$ = 4.8 Hz, $J_{H\text{-}5'\text{-}H\text{-}4'}$ = 9.9 Hz, H-5′), 4.04 (dd, 1H, $J_{H\text{-}6'\text{-}H\text{-}5'}$ = 2.1 Hz, $J_{H\text{-}6'\text{-}H\text{-}6''}$ = 12.6 Hz, H-6′), 4.19 (dd, 1H, $J_{H\text{-}6''\text{-}H\text{-}5'}$ = 4.8 Hz, $J_{H\text{-}6''\text{-}H\text{-}6'}$ = 12.6 Hz, H-6″), 5.12 (dt, 1H, $J_{H\text{-}4'\text{-}H\text{-}2'}$ = 2.1 Hz, $J_{H\text{-}4'\text{-}H\text{-}3'}$ = 6.6 Hz, $J_{H\text{-}4'\text{-}H\text{-}5'}$ = 9.9 Hz, H-4′), 5.25 (dt, 1H, $J_{H\text{-}3'\text{-}H\text{-}1'}$ = 2.1 Hz, $J_{H\text{-}3'\text{-}H\text{-}4'}$ = 6.6 Hz, H-3′), 5.28 (dt, 1H, $J_{H\text{-}2'\text{-}H\text{-}4'}$ = 2.1 Hz, $J_{H\text{-}2'\text{-}H\text{-}1'}$ = 6.6 Hz, H-2′), 5.73 (dd, 1H, $J_{H\text{-}1'\text{-}H\text{-}3'}$ = 2.1 Hz, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 6.6 Hz, H-1′), 7.38 (m, 3H, ArH), 7.74 (dd, 2H, J = 1.6, 6.7 Hz, ArH), 13.70 (br, 1H, D_2O exchangeable NH). Anal. Calcd for $C_{24}H_{25}F_3N_4O_{10}$ (586.1): C, 49.15; H, 4.30; N, 9.55. Found: C, 48.97; H, 4.27; N, 9.39.

*3.1.1.2.2. 2-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-4-(4-methylphenylhydrazono)-5-trifluoromethyl-2,4-dihydropyrazol-3-one (**5b**).* Yield 744 mg (59%, A), 63 mg (5%, B); yellow crystals, mp 147–149 °C. (R_f = 0.16). IR: 3236, 3031, 3006, 2959, 2757, 1755, 1651, 1621, 1616, 1558, 1500, 1439, 1373, 1230, 1142, 1038, 980, 906, 825, 756, 710, 602, 555, 478; ^{1}H NMR ($CDCl_3$) δ 1.989, 1.997, 2.048, 2.050 (4s, 12H, CH_3CO), 2.42 (s, 3H, CH_3), 3.89 (ddd, 1H, $J_{H\text{-}5'\text{-}H\text{-}6'}$ = 2.1 Hz, $J_{H\text{-}5'\text{-}H\text{-}6''}$ = 4.8 Hz, $J_{H\text{-}5'\text{-}H\text{-}4'}$ = 9.6 Hz, H-5′), 4.14 (dd, 1H, $J_{H\text{-}6'\text{-}H\text{-}5'}$ = 2.1 Hz, $J_{H\text{-}6'\text{-}H\text{-}6''}$ = 12.6 Hz, H-6′), 4.28 (dd, 1H, $J_{H\text{-}6''\text{-}H\text{-}5'}$ = 4.8 Hz, $J_{H\text{-}6''\text{-}H\text{-}6'}$ = 12.6 Hz, H-6″), 5.21 (dt, 1H, $J_{H\text{-}4'\text{-}H\text{-}2'}$ = 2.1 Hz, $J_{H\text{-}4'\text{-}H\text{-}3'}$ = 9.3 Hz, $J_{H\text{-}4'\text{-}H\text{-}5'}$ = 9.6 Hz, H-4′), 5.35 (dt, 1H, $J_{H\text{-}3'\text{-}H\text{-}1'}$ = 2.1 Hz, $J_{H\text{-}3'\text{-}H\text{-}4'}$ = 9.3 Hz, H-3′), 5.36 (dt, 1H, $J_{H\text{-}2'\text{-}H\text{-}4'}$ = 2.1 Hz, $J_{H\text{-}2'\text{-}H\text{-}1'}$ = $J_{H\text{-}2'\text{-}H3'}$ = 6.1 Hz, H-2′), 5.80 (dd, 1H, $J_{H\text{-}1'\text{-}H\text{-}3'}$ = 2.1 Hz, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 6.1 Hz, H-1′), 7.29 (d, 2H, J = 8.2 Hz,

ArH), 7.74 (d, 2H, J = 8.2 Hz, ArH), 13.77 (br, 1H, D_2O exchangeable NH). Anal. Calcd for $C_{25}H_{27}F_3N_4O_{10}$ (600.2): C, 50.00; H, 4.53; N, 9.33. Found: C, 49.94; H, 4.61; N, 9.24.

*3.1.1.2.3. 2-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-4-(4-methoxyphenylhydrazono)-5-trifluoromethyl-2,4-dihydropyrazol-3-one (**5c**).* Yield 815 mg (63%, A); yellow crystals, mp 146–148 °C. (R_f = 0.18). ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 2.05 (s, 6H, CH_3CO), 3.88 (s, 3H, OCH_3), 3.87 (ddd, 1H, $J_{H\text{-}5'\text{-}H\text{-}6'}$ = 2.1 Hz, $J_{H\text{-}5'\text{-}H\text{-}6''}$ = 4.8 Hz, $J_{H\text{-}5'\text{-}H\text{-}4'}$ = 9.6 Hz, H-5′), 4.15 (dd, 1H, $J_{H\text{-}6'\text{-}H\text{-}5'}$ = 2.1 Hz, $J_{H\text{-}6'\text{-}H\text{-}6''}$ = 12.6 Hz, H-6′), 4.28 (dd, 1H, $J_{H\text{-}6''\text{-}H\text{-}5'}$ = 4.8 Hz, $J_{H\text{-}6''\text{-}H\text{-}6'}$ = 12.6 Hz, H-6″), 5.20 (dt, 1H, $J_{H\text{-}4'\text{-}H\text{-}2'}$ = 2.1 Hz, $J_{H\text{-}4'\text{-}H\text{-}3'}$ = 9.3 Hz, $J_{H\text{-}4'\text{-}H\text{-}5'}$ = 9.6 Hz, H-4′), 5.33 (dt, 1H, $J_{H\text{-}3'\text{-}H\text{-}1'}$ = 2.1 Hz, $J_{H\text{-}3'\text{-}H\text{-}4'}$ = 9.3 Hz, H-3′), 5.36 (dt, 1H, $J_{H\text{-}2'\text{-}H\text{-}4'}$ = 2.1 Hz, $J_{H\text{-}2'\text{-}H\text{-}1'}$ = $J_{H\text{-}2'\text{-}H3'}$ = 6.1 Hz, H-2′), 5.74 (dd, 1H, $J_{H\text{-}1'\text{-}H\text{-}3'}$ = 2.1 Hz, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 6.1 Hz, H-1′), 6.99 (d, 2H, J = 9.0 Hz, ArH), 7.84 (d, 2H, J = 9.0 Hz, ArH), 13.92 (br, 1H, D_2O exchangeable NH). Anal. Calcd for $C_{25}H_{27}F_3N_4O_{11}$ (616.2): C, 48.71; H, 4.41; N, 9.09. Found: C, 48.59; H, 4.37; N, 9.03.

*3.1.1.2.4. 2-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-4-(4-chlorophenylhydrazono)-5-trifluoromethyl-2,4-dihydropyrazol-3-one (**5d**).* Yield 938 mg (72%, A); yellow crystals, mp 160–2 °C. (R_f = 0.19). ^{1}H NMR ($CDCl_3$) δ 1.987, 2.018, 2.045, 2.048 (4s, 12H, CH_3CO), 3.88 (ddd, 1H, $J_{H\text{-}5'\text{-}H\text{-}6'}$ = 2.1 Hz, $J_{H\text{-}5'\text{-}H\text{-}6''}$ = 4.8 Hz, $J_{H\text{-}5'\text{-}H\text{-}4'}$ = 9.6 Hz, H-5′), 4.17 (dd, 1H, $J_{H\text{-}6'\text{-}H\text{-}5'}$ = 2.1 Hz, $J_{H\text{-}6'\text{-}H\text{-}6''}$ = 12.6 Hz, H-6′), 4.27 (dd, 1H, $J_{H\text{-}6''\text{-}H\text{-}5'}$ = 4.8 Hz, $J_{H\text{-}6''\text{-}H\text{-}6'}$ = 12.6 Hz, H-6″), 5.20 (dt, 1H, $J_{H\text{-}4'\text{-}H\text{-}2'}$ = 2.1 Hz, $J_{H\text{-}4'\text{-}H\text{-}3'}$ = 9.3 Hz, $J_{H\text{-}4'\text{-}H\text{-}5'}$ = 9.6 Hz, H-4′), 5.33 (dt, 1H, $J_{H\text{-}3'\text{-}H\text{-}1'}$ = 2.1 Hz, $J_{H\text{-}3'\text{-}H\text{-}4'}$ = 9.3 Hz, H-3′), 5.35 (dt, 1H, $J_{H\text{-}2'\text{-}H\text{-}4'}$ = 2.1 Hz, $J_{H\text{-}2'\text{-}H\text{-}1'}$ = $J_{H\text{-}2'\text{-}H3'}$ = 6.1 Hz, H-2′), 5.70 (dd, 1H, $J_{H\text{-}1'\text{-}H\text{-}3'}$ = 2.1 Hz, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 6.1 Hz, H-1′), δ 7.47 (d, 2H, J = 9.0 Hz, ArH), 7.79 (d, 2H, J = 9.0 Hz, ArH), 13.71 (br, 1H, D_2O exchangeable NH). Anal. Calcd for $C_{24}H_{24}ClF_3N_4O_{10}$ (620.1): C, 46.42; H, 3.90; N, 9.02. Found: C, 46.55; H, 3.96; N, 8.87.

*3.1.1.2.5. 1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-3-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyloxy)-4-phenylhydrazono-5-trifluoromethyl-1H-pyrazole (**6a**).* Yield: 115 mg (6%, A), 1.31 (68%, B) mp 121–123 °C (R_f = 0.07). ^{1}H NMR ($CDCl_3$) δ 1.9–2.12 (overlapped singlets, 24H, CH_3CO), 3.89 (ddd, 1H, $J_{H\text{-}5'\text{-}H\text{-}6'}$ = 2.1 Hz, $J_{H\text{-}5'\text{-}H\text{-}6''}$ = 4.8 Hz, $J_{H\text{-}5'\text{-}H\text{-}4'}$ = 9.6 Hz, H-5′ of either *N*- or *O*-gluc.), 3.95 (ddd, 1H, $J_{H\text{-}5'\text{-}H\text{-}6'}$ = 2.1 Hz, $J_{H\text{-}5'\text{-}H\text{-}6''}$ = 4.8 Hz, $J_{H\text{-}5'\text{-}H\text{-}4'}$ = 9.6 Hz, H-5′ of either *N*- or *O*-gluc.), 4.16 (dd, 1H, $J_{H\text{-}6'\text{-}H\text{-}5'}$ = 2.1 Hz, $J_{H\text{-}6'\text{-}H\text{-}6''}$ = 12.6 Hz, H-6′ of either *N*- or *O*-gluc.), 4.256 (dd, 1H, $J_{H\text{-}6'\text{-}H\text{-}5'}$ = 2.1 Hz, $J_{H\text{-}6'\text{-}H\text{-}6''}$ = 12.6 Hz, H-6′ of either *N*- or *O*-gluc.), 4.265 (dd, 1H, $J_{H\text{-}6''\text{-}H\text{-}5'}$ = 4.2 Hz, $J_{H\text{-}6''\text{-}H\text{-}6'}$ = 12.6 Hz, H-6″ either *N*- or *O*-gluc.), 4.38 (dd, 1H, $J_{H\text{-}6''\text{-}H\text{-}5'}$ = 4.8 Hz, $J_{H\text{-}6''\text{-}H\text{-}6'}$ = 12.6 Hz, H-6″ of either *N*- or *O*-gluc.), 5.07 (d, 1H, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 9.3 Hz, H-1′ of *O*-gluc.), 5.17 (t, 1H, J = 7.8 Hz), 5.28 (2dt, 2H, J = 2.1, 9.6 Hz), 5.30 (t, 1H, J = 7.5 Hz), 5.36 (t, 1H, J = 7.3 Hz), 5.51 (d, 1H, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 9.0 Hz, H-1′ of *N*-gluc.), 5.92 (t, 1H, J = 9.0 Hz), 7.38 (m, 3H, ArH), 7.78 (dd, 2H, J = 1.8, 8.1 Hz, ArH). Anal. Calcd for $C_{38}H_{43}F_3N_4O_{19}$ (916.2): C, 49.78; H, 4.73; N, 6.11. Found: C, 49.76; H, 4.58; N, 5.99.

*3.1.1.2.6. 1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-3-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyloxy)-4-(4-methylphenylhydrazono)-5-trifluoromethyl-1H-pyrazole (**6b**).* Yield 117 mg (6%, A), 1.39 g (71%, A); yellow crystals, mp 131–3 °C. (R_f = 0.06). IR: 2959, 2739, 1755, 1639, 1620, 1504, 1435, 1373, 1227, 1142, 1073, 1038, 984, 906, 829, 748, 602, 474. ^{1}H NMR ($CDCl_3$) δ 1.81–2.02 (overlapped singlets, 24H, CH_3CO), 2.42 (s, 3H, CH_3), 3.89 (ddd, 1H, $J_{H\text{-}5'\text{-}H\text{-}6'}$ = 2.1 Hz, $J_{H\text{-}5'\text{-}H\text{-}6''}$ = 4.8 Hz, $J_{H\text{-}5'\text{-}H\text{-}4'}$ = 9.6 Hz, H-5′ of either *N*- or *O*-gluc.), 3.95 (ddd, 1H, $J_{H\text{-}5'\text{-}H\text{-}6'}$ = 2.1 Hz, $J_{H\text{-}5'\text{-}H\text{-}6''}$ = 4.8 Hz, $J_{H\text{-}5'\text{-}H\text{-}4'}$ = 9.6 Hz, H-5′ of either *N*- or *O*-gluc.), 4.08 (dd, 1H, $J_{H\text{-}6'\text{-}H\text{-}5'}$ = 2.1 Hz, $J_{H\text{-}6'\text{-}H\text{-}6''}$ = 12.6 Hz, H-6′ of either *N*- or *O*-gluc.), 4.156 (dd, 1H, $J_{H\text{-}6'\text{-}H\text{-}5'}$ = 2.1 Hz, $J_{H\text{-}6'\text{-}H\text{-}6''}$ = 12.6 Hz, H-6′ of either *N*- or *O*-gluc.), 4.162 (dd, 1H, $J_{H\text{-}6''\text{-}H\text{-}5'}$ = 4.2 Hz, $J_{H\text{-}6''\text{-}H\text{-}6'}$ = 12.6 Hz, H-6″ either *N*- or *O*-gluc.), 4.30 (dd, 1H, $J_{H\text{-}6''\text{-}H\text{-}5'}$ = 4.8 Hz, $J_{H\text{-}6''\text{-}H\text{-}6'}$ = 12.6 Hz, H-6″ of either *N*- or *O*-gluc.), 5.16 (d, 1H, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 9.3 Hz, H-1′ of *O*-gluc.), 5.26 (t, 1H, J = 8.8 Hz), 5.35 (2dt, 2H, J = 2.1, 9.2 Hz), 5.39 (t, 1H, J = 7.5 Hz), 5.44 (t, 1H, J = 7.3 Hz), 5.59 (d, 1H, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 9.3 Hz, H-1′ of *N*-gluc.), 6.00 (t, 1H, J = 9.3 Hz), 7.28 (d, 2H, J = 7.9 Hz, ArH), 7.79 (d, 2H, J = 7.9 Hz, ArH). Anal. Calcd for $C_{39}H_{45}F_3N_4O_{19}$ (930.3): C, 50.32; H, 4.87; N, 6.02. Found: C, 50.27; H, 4.94; N, 5.97.

*3.1.1.2.7. 1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-3-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyloxy)-4-(4-methoxyphenylhydrazono)-5-trifluoromethyl-1H-pyrazole (**6c**).* Yield 119 mg (6%, A); yellow crystals, mp 137 °C. (R_f = 0.07). ^{1}H NMR ($CDCl_3$) δ 1.80–2.03 (overlapped singlets, 24H, CH_3CO), 3.89 (ddd, 1H, $J_{H\text{-}5'\text{-}H\text{-}6'}$ = 2.1 Hz, $J_{H\text{-}5'\text{-}H\text{-}6''}$ = 4.8 Hz, $J_{H\text{-}5'\text{-}H\text{-}4'}$ = 9.6 Hz, H-5′ of either *N*- or *O*-gluc.), 3.91 (s, 3H, OCH_3), 3.94 (ddd, 1H, $J_{H\text{-}5'\text{-}H\text{-}6'}$ = 2.1 Hz, $J_{H\text{-}5'\text{-}H\text{-}6''}$ = 4.8 Hz, $J_{H\text{-}5'\text{-}H\text{-}4'}$ = 9.5 Hz, H-5′ of either *N*- or *O*-gluc.), 4.07 (dd, 1H, $J_{H\text{-}6'\text{-}H\text{-}5'}$ = 2.1 Hz, $J_{H\text{-}6'\text{-}H\text{-}6''}$ = 12.6 Hz, H-6′ of either *N*- or *O*-gluc.), 4.157 (dd, 1H, $J_{H\text{-}6'\text{-}H\text{-}5'}$ = 2.1 Hz, $J_{H\text{-}6'\text{-}H\text{-}6''}$ = 12.6 Hz, H-6′ of either *N*- or *O*-gluc.), 4.163 (dd, 1H, $J_{H\text{-}6''\text{-}H\text{-}5'}$ = 4.2 Hz, $J_{H\text{-}6''\text{-}H\text{-}6'}$ = 12.6 Hz, H-6″ either *N*- or *O*-gluc.), 4.29 (dd, 1H, $J_{H\text{-}6''\text{-}H\text{-}5'}$ = 4.8 Hz, $J_{H\text{-}6''\text{-}H\text{-}6'}$ = 12.6 Hz, H-6″ of either *N*- or *O*-gluc.), 5.17 (d, 1H, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 9.2 Hz, H-1′ of *O*-gluc.), 5.26 (t, 1H, J = 9.0 Hz), 5.36 (2dt, 2H, J = 2.1, 9.2 Hz), 5.40 (t, 1H, J = 7.4 Hz), 5.43 (t, 1H, J = 7.3 Hz), 5.58 (d, 1H, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 9.3 Hz, H-1′ of *N*-gluc.), 6.01 (t, 1H, J = 9.3 Hz), 7.00 (d, 2H, J = 9.0 Hz, ArH), 7.83 (d, 2H, J = 9.0 Hz, ArH). Anal. Calcd for $C_{39}H_{45}F_3N_4O_{20}$ (946.3): C, 49.47; H, 4.79; N, 5.92. Found: C, 49.44; H, 4.65; N, 5.81.

*3.1.1.2.8. 1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-3-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyloxy)-4-(4-chlorophenylhydrazono)-5-trifluoromethyl-1H-pyrazole (**6d**).* Yield 140 mg (7%, A); yellow crystals, mp 129–131 °C. (R_f = 0.07). ^{1}H NMR ($CDCl_3$) δ 1.82–2.02 (overlapped singlets, 24H, CH_3CO), 3.89 (ddd, 1H, $J_{H\text{-}5'\text{-}H\text{-}6'}$ = 2.1 Hz, $J_{H\text{-}5'\text{-}H\text{-}6''}$ = 4.8 Hz, $J_{H\text{-}5'\text{-}H\text{-}4'}$ = 9.6 Hz, H-5′ of either *N*- or *O*-gluc.), 3.96 (ddd, 1H, $J_{H\text{-}5'\text{-}H\text{-}6'}$ = 2.1 Hz, $J_{H\text{-}5'\text{-}H\text{-}6''}$ = 4.8 Hz, $J_{H\text{-}5'\text{-}H\text{-}4'}$ = 9.6 Hz, H-5′ of either *N*- or *O*-gluc.), 4.07 (dd, 1H, $J_{H\text{-}6'\text{-}H\text{-}5'}$ = 2.1 Hz, $J_{H\text{-}6'\text{-}H\text{-}6''}$ = 12.6 Hz, H-6′ of either *N*- or *O*-gluc.), 4.153 (dd, 1H, $J_{H\text{-}6'\text{-}H\text{-}5'}$ = 2.1 Hz, $J_{H\text{-}6'\text{-}H\text{-}6''}$ = 12.6 Hz, H-6′ of either *N*- or *O*-gluc.), 4.159 (dd, 1H, $J_{H\text{-}6''\text{-}H\text{-}5'}$ = 4.2 Hz, $J_{H\text{-}6''\text{-}H\text{-}6'}$ = 12.6 Hz, H-6″ either *N*- or *O*-gluc.), 4.32 (dd, 1H, $J_{H\text{-}6''\text{-}H\text{-}5'}$ = 4.8 Hz, $J_{H\text{-}6''\text{-}H\text{-}6'}$ = 12.6 Hz, H-6″ of either *N*- or *O*-gluc.), 5.18 (d, 1H, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 9.3 Hz, H-1′ of *O*-gluc.), 5.27 (t, 1H, J = 8.8 Hz), 5.35 (2dt, 2H, J = 2.1, 9.3 Hz), 5.42 (t, 1H, J = 7.5 Hz), 5.44 (t, 1H, J = 7.4 Hz), 5.58 (d, 1H, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 9.3 Hz, H-1′ of *N*-gluc.), 6.05 (t, 1H, J = 9.3 Hz), 7.47 (d, 2H, J = 9.0 Hz, ArH), 7.80 (d, 2H, J = 9.0 Hz, ArH) Anal. Calcd for $C_{38}H_{42}ClF_3N_4O_{19}$ (950.2): C, 47.98; H, 4.45; N, 5.89. Found: C, 48.04; H, 4.33; N, 5.77.

3.1.1.3. General procedure for deacetylation of compounds 5a and 6a. To a stirred solution of compounds **5a** and **6a** (1 mmol) in anhydrous MeOH (10 mL) was added portion wise NaOMe [0.054 g (1 mmol) and 0.108 g (2 mmol), for compounds **5a** and **6a**, respectively] in anhydrous MeOH (10 mL) at room temperature and the solution was stirred overnight. After evaporation of solvent in vacuo, H_2O (15 mL) was added and the mixture was extracted several times with DCM to remove the ester formed during the deprotection. To the resulting aqueous solution was added an ion exchange resin (Dowex 50W × 2, H^+ form), previously washed with MeOH. After stirring for five minutes, the solution was filtered, evaporated in vacuo and the residue chromatographed on silica gel with the gradient 0–10% MeOH in $HCCl_3$ to give compounds **7** and **8**. R_f values of the latter compounds were determined using TLC aluminum sheets and $HCCl_3$/MeOH (60:40, v/v) as a developing system.

*3.1.1.3.1. 2-β-D-Glucopyranosyl-4-phenylhydrazono-5-trifluoromethyl-2,4-dihydropyrazol-3-one (**7**).* Yield 376 mg (90%); yellow crystals, mp 210–212 °C (R_f = 0.29). MS: m/z = 418 (M^+). ^{1}H NMR (DMSO-d_6) δ 3.11–3.39 (m, 4H, H-2′, H-3′, H-4′, H-5′), 3.45 (dd, 1H, $J_{H\text{-}6'\text{-}OH}$ = 5.7 Hz, $J_{H\text{-}6'\text{-}H\text{-}6''}$ = 11.7 Hz,), 3.64 (dd, 1H, $J_{H\text{-}6''\text{-}H\text{-}5'}$ = 2.3 Hz, $J_{H\text{-}6''\text{-}H\text{-}6'}$ = 11.7 Hz, H-6″), 4.55 (br, 1H, D_2O-

exchangeable OH), 5.00 (d, 1H, J = 5.4 Hz, D_2O-exchangeable OH), 5.14 (d, 1H, J = 4.8 Hz, D_2O-exchangeable OH), 5.52 (br, 1H, D_2O-exchangeable OH), 5.56 (d, 1H, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 9.3 Hz, H-1′), 7.47 (m, 3H, ArH), 7.82 (dd, 2H, J = 1.5, 7.0 Hz, ArH), 13.53 (br, 1H, D_2O-exchangeable NH). Anal. Calcd for $C_{16}H_{17}F_3N_4O_6$ (418.1): C, 45.94; H, 4.10; N, 13.39. Found: C, 45.88; H, 4.09; N, 13.22.

*3.1.1.3.2. 1-β-D-Glucopyranosyl-3-β-D-glucopyranosyloxy-4-phenylhydrazono-5-trifluoromethyl-1H-pyrazole (**8**).* Yield 435 mg (75%); yellow crystals, mp 202–204 °C (R_f = 0.1). MS: m/z = 580 (M^+). ^1H NMR (DMSO-d_6) δ 3.08–3.42 (m, 8H, H-2′, H-3′, H-4′, H-5′ of both *N*- and *O*-glucs.), 3.45 (dd, 1H, $J_{H\text{-}6'\text{-}OH}$ = 5.7 Hz, $J_{H\text{-}6'\text{-}H\text{-}6''}$ = 11.7 Hz, H-6′ of either *N*- or *O*-gluc.), 3.48 (dd, 1H, $J_{H\text{-}6'\text{-}OH}$ = 5.1 Hz, $J_{H\text{-}6'\text{-}H\text{-}6''}$ = 12.0 Hz, H-6′ of either *N*- or *O*-gluc.), 3.64 (dd, 1H, $J_{H\text{-}6''\text{-}H\text{-}5'}$ = 2.3 Hz, $J_{H\text{-}6''\text{-}H\text{-}6'}$ = 11.7 Hz, H-6″ of either *N*- or *O*-gluc.), 3.66 (dd, 1H, $J_{H\text{-}6''\text{-}H\text{-}5'}$ = 2.5 Hz, $J_{H\text{-}6''\text{-}H\text{-}6'}$ = 12.0 Hz, H-6″ of either *N*- or *O*-gluc.), 4.55 (br, 2H, D_2O-exchangeable OH), 4.89 (d, 1H, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 8.7 Hz, H-1′ of *O*-gluc.), 5.00 (d, 1H, J = 5.4 Hz, D_2O-exchangeable OH), 5.02 (br s, 1H, D_2O-exchangeable OH), 5.11 (br s, 1H, D_2O-exchangeable OH) , 5.14 (d, 1H, J = 4.8 Hz, D_2O-exchangeable OH), 5.52 (br, 2H, D_2O-exchangeable OH), 5.55 (d, 1H, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 9.3 Hz, H-1′). 7.46 (m, 3H, ArH), 7.78 (dd, 2H, J = 1.8, 8.1 Hz, ArH). Calcd for $C_{22}H_{27}F_3N_4O_{11}$ (580.16): C, 45.52; H, 4.69; N, 9.65. Found: C, 45.71; H, 4.66; N, 9.77.

3.2. Antimicrobial activity

Antimicrobial screening of compounds **1, 3a–d, 5a,b**, and **6a** was carried out using the diffusion agar technique.[50] The test organisms were obtained from the culture of the Regional Center for Mycology and Biotechnology, Faculty of Science, Al-Azhar University, Cairo, Egypt. Compounds, **1**, **3a–d**, **5a,b**, **6a** and standard antimicrobial agents (chloramphenicol and terbinafin were used as standard antibacterial and antifungal agents, respectively) were dissolved in DMF (5 mg/mL). Further dilutions of the compounds and standard drugs were prepared at the required quantities of 2.5 and 1 mg/mL concentrations. All the compounds were tested for their in vitro growth inhibitory activity against two Gram-positive bacteria (*B. subtilis* RCMB 101-001 and *S. aureus* RCMB 106-001 (1)), two Gram-negative bacteria (*P. aeruginosa* RCMB 102-002 and *E. coli* RCMB 103-001), one yeast (*C. albicans* RCMB 005003), and three fungal strains (*A. fumigatus* RCMB 002008 (1), *P. italicum* RCMB 001018 (1), and *S. racemosum* RCMB 016001). The minimum inhibitory concentrations (MICs) of compounds **3a** and **5a** were determined via further twofold serial dilutions in the test medium to prepare the required quantities of 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 μg/mL concentrations. The antimicrobial activities were expressed as the diameter of the inhibition zones (Table 1).

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