Design, synthesis and antimicrobial evaluation of novel N-, O- and S- glycosides based 3,5-Pyrazolidinedione scaffolds

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Received: 04 Aug. 2022, Revised: 27 Aug. 2022. Accepted: 30 Aug. 2022.
Published online: 1 Sept. 2022

Abstract: 2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide were reacted with 4-arylidene-1-phenylpyrazolidine-3,5-diones 1ab under phase transfer catalysis (PTC) conditions to yield unseparated products namely; N-(2’,3’,4’,6’-tetra-O-acetyl-β-D-glucopyranosyl)-4-arylidene-1-phenyl-3,5-pyrazolidinediones 2 & 4 and 3-(2’,3’,4’,6’-tetra-O-acetyl-β-D-glucopyranosyloxy)-4-arylidene-1-phenyl-1H-pyrazol-5-ones 3 & 5, respectively. Also, compounds 1ab were treated with a mixture of carbon disulphide and 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide to give unisolated products namely; (2’,3’,4’,6’-tetra-O-acetyl-β-D-glucopyranosyl)N-(1-phenyl-4-arylidene)-pyrazolidin-3,5-diones-carbodithioates 6 & 8 and (2’,3’,4’,6’-tetra-O-acetyl-β-D-glucopyranosyl)3-oxo-(1-phenyl-4-arylidene)-pyrazol-5-one-carbodithioates 7 & 9, respectively.

Keywords: 1-phenylpyrazolidine-3,5-dione, PTC, β-D-glucopyranosyl, glycosides.

1 Introduction

The biological and pharmacological activities of pyrazoles as important class of heterocycles owing as anti-inflammation [1–5], antitumour [6,7], antimicrobial [8,9], antiviral [10], antimalarial activities [11], anticancer agents [12] and treating Alzheimer’s disease [13] have reported. Glycosyl sulfanyl heterocycles have been regarded as good glycosyl donors in addition to their biological activities such as the inhibition of enzyme activity [14], fungicides in pesticides and fumigants [15,16] and anti corona virus[17,18]. On the basis of above mentioned findings, the purpose of the present work was to design synthesize and investigate the antimicrobial activity of some novel 3,5-Pyrazolidinedione Scaffolds carrying carbohydrate residues through N-, O- and S-glycosidic bond formation.

2 Results and Discussion

1- Chemistry part:

4-(Arylidene)-1-phenylpyrazolidine-3,5-diones 1ab [19] were reacted with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide under PTC conditions [dioxane / anhydrous K2CO3 / tetrabutyl ammonium bromide (TBABr)] at room temperature to yield N-(2’,3’,4’,6’-tetra-O-acetyl-β-D-glucopyranosyl)-4-arylidene-1-phenyl-3,5-pyrazolidinediones 2 & 4 and 3-(2’,3’,4’,6’-tetra-O-acetyl-β-D-glucopyranosyloxy)-4-arylidene-1-phenyl-1H-pyrazol-5-ones 3 & 5, respectively (Scheme 1).

Their IR spectra showed ν 1758 cm⁻¹ corresponding to C=Oacetyl groups. 1H NMR spectrum of a mixture of compound 2 + 3 showed the following signals: 2.02 (s, 2H, 8CH₂CO), 4.11-4.23 (dd, 4H, 6a, 6b, H-6a*, H-6b*), 4.38 (t, 1H, H-4), 5.1(d, 2H, H-3), 5.25 (q, 1H, H-5), 5.58 (t, 2H, H-2*), 6.30 (d, 1H, H-1, J=8.00 Hz), 6.46 (d, 1H, H-1*, J=8.00 Hz), 7.23-8.65 (m, 24H, 4Ar-H) and, 9.03 (s, 2H,2=CH). It was shown from 1H NMR spectrum that; two unseparated compounds 2 & 3 with their anomeric protons (H-1), (H-1*) as a doublets at δ = 6.30 and δ = 6.46 ppm with a J₁,₂ = 8 Hz, which corresponds to the diaxial orientation of the H-1 and H-2 protons indicating the β-configuration.

Similarily, A mixture of carbon disulphide and 2,3,4,6-
tetra-O-acetyl-α-D-glucopyranosyl bromide was reacted with compounds 1a,b to yield also unisolated products namely; (2’,3’,4’,6’-tetra-O-acetyl-β-D-glucopyranosyl) [N-(1-phenyl-4-arylidene)-pyrazolidin-3,5-dione]-carbodithioates 

6&8 and (2’,3’,4’,6’-tetra-O-acetyl-β-D-glucopyranosyl)[3-oxy-(1-phenyl-4-arylidene)-pyrazol-5-one]-carbodithioates 7 & 9, respectively (Scheme 2).

1H NMR spectrum of a mixture of compounds 8 + 9 showed the following signals: 1.92,1.99,2.03(s, 24H, H-5*), 3.08(s,6H,2CH3), 5.06-5.10 (m, 2H, H-3*, H-5*), 5.48-5.52 (m, 1H, H-2*), 6.10 (d, 1H, H-1*,J=8.00 Hz), 6.77-7.90 (m,9H, Ar-H), 8.46(s, 1H, H-3*).

From 1H NMR spectrum of 8 + 9 it was shown that; two unseparated compounds with their anomic protons (H-1, H-1*) as a doublet signals at δ = 6.19 and δ = 6.35 ppm with a J 1,2 = 8 Hz, which corresponds to the diaxial orientation of the H-1 and H-2 protons indicating the β-configuration.

13C NMR spectrum of a mixture of compounds 8 + 9 exhibited a two signals at δ 95.31 and 96.05 corresponding to two anomic carbons (C-1),(C-1*),also showed two C=S group signals at 8C = 170.05, 170.48, whereas the three C-O groups appeared at 8C = 160.98 and 163.29. Column chromatography separation of products 8 & 9 mixture by using (ethyl acetate/ hexane) has been done to yield the separated compounds 8 and 9 in low yields.

IR spectrum of compound 8 showed a new absorption band corresponding to (CH3CO) at ν 1758.58; Its 1H NMR spectrum showed the following signals: 1.96, 1.99(s, 12H, 4CH3CO), 3.08(s,6H,2CH3), 5.06-5.10 (m, 2H, H-6a, H-6b), 5.14-5.17 (t, 1H, H-4), 5.28-5.31(m, 2H, H-3, H-5 ), 5.48-5.52 (m, 1H, H-2* ), 6.10 (d, 1H, H-1*,J=8.00 Hz), 6.77-7.90 (m,9H, Ar-H), 8.46(s, 1H, H-3*).

IR spectrum of compound 9 showed a new absorption band corresponding to (CH3CO) at ν 1758.58. Its 1H NMR spectrum showed the following signals at: 1.99,2.03(s, 12H, 4CH3CO), 3.16(s,6H,2CH3), 4.11-4.18 (m, 2H, H-6a*, H-6b*), 4.20-4.23 (t, 1H, H-4*), 5.07-5.17(m, 2H, H-3*, H-5*), 5.54-5.57 (m, 1H, H-2*), 6.19-6.21 (d, 1H, H-1*,J=8.00 Hz), 6.85-7.97 (m,9H, Ar-H), 8.56(s, 1H, H-3*).

II- Biological Activity:

II- 1 In Vitro Antimicrobial Activity of some prepared glycoside derivatives:

The antimicrobial potential of some prepared compounds were performed against four isolates belonging to four species of bacteria and fungi (each, two isolates). The bacterial isolates were, Escherichia coli (ATCC6538, Gram –ve, pathogen) and Staphylococcus aureus (ATCC9027, Gram+ve, pathogen). Whereas, the fungal isolates were: Candida albicans (ATCC10231, pathogen) and Aspergillus fumigatus (local isolates, saprophyte). The isolates of bacteria (E. coli and S. aureus) were pregerminated and grown on nutrient agar, whreas, Calbicans on sabouraud and A. fumigatus on 1% glucose-Czapek's agar media, respectively.

Method:

The pregermination was done on the suitable media to have a mass of cells (bacteria) or conidia and mycelia (fungi) at 37°C, 37°C and 28°C of bacteria, Calbicans and A. fumigatus isolates, respectively. At the end of incubation period, distilled water (10 ml) was added onto the surface of organism growth, carefully crushed by needle with loop and transferred to Erlenmeyer flask (100 ml) containing distilled water (50 ml) to have approx.1× 1010 - 1× 1011 cells and 1× 105 - 1× 106 conidia of bacteria and fungi, respectively. The final dilution (1 ml) was used to inoculate the medium in petri dish (9 cm), carefully moved with swabbed on the solidified medium. The titled compounds and standard (Ciprofloxacine and clotrimazole as antibacterial and antifungal, respectively) were dissolved in dimethylsulfoxide (DMSO, 10 ml). the discs (10mm) of filter paper (Whatman No.1) were sterilized by dry heat at 140°C for 1h, soaked in dissolving solvent paper (Whatman No.1) and air dried (4-5h) to exclude the effect of DMSO. The loaded discs were fixed on the surface of cultured (4 discs per Petri dish), kept at 40°C for 2h to allow the diffusion of dissolved compounds into agar media, and incubated under suitable conditions as previously described.

II-2 In Vitro Antimicrobial Activity of separated compounds 8&9:

II- 3 Antimicrobial evaluation:

All tested glycoside derivatives had a clear activities against both the Gram-positive (S. aureus) and the Gram-negative (E. Coli) bacteria and some of them had a good activities against the fungal strains (Table 1,2). Compound 8 showed the highest inhibition zone 28 and 30 mm, and lowest MIC 1.6 and 2.2 μg ml-1 against both the Gram-positive (S. aureus) and the Gram-negative (E.coli)
bacteria respectively, with comparable values to the standard Ciprofloxacin drug. In addition to its low antifungal activity against *C. albicans* strain with inhibition zone of 10 mm. However it showed no reactivity against the *A. fumigatus* Compound 9 showed strong antibacterial effect against the Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria with inhibition zone of 26 and 28 mm, respectively and low MIC 2.4 and 2.8 μg ml⁻¹ against both the Gram-positive (*S. aureus*) and the Gram-negative (*E. coli*) bacteria respectively. However it showed no antifungal activity against *C. albicans* strain nor *A. fumigatus*.

III- Molecular docking study: *E. coli* DNA gyrase B

Docking simulations were performed to study the binding pattern of the newly synthesized compounds in *E. coli* DNA gyrase B active site to predict their binding pattern and to investigate their ability to satisfy the required structural features for binding interactions [20]. The docking setup was first validated by performing self-docking of the co-crystallized thiazole inhibitor in the active site of *E. coli* DNA gyrase B (PDB ID: 4DUH) [21]. The self-docking validation reproduced the co-crystallized thiazole indicating that the docking protocol used is suitable for the intended docking study. This is shown by the small RMSD between the experimental co-crystallized inhibitor pose and the docked pose of 1.00 Å; and by the capability of the docking pose to reproduce all the key interactions achieved by the co-crystallized ligands in the active site. the docking energy score was S = -9.25 kcal/mol. Arg73, Arg136 interact with Ph-COO⁻ through H-bonding. Lys103 interacts with thiazole moiety by arene-cation interaction. Gly101 interact with Ph-NH and sulfur atom by H-bonding. Asp73 interact with NH of NHCOEt by H-bonding. Gly77 with Asp73, and Thr165 with N of thiazole by H-bonding through water bridge (Table 3 & Figure 1a, b).

Table 1: The inhibitory effect (inhibition Zone, mm) of some prepared glycoside derivatives in addition to standard (antibiotics) compounds (100mg) against isolate of bacteria and fungi (each, 2 isolate of 2 species), where a, b are standard drugs.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (μg ml⁻¹)</th>
<th>Inhibition Zone (mm)</th>
<th>Fungal Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Bacteria</strong></td>
<td><strong>Fungal Strains</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>S. aureus</em></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td><strong>8</strong></td>
<td>100</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td><strong>9</strong></td>
<td>100</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td><strong>aCiprofloxacin</strong></td>
<td>100</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td><strong>bClotrimazole</strong></td>
<td>100</td>
<td>N. A.</td>
<td>N. A.</td>
</tr>
</tbody>
</table>

Figure 1a: 2D diagram representation of thiazole inhibitor docked into *E col* DNA gyrase B active site showing his binding interactions with the amino acids binding site.

Figure 1b: 3D diagram representation of thiazole inhibitor docked into *E col* DNA gyrase B active site.

Experimental:

Melting points were determined with an electronic melting point apparatus (Stuart) in open capillaries and are uncorrected. TLC was performed on E. Merck Silica Gel 60 F254 with detection by UV light absorption. IR spectra were recorded with a Bruker infrared spectro-photometer.
Table 2: The inhibitory effect (minimum inhibitory concentration (MIC), μg ml⁻¹) of the most active glycoside derivatives 8&9 in addition to standard (antibiotics) compounds against isolate of bacteria and fungi (each, 2 isolate of 2 species), where  a, b are atndded drugs .

<table>
<thead>
<tr>
<th>Compound</th>
<th>Minimum inhibitory concentration (MIC) (μg ml⁻¹)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>S. aureus</td>
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<tr>
<td>8</td>
<td>1.6</td>
</tr>
<tr>
<td>9</td>
<td>2.4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
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<tr>
<td>Clotrimazole</td>
<td>-</td>
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</table>

Table: 3

<table>
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<tr>
<th>Compound</th>
<th>Binding scores (kcal/mol)</th>
<th>Ligand atom</th>
<th>Residue</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>-8.3</td>
<td>Sulfur atom of carboxithioate</td>
<td>Arg136</td>
<td>H-bond</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O of CO of pyrazole ring</td>
<td>Arg136</td>
<td>H-bond</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O of Pyrine ring of sugar</td>
<td>Arg76</td>
<td>H-bond</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O of CO of O-Acetyl group</td>
<td>His55</td>
<td>H-bond</td>
</tr>
<tr>
<td>9</td>
<td>-7.85</td>
<td>Sulfur atom of carboxithioate</td>
<td>Arg76</td>
<td>H-bond</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benzene ring of pyrazole</td>
<td>Gly101</td>
<td>H-bond</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O of CO of O-Acetyl group</td>
<td>H2O</td>
<td>H-bond</td>
</tr>
</tbody>
</table>


Figure 2a: 2D diagram representation of glycoside 8 docked into E coli DNA gyrase B active site.

Figure 2b: 3D diagram representation of glycoside 8 docked into E coli DNA gyrase B active site.

(KBr technique). $^1$H and $^{13}$C NMR spectra were recorded on Bruker Avance NMR spectrometer at 400 and 100 MHz, with TMS as the internal standard. Solvents used were purified by simple distillation.

**Synthesis of compounds 2-5:**

To a solution of the corresponding compound 1a,b (0.05 mol) in dioxane (40 ml), anhydrous potassium carbonate (3 g) and tetrabutylammonium bromide (TBABr)(0.02 g) were added. The reaction mixture was stirred at room temperature for 30 mints, and then 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide was added portion wise (5 mints). Continues the stirring for the mixture at same temperature for overnight (TLC). Filtered the carbonate layer and the filtrate was evaporated, the residue was treated with water, dried and crystallized from ethanol.
& 3-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-4-(4-N,N-dimethylaminobenzylidene)-1-phenyl-1H-pyrazol-5-one (5)

Orange solid, m.p 168-170 °C, IR (KBr, cm⁻¹): 1758.51 (CH₃C=O); ¹H NMR (400 MHz, DMSO): 1.92, 1.99, 2.03 (s, 2H), 3.14 (s, 12H, 4CH₃) 4.09-4.22 (m, 4H, H-6a, H-6b, H-6a*, H-6b*), 4.33 (t, 2H, H-4, H-4*), 5.05-5.33 (m, 4H, H-3, H-3*, H-5, H-5*) 5.52-5.59 (m, 2H, H-2, H-2*), 6.19 (d, 1H, H-1,J₈=8.00), 6.36 (d, 1H, H-1*,J₈=8.00), 6.8-8.00 (m, 18H, Ar-H), 8.55 (s, 2H, 2=CH).

Synthesis of compounds 6-9:
To a solution of the corresponding compounds 1₄₁₈ (0.05 mol) in dioxane (40 ml), anhydrous potassium carbonate (3g), carbodiisulphide (0.05mol, 3ml) and tetrabutyl ammonium bromide (TBABr) (0.02 g) were added and was stirred at 0-5 °C for 30 mins to the reaction mixture, 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide was added portion wise (5 mins). Continues the stirring for the mixture at room temperature for overnight (TLC). Filtered the carbonate layer and the filtrate was evaporated, the residue was treated with water, the precipitated product was filtered, dried and crystallized from ethanol.

A mixture of:
(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)[N-(1-phenyl-4-(2-naphthylidene)]-pyrazolidin-3,5-dione]-carbdithioate (6)
& (2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)[3-oxo-(1-phenyl-4-(2-naphthylidene)]-pyrazol-5-one]-carbdithioate (7)
Brown solid , m.p 216-218 °C, IR (KBr, cm⁻¹): 1761 (CH₃C=O); ¹H NMR: 1.90, 1.95, 1.98, 1.99, 2.01, 2.03 (s, 2H), 3.14 (s, 12H, 4CH₃) 4.09-4.24 (m, 4H, H-6a, H-6b, H-6a*, H-6b*), 4.38 (t, 2H, H-4, H-4*), 5.07-5.09 (m, 2H, H-3, H-3*) 5.22-5.28 (m, 2H, H-5, H-5*) 5.55-5.6 (m, 2H, H-2, H-2*), 6.28 (d, 1H, H-1,J₈=8.00 Hz), 6.44 (d, 1H, H-1*,J₈=8.00 Hz), 7.23-8.11, 8.7 (m, 24H, Ar-H), 8.66 (s, 1H, =CH*).

Amixture of:
(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)[N-(1-phenyl-4-(4-N,N-dimethylaminobenzylidene)]-pyrazolidin-3,5-dione]-carbdithioate (8)
& (2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)[3-oxo-(1-phenyl-4-(4-N,N-dimethylaminobenzylidene)]-pyrazol-5-one]-carbdithioate (9)
Orange solid , m.p 182-184 °C, IR (KBr, cm⁻¹): 1758.82 (CH₃C=O); ¹H NMR: 1.92, 1.99, 2.03 (s, 2H), 3.14 (s, 12H, 4CH₃) 4.08-4.25 (m, 4H, H-6a, H-6b, H-6a*, H-6b*), 4.30-4.33 (t, 2H, H-4, H-4*), 5.04-5.31 (m, 4H, H-3, H-3*, H-5, H-5*) 5.52-5.57 (m, 2H, H-2, H-2*), 6.19 (d, 1H, H-1,J₈=8.00 Hz), 6.35 (d, 1H, H-1*,J₈=8.00 Hz), 6.8-8.01 (m, 18H, Ar-H), 8.54 (s, 2H, 2=CH). ¹³CNMR: 20.75 (8CH₃CO), 40.52 (4NCH₃), 62.09 (C-6, 6*), 68.48 (C-4, 4*), 71.04 (C-3), 71.35 (C-3*), 72.08 (C-2), 72.25 (C-2*), 72.71 (C-5, 5*), 95.31 (1-C), 96.05 (1-C*)
(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)[N-(1-phenyl-4-(4-N,N-dimethylaminobenzylidene)-
pyrazolidin-3,5-dione]-carbodithioate (8)

Orange solid, m.p182-184 °C, IR(KBr, cm⁻¹): ν=1758.58 (CH=O), ν=1703.73 (C=O), ν=1585.54 (C=C), ν=763.54 (C–N), ν=722.23 (C–C).

Acknowledgments

The authors are acknowledging Kaoud Salama, teaching assistant in chemistry department, Faculty of science, Sohag university, for providing the molecular docking studies.

References


