Microwave Assisted Synthesis Of Some New Purines And Their N-Alkyl Derivatives (Hydrazones And Their Acyclo C-Nucleosides) Of Expected Antimicrobial And Antifungal Activity

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Abstract - Microwave assisted synthesis of new purines and their N-alkyl derivatives was described. Also some new purines such as sugar 7-alkyl-8-theophyllinyl-hydrazones (7,8\textsubscript{a-e}) and their acyclo C-nucleosides (5,7,9-trimethyl-3-(D-pentitol and tetritol-1-yl)-5,9-dihydro-6H-[1,2,4]triazolo[4,3-e]purine-6,8(7H)-dione) (9,10\textsubscript{a-e}) have been synthesized. Structures of the products have been deduced from their elemental analysis and spectral data (IR, $^{1}$H-NMR, $^{13}$C-NMR). Selected new synthesized compounds were screened as antimicrobial agents and showed some antibacterial against three bacteria species, namely \textit{Escherichia coli} (EC), \textit{Pseudomonas aeruginosa} (PA) and \textit{Staphylococcus aureus} (SA) and antifungal activities against three fungal species, namely \textit{Aspergillus fumigatus} (AF), \textit{Aspergillus niger} (AN) and \textit{Candida albicans} (CA).

Keywords: Purines, theophylline, caffeine, hydrazones, cyclonucleosides, synthesis, microwave, biological activity.

1. INTRODUCTION

Because the purine scaffold is present in many natural products and analogs capable of mediating biochemical processes, several methods for the high-throughput synthesis of purine derivatives have been developed. Many of the reported protocols share in common the nucleophilic displacement of purines, a reaction requiring high temperature and long exposure. While developing a method for the synthesis of purin-9-yl-substituted derivatives was most troublesome. Employment of microwave irradiation to not only heat but accelerate chemical reactions rates and decrease the reaction time is now common practice in modern chemistry.
Microwaves are defined as electromagnetic waves with vacuum wavelengths ranging between 0.1 to 100 cm or, equivalently, with frequencies between 0.3- 300 GHz. Microwave dielectric heating uses the ability of some liquids and solids to transform electromagnetic radiation into heat to drive chemical reactions. This technology opens up new opportunities to the synthetic chemist in the form of new reactions that are not possible using conventional heating\(^{(1-6)}\).

A purine is a heterocyclic aromatic organic compound, consisting of a pyrimidine ring fused to an imidazole ring. Purines, including substituted purines and their tautomers, are the most widely distributed kind of nitrogen-containing heterocycle in nature. The quantity of naturally occurring purines produced on earth is huge, as 50 percent of the bases in nucleic acids, adenine and guanine are purines. In DNA, these bases form hydrogen bonds with their complementary pyrimidines thymine and cytosine, respectively. This is called complementary base pairing. In RNA, the complement of adenine is uracil instead of thymine\(^{(7)}\). Purine derivatives constitute an enormous class of compounds, some of which are well-known as therapeutic agents. These fused planar heterocycles present key hydrogen bond donating/accepting functionalities, making them interesting scaffolds for targeting many biosynthetic, regulatory and signal transduction proteins including cellular kinases, G proteins, and polymerases\(^{(8)}\).

The naturally occurring methylxanthine, caffeine and theophylline are the classical adenosine receptor antagonists. Caffeine is widely consumed in beverages, theophylline is used as a drug in the treatment of bronchial asthma and several other xanthines derived from caffeine and theophylline are therapeutically used as analeptics, antiasthmatics, vasodilators, antihypertensive and diuretics\(^{(9)}\).
It has been reported that 8-substituted xanthine derivatives and fused purines as examples shown in Scheme, which known as A$_2$A and A$_{2B}$-selective antagonists and anticancer agents are synthesized by microwave technique and discovered$^{(10-13)}$.

Scheme: Purines as A$_2$A and A$_{2B}$-selective antagonists and anticancer agents

II. RESULTS AND DISCUSSION
II.1. CHEMISTRY

The required starting 8-hydrazinocaffeine 4 and 7-ethyl-8-hydrazinotheophyllines 5 have been obtained according to the reported literature$^{(14)}$. They were prepared by alkylation of 8-chlorotheophylline 1 with iodomethane in DMF containing potassium carbonate to afford 8-
chlorocaffeine 2 and 8-chloro-7-ethylheophylline 3\(^{(15)}\), respectively, followed by treatment of 2 and 3 with hydrazine hydrate in boiling ethanol. In our previous paper\(^{(16)}\), it was reported that the condensation of equimolar amounts of 7-ethyl-8-hydrazinotheophylline 5 with aldohexoses such as, D-glucose 6\(\text{a}\), D-galactose 6\(\text{b}\); D-mannose 6\(\text{c}\), and aldopentoses, such as D-ribose 6\(\text{d}\), D-arabinose 6\(\text{e}\), by heating for 2-6 h in an aqueous ethanolic solution and in the presence of a catalytic amount of acetic acid gave the corresponding aldehydo-sugar (7-ethyl-8-theophyllinyl)hydrazones 8\(\text{a-e}\) (Scheme 1). By the same manner, the hydrazone derivatives 7\(\text{a-e}\) were synthesized by reaction of 8-hydrazinocaffeine 4 with aldohexoses and aldopentoses 6. The structures of obtained hydrazones 7\(\text{a-e}\) and 8\(\text{a-e}\) were confirmed by their elemental analyses and spectral (IR, 1H NMR and MS) data (see Experimental Section).

Under environmentally friendly, time saving microwave-assisted conditions, we re-synthesized the previously described sugar hydrazone compounds 7\(\text{a-e}\) and 8\(\text{a–e}\) under microwave conditions, aiming to increase reaction yields and reduce the reaction times. The results of these preparations, which are depicted in table 1, indicated that reaction yields were increased by 10–20% compared to the conventional conditions. Reaction times were also significantly reduced.
Scheme 1: Microwave assisted synthesis of purine hydrazones and their C-Nucleosides
Table 1: The benefits of using microwave conditions for the synthesis of the above-mentioned compounds 7,8a-e.

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>Reaction Yield %</th>
<th>Reaction Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Microwave</td>
<td>Conventional Method</td>
</tr>
<tr>
<td>7a</td>
<td>78</td>
<td>60</td>
</tr>
<tr>
<td>7b</td>
<td>80</td>
<td>63</td>
</tr>
<tr>
<td>7c</td>
<td>80</td>
<td>70</td>
</tr>
<tr>
<td>7d</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>7e</td>
<td>65</td>
<td>50</td>
</tr>
<tr>
<td>8a</td>
<td>80</td>
<td>70</td>
</tr>
<tr>
<td>8b</td>
<td>85</td>
<td>73</td>
</tr>
<tr>
<td>8c</td>
<td>86</td>
<td>78</td>
</tr>
<tr>
<td>8d</td>
<td>70</td>
<td>55</td>
</tr>
<tr>
<td>8e</td>
<td>65</td>
<td>55</td>
</tr>
</tbody>
</table>

Thermal attempt of the dehydrogenative cyclization of the formed sugar hydrazones 7a-e and 8a-e with bromine in acetic acid/sodium acetate(17), ferric chloride in ethanol(18,19), copper dichloride in dimethylformamide(20), bromine in water(21) or in acetic acid(22), bromine in acetic acid, sodium acetate and acetic anhydride(23) or thionyl chloride(24) as previously used for the cyclization of related sugar hydrazones, was failed and no crystalline derivatives of the acyclo C-nucleosides (R= sugar) 9a-e and 10a-e (Scheme 1) could be isolated. So the microwave technique was used, hence, microwave irradiation of the
sugar hydrazones 7a-e and 8a-e in ethanol containing 2M solution of iron (III) chloride yielded the new cyclized sugar hydrazones, acyclo C-nucleosides 9a-e and 10a-e, respectively.

According to our knowledge, none of acyclo C-nucleosides 9a-e and 10a-e has been reported hitherto. The structures of obtained acyclo C-nucleosides 9a-e and 10a-e were confirmed by their elemental analyses and spectral (IR, $^1$H and $^{13}$C NMR and MS) data (see Experimental Section). For example, their $^1$H NMR spectra in DMSO-$d_6$ revealed in each case, absence of signals assignable to the azamethine N=CH proton and NH of hydrazone group =N-NH-. This finding indicates the cyclization of 7a-e and 8a-e to acyclo C-nucleosides 9a-e and 10a-e. $^1$H NMR spectra revealed methyl groups at $\delta = 3.17, 3.31, 3.42$ for 9a-e, and an ethyl group signals at $\delta = 1.20$ (CH$_3$) and $4.2$ (CH$_2$) for 10-a-e. The alditolyl group protons (CHOH proton signals of the sugar chain) were associated with the solvent absorption (DMSO-$d_6$) forming a broad signal at $\delta = 3.20$-4.06 (CH protons) and 4.20-5.79 (OH protons).

$^{13}$C NMR spectra of 9a-e and 10a-e revealed characteristic alditolyl group carbons at $\delta$ 61.5-77.5 and cyclised carbon (C3=N as in 9a) at $\delta$ 154.0-156.0.

The IR spectra of 9a-e and 10a-e showed bands at 3243-3462 cm$^{-1}$ due to the OH groups bands at 1629-1699 cm$^{-1}$ due to the C=O groups. The mass spectra of the nucleosides 9a-e and 10a-e by using
electron ion technique revealed M⁺ and M⁺+1 peaks with intensities of 25-45%.

II.2. ANTIMICROBIAL ACTIVITY

Ten products, 8a-e and 9a-e were evaluated for their antibacterial and antifungal activities in vitro against two gram negative anaerobic bacteria *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA) and one gram positive bacteria *Staphylococcus aureus* (SA) as well as three fungal species, namely *Aspergillus fumigatus* (AF), *Aspergillus niger* (AN) and *Candida albicans* (CA). The reference antibiotics tetracycline and amphotericin B were used as references to evaluate the potency of the tested compounds under the same conditions. The test results are depicted in table 2. The solvent used was dimethylsulfoxide. Concentration of the sample is 100 μg/mL. The test results revealed that all compounds exhibited moderate activity against the three bacterial species and all compounds, except (9b), (9c), and (10c) and (10d), showed moderate activity against one fungal species, *Candida albicans*. 
### Table 2: Antibacterial and Antifungal Activities of some of the Synthesized Compounds.

Inhibition Zone Diameter (IZD*) (mm/mg Compound Tested)

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>(EC) G&lt;sup&gt;-&lt;/sup&gt;</th>
<th>(PA) G&lt;sup&gt;+&lt;/sup&gt;</th>
<th>(SA) G&lt;sup&gt;-&lt;/sup&gt;</th>
<th>(AF) Fungus</th>
<th>(AN) Fungus</th>
<th>(CA) Fungus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control: DMSO</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Tetracycline Antibacterial agent</td>
<td>28</td>
<td>28</td>
<td>26</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amphotericin B Antifungal agent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>9a</td>
<td>11</td>
<td>13</td>
<td>12</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>9b</td>
<td>11</td>
<td>15</td>
<td>16</td>
<td>0.0</td>
<td>0.0</td>
<td>11</td>
</tr>
<tr>
<td>9c</td>
<td>11</td>
<td>15</td>
<td>15</td>
<td>0.0</td>
<td>0.0</td>
<td>11</td>
</tr>
<tr>
<td>9d</td>
<td>9</td>
<td>14</td>
<td>14</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>9e</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>10a</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>10b</td>
<td>12</td>
<td>11</td>
<td>12</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>10c</td>
<td>11</td>
<td>10</td>
<td>11</td>
<td>0.0</td>
<td>0.0</td>
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</tr>
<tr>
<td>10d</td>
<td>11</td>
<td>15</td>
<td>11</td>
<td>0.0</td>
<td>0.0</td>
<td>10</td>
</tr>
<tr>
<td>10e</td>
<td>10</td>
<td>9</td>
<td>11</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*IZD = 2-10 mm beyond control = + (low activity).
IZD = 11-24 mm beyond control = ++ (moderate activity).
IZD = 25-35 mm beyond control = +++ (high activity)
III. EXPERIMENTAL

III.1. GENERAL

Chemical were purchased from Sigma (NY, USA) company in high purity. All the materials were of commercial reagent grade and the solvents were purified by standard procedures. All melting points were taken on an Electrothermal IA 9100 series digital melting point apparatus. The IR spectra (KBr) discs were recorded on a Perkin-Elmer 1650 spectrometer. $^1$H- and $^{13}$C-NMR spectra were recorded on a Bruker AC-300 Hz instrument. Chemical shifts were expressed as δ (ppm) relative to TMS as internal standard and DMSO-d$_6$ as solvent. The elemental analysis were performed at the Micro-analytical Center, Cairo University. Mass spectra were recorded on a Shimadzu GC-MS-QP 1000 EX spectrometer. Microwave reactions were performed with a Millstone Organic Synthesis Unit (MicroSYNTH with touch control terminal, 2450MHz, 800W) with a continuous focused microwave power delivery system in a pressure glass vessel (10 mL) sealed with a septum under magnetic stirring. The temperature of the reaction mixture was monitored using a calibrated infrared temperature control under the reaction vessel, and control of the pressure was performed with a pressure sensor connected to the septum of the vessel.

III.2. General procedure for synthesis of sugar 7-alkyl-8-theophyllinyl-hydrazones 7a-e and 8a-e

Method B: To a suspension of 7-alkyl-8-hydrazone of theophylline 4 or 5 (10 mmol) in ethanol (30 ml), was added a solution of the appropriate sugar 6a-e (10 mmol) in water (10 ml) and few drops of glacial acetic acid. The mixture was heated at reflux until reaction was judged complete by TLC (2-6 h). The solid product formed upon cooling was filtered off, washed
with the minimum amount of ethanol, dried and finally recrystallized from ethanol to afford the respective hydrazones 7a-e and 8a-e.

**Method B (Microwave Method):**

The same reactants of method A were heated at 140 °C in microwave oven for 5-15 min. The reaction mixture was treated in a similar manner to method A to obtain compounds 7a-e and 8a-e.

The physical constants and the spectral data of the products 8a-e are in the match with the reported data [15], while that of products 7a-e, 9a-e, 10a-e are listed below:

**D-Glucose-7-methyl-8-theophyllinylhydrazone 7a** (D-Glucose-8-caffeinythydrazone 7a).

Yield 60%; white crystals, mp 228-230 °C; IR (KBr) ν 3446 (OH), 3248 (NH), 1697, 1655 (C=O) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆) δ 3.02 (m, 2H, alditolyl 2H), 3.17-3.48 (m, 11H, alditolyl 2H, 3N-CH₃), 3.65-3.89 (2m, 2H, alditolyl 2H), 4.22-4.50 (m, 2H, 2OH, exchangeable), 4.89 (t, 1H, OH, exchangeable), 5.22, 5.79 (2d, 2H, 2OH, exchangeable), 7.43 (d, 1H, N=CH), 8.41 (s, 1H, exchangeable, =N-NH); ¹³C NMR (75 MHz, DMSO-d₆) δ 28.9 (C-3), 38.8 (C-1), 41.0 (N-7 CH₃), 61.2-77.5 (C-alditol), 101.5 (C-5), 144.5 (C-4), 147.4 (C-8), 150.6 (N=CH), 152.5 (C-2), 153.9 (C-6); MS: m/z (%) = 387 (M+1⁺, 23), 386 (M⁺, 45), 303 (33), 287 (40), 277 (14), 265 (8.0), 236 (16), 220 (100), 214 (5.0). Anal. calcd. for C₁₄H₂₂N₆O₇ (386): C, 43.52; H, 5.74; N, 21.75. Found: C, 43.33; H, 5.20; N, 21.70%.

**D-Galactose-7-methyl-8-theophyllinylhydrazone 7b** (D-Galactose-8-caffeinythydrazone 7b).

Yield 63%; white crystals, mp 190-193 °C; IR (KBr) ν 3462 (OH), 3235 (NH), 1696, 1629 (C=O) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆) δ 3.18 (s, 3H, N-CH₃), 3.24-3.41 (m, 5H, alditolyl 2H, N-CH₃), 3.45 (s, 3H, N7-CH₃), 3.53, 3.83 (2m., 4H, alditolyl 4H), 4.23-4.28 (m, 1H, OH, exchangeable), 4.47 (t, 1H, OH, exchangeable), 4.69,
5.21, 5.74 (3d, 3H, 3OH, exchangeable), 7.54 (d, 1H, N=CH), 8.46 (s,1H, exchangeable, =N-NH-); \(^{13}\text{C}\) NMR (75 MHz, DMSO-d6) δ 29.2 (C-3), 39.1 (C-1), 40.5 (N7-CH\(_3\)), 60.7-76.6 (C-alditol), 101.7 (C-5), 143.5 (C-4), 150.9 (C-8), 152.8 (N=CH), 152.9 (C-2), 154.5 (C-6); MS: m/z (%) = 387 (M+1\(^+\), 27), 386 (M\(^+\), 40), 300 (33), 285 (40), 270 (14), 260 (8.0), 230 (16), 222 (100). Anal. calcd. for C\(_{14}\)H\(_{22}\)N\(_6\)O\(_7\) (387): C, 43.52; H, 5.74; N, 21.75. Found: C, 43.50; H, 5.50; N, 21.9%.

**D-Mannose-7-methyl-8-theophyllinylhydrazone 7c (D-Mannose-8-caffeinyldrazone 7c).** Yield 70%; yellowish white crystals, mp 216-218 \(^\circ\)C; IR (KBr) ν 3407 (OH), 3245 (NH), 1684, 1657 (C=O) cm\(^{-1}\); 1H NMR (300 MHz, DMSO-d6) δ 3.19-3.71 (m, 14H, alditolyl 5H, 3N -CH\(_3\)), 4.05 (m, 1H, alditolyl H), 4.23-4.34 (m, 3H, 3OH, exchangeable), 4.43 (t, 1H, OH, exchangeable), 5.16 (d,1H, OH, exchangeable), 7.43 (d, 1H, N=CH), 10.92 (s, 1H, exchangeable, =N-NH); \(^{13}\text{C}\) NMR (75 MHz, DMSO-d6) δ 28.9 (C-3), 38.8 (C-1), 40.0 (N7-CH\(_3\)), 63.4-70.8 (Calditol),101.7 (C-5), 146.0 (C-4), 148.7 (C-8), 149.6 (N=CH), 150.6 (C-2), 152.6 (C-6); MS: m/z (%) = 387 (M\(^+\) +1, 30), 386 (M\(^+\), 19), 308 (22), 307 (100), 289 (45), 273 (7.0), 224 (7.0), 222 (42), Anal. calcd. for C\(_{14}\)H\(_{22}\)N\(_6\)O\(_7\) (386): C, 43.52; H, 5.74; N, 21.75. Found: C, 43.30; H, 5.25; N, 21.75%.

**D-Ribose-7-methyl-8-theophyllinylhydrazone 7d (D-Ribose-8-caffeinyldrazone 7d).** Yield 40%; white crystals, mp 115-116 \(^\circ\)C; IR (KBr) ν 3356 (OH), 3235 (NH), 1699, 1638 (C=O) cm\(^{-1}\); 1H NMR (300 MHz, DMSO-d6) δ 3.19 (s, 3H, N-CH\(_3\)), 3.31-3.61 (m, 10H, alditolyl 4H, 2 N-CH\(_3\)), 4.20-4.34 (m, 2H, alditolyl H, OH, exchangeable), 4.52, 4.85, 5.16 (3d, 3H, 3OH, exchangeable), 7.46 (d, 1H, N=CH), 10.90 (s, 1H, exchangeable, =N-NH-); \(^{13}\text{C}\) NMR (75 MHz, DMSO-d6) δ 29.2 (C-3), 38.8 (C-1), 40.0 (N7-CH\(_3\)), 63.1-74.1 (C-alditol), 101.9 (C-5), 147.8 (C-4), 148.0 (C-8), 149.9 (N=CH), 150.9 (C-2), 152.9 (C-6); MS: m/z
(%) = 357 (M$^+$ +1, 29), 356 (M$^+$, 15), 305 (100), 288 (50), 270 (9.0), 260 (5.0), 245 (6.0), 220 (26), 215 (5.0). Anal. calcd. for C$_{13}$H$_{20}$N$_6$O$_6$ (356): C, 43.82; H, 5.66; N, 23.58. Found: C, 43.55; H, 5.44; N, 23.70%

D-Arabinose-7-methyl-8-theophyllinylhydrazone 7e (D-Arabinose-8-
caffeinylhydrazone 7e). Yield 50%; white crystals, mp 238-239 °C; IR
(KBr) v 3356 (OH), 3208 (NH), 1688, 1655 (C=O) cm$^{-1}$; $^1$H NMR (300
MHz, DMSO-d6) $\delta$ 3.19 (s, 3H, N-CH$_3$), 3.31-3.58 (m, 10H, alditolyl
4H, 2N-CH$_3$), 4.28-4.34 (m, 2H, alditolyl H, OH, exchangeable), 4.57
(m, 2H, 2OH, exchangeable), 4.96 (d, 1H, OH, exchangeable), 7.51 (d,
1H, N=CH), 10.91 (s, 1H, exchangeable, $\equiv$N-NH-); $^{13}$C NMR (75 MHz,
DMSO-d6) $\delta$ 29.1 (C-3), 38.7 (C-1), 40.0 (N7-CH$_3$), 63.2-73.3 (Calditol),
101.9 (C-5), 147.8 (C-4), 148.8 (C-8), 149.8 (N=CH), 150.8 (C-2), 152.8
(C-6); MS: m/z (%) = 357 (M$^+$ +1, 38), 305 (100), 286 (54), 256 (8.0),
220 (29). Anal. calcd. for C$_{13}$H$_{20}$N$_6$O$_6$ (356): C, 43.82; H, 5.66; N, 23.58.
Found: C, 43.50; H, 5.33; N, 23.40%

III.3. General procedure for cyclization of sugar 7-alkyl-8-
theophyllinyl-hydrazones 7a-e and 8a-e: Formation of acyclo C-
nucleosides 9a-e and 10a-e.

To a solution of sugar 7-alkyl-8-hydrazinotheophylline hydrazones 7a-e
or 8a-e (1 mmol) in ethanol (30 ml), was added a solution of 2M FeCl$_3$ 2
ml). The mixture was irradiated for 5-10 min in a domestic microwave
oven (2450 MHz, 800 W). The precipitate formed upon cooling was
filtered off, washed with water and then ethanol, dried and finally
recrystallized from ethanol-DMF (v:v, 2:1) to afford the respective acyclo
C-nucleosides 9a-e and 10a-e.

The physical constants and the spectral data of the products 9a-e and
10a-e are listed below:
5,7,9-trimethyl-3-(D-gluco-pentitol-1-yl)-5,9-dihydro-6H-
[1,2,4]triazolo[4,3-e]purine-6,8(7H)-dione 9a. Yield 60%; pale yellow
crystals, mp 185 °C; IR (KBr) v 3440 (OH), 3240 (NH), 1680, 1650
(C=O) cm\(^{-1}\); \(^1\)H NMR (300 MHz, DMSO-d6) \(\delta\) 3.02 (m, 2H, alditolyl
2H), 3.17-3.48 (m, 11H, alditolyl 2H, 3N-CH\(_3\)), 3.60-3.90 (2m, 2H,
arditolyl 2H), 4.22-4.50 (m, 2H, 2OH, exchangeable), 4.89 (t, 1H,OH,
exchangeable), 5.22, 5.79 (2d, 2H, 2OH, exchangeable); \(^{13}\)C NMR (75
MHz, DMSO-d6) \(\delta\) 28.5 (C7), 36.5 (C5), 38.0 (C9), 61.5-77.5 (C-alditol),
101.0 (C8a), 119.0 (C4a), 147.0 (C9a), 155.5 (C3), 157.0 (C6), 159.0
(C8); MS: m/z (%) = 385 (M+1\(^+\), 20), 384 (M+, 45), 300 (33), 285 (40),
275 (14), 265 (8.0), 233 (16), 220 (80), 214 (5.0). Anal. calcd. for
C\(_{14}\)H\(_{20}\)N\(_6\)O\(_7\) (384): C, 43.75; H, 5.24; N, 21.87. Found: C, 43.40; H,
5.15; N, 21.50%.

5,7,9-trimethyl-3-(D-galacto-pentitol-1-yl)-5,9-dihydro-6H-
[1,2,4]triazolo[4,3-e]purine-6,8(7H)-dione 9b. Yield 49%; buff
crystals, mp 224-226 °C; IR (KBr) IR (KBr) v 3442 (OH), 3442 (OH),
3245 (NH), 1685, 1653 (C=O) cm\(^{-1}\); \(^1\)H NMR (300 MHz, DMSO-d6) \(\delta\) 3.05 (m, 2H,
arditolyl 2H), 3.19-3.50 (m, 11H, alditolyl 2H, 3N-CH\(_3\)), 3.60-3.90 (2m,
arditolyl 2H), 4.22-4.52 (m, 2H, 2OH, exchangeable), 4.90 (t,
1H,OH, exchangeable), 5.20, 5.75 (2d, 2H, 2OH, exchangeable); \(^{13}\)C NMR (75
MHz, DMSO-d6) \(\delta\) 28.8 (C7), 36.6 (C5), 38.2 (C9), 61.5-77.0
(C-alditol), 101.5 (C8a), 112.0 (C4a), 147.2 (C9a), 154.5 (C3), 157.0
(C6), 160.0 (C8); MS: m/z (%) = 385 (M+1\(^+\), 24), 384 (M+, 25), 300
(30), 285 (35), 275 (15), 265 (10), 233 (15), 220 (30), 214 (1.0). Anal.
calcd. for C\(_{14}\)H\(_{20}\)N\(_6\)O\(_7\) (384): C, 43.75; H, 5.24; N, 21.87. Found: C,
43.50; H, 5.20; N, 21.62%.

5,7,9-trimethyl-3-(D-manno-pentitol-1-yl)-5,9-dihydro-6H-
[1,2,4]triazolo[4,3-e]purine-6,8(7H)-dione 9c. Yield 50%; buff crystals,
mp 218-219 °C; IR (KBr) IR (KBr) v 3441(OH), 3245 (NH), 1688, 1655
(C=O) cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-d6) δ 3.04 (m, 2H, alditolyl 2H), 3.17-3.50 (m, 11H, alditolyl 2H, 3N-CH$_3$), 3.62-3.91 (2m, 2H, alditolyl 2H), 4.22-4.50 (m, 2H, 2OH, exchangeable), 4.89 (t, 1H,OH, exchangeable), 5.20, 5.70 (2d, 2H, 2OH, exchangeable); $^{13}$C NMR (75 MHz, DMSO-d6) δ 28.9 (C7), 36.5 (C5), 38.5 (C9), 62.0-77.0 (C-alditol), 101.3 (C8a), 146.0 (C9a), 156.0 (C3), 157.1 (C6), 160.5 (C8); MS: m/z (%) = 385 (M$^{+}$ +1, 20), 384 (M+, 20), 300 (15), 285 (30), 275 (15), 265 (10), 230 (15), 225 (30), 210 (1.5). Anal. calcd. for C$_{14}$H$_{20}$N$_6$O$_7$ (384): C, 43.75; H, 5.24; N, 21.87. Found: C, 43.45; H, 5.10; N, 21.70%.

5,7,9-trimethyl-3-(D-ribo-tetritol-1-yl)-5,9-dihydro-6H-[1,2,4]triazolo[4,3-e]purine-6,8(7H)-dione 9d. Yield 40%; pale yellow crystals, mp 202-203 ºC; IR (KBr) v 3356 (OH), 3235 (NH), 1690, 1635 (C=O) cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-d6) δ 3.19 (s, 3H, N-CH$_3$), 3.31-3.61 (m, 10H, alditolyl 4H, 2 N-CH$_3$), 4.20-4.34 (m, 2H, alditolyl H, OH, exchangeable), 4.52, 4.85, 5.16 (3d, 3H, 3OH, exchangeable); $^{13}$C NMR (75 MHz, DMSO-d6) δ 26.5 (C7), 35.0 (C5), 37.5 (C9), 61.0-76.0 (C-alditol), 100.5 (C8a), 145.0 (C9a), 155.0 (C3), 157.5 (C6), 159.0 (C8); MS: m/z (%) = 355 (M$^{+}$ +1, 29), 354 (M$^{+}$, 15), 304 (80), 285 (50), 275 (9.0), 260 (5.0), 245 (6.0), 220 (26), 215 (5.0). Anal. calcd. for C$_{13}$H$_{18}$N$_6$O$_6$ (354): C, 44.07; H, 5.12; N, 23.72. Found: C, 44.15; H, 5.00; N, 23.50%.

5,7,9-trimethyl-3-(D-arabino-tetritol-1-yl)-5,9-dihydro-6H-[1,2,4]triazolo[4,3-e]purine-6,8(7H)-dione 9e. Yield 40%; buff crystals, mp 190-191 ºC; IR (KBr) v 3350 (OH), 3238 (NH), 1690, 1635 (C=O) cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-d6) δ 3.15 (s, 3H, N-CH$_3$), 3.30-3.65 (m, 10H, alditolyl 4H, 2 N-CH$_3$), 4.25-4.35 (m, 2H, alditolyl H, OH, exchangeable), 4.50, 4.80, 5.20 (3d, 3H, 3OH, exchangeable); $^{13}$C NMR (75 MHz, DMSO-d6) δ 26.0 (C7), 35.0 (C5), 37.0 (C9), 61.5-76.5 (C-alditol), 102.5 (C8a), 145.0 (C9a), 154.0 (C3), 156.5 (C6), 158.5 (C8);
MS: m/z (%) = 355 (M⁺ +1, 25), 354 (M⁺, 20), 304 (50), 285 (30), 275 (9.0), 260 (1.0), 245 (6.0), 220 (26), 215 (1.0). Anal. calcd. for C₁₃H₁₅N₆O₆ (354): C, 44.07; H, 5.12; N, 23.72. Found: C, 43.80; H, 4.95; N, 23.55%.

9-Ethyl-5,7-dimethyl-3-(D-gluco-pentitol-1-yl)-5,9-dihydro-6H-[1,2,4]triazolo[4,3-e]purine-6,8(7H)-dione 10a. Yield 45%; colorless crystals, mp 204 °C; IR (KBr) ν 3440 (OH), 3242 (NH), 1682, 1650 (C=O) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆) δ 1.3 (t, 3H, C-CH₃), 3.10 (m, 2H, alditolyl 2H), 3.15-3.45 (m, 8H, alditolyl 2H, 2N-CH₃), 3.60-3.90 (2m, 2H, alditolyl 2H), 4.22-4.55 (m, 4H, 2OH, N-CH₂, exchangeable), 4.90 (t, 1H,OH, exchangeable), 5.20, 5.75 (2d, 2H, 2OH, exchangeable); ¹³C NMR (75 MHz, DMSO-d₆) δ 22.5 (C-9 CH₂), 35.5 (C5), 37.0 (C9), 62.0-77.0 (C-alditol), 102.0 (C8a), 118.0 (C4a), 145.0 (C9a), 152.5 (C3), 155.0 (C6), 158.0 (C8); MS: m/z (%) = 398 (M+1⁺, 15), 384 (M⁺, 35), 300 (30), 285 (40), 275 (15), 265 (10), 233 (16), 220 (55), 214 (8.0). Anal. calcd. for C₁₅H₂₂N₆O₇ (398): C, 45.22; H, 5.57; N, 21.50. Found: C, 44.90; H, 5.20; N, 21.50%.

9-Ethyl-5,7-dimethyl-3-(D-galacto-pentitol-1-yl)-5,9-dihydro-6H-[1,2,4]triazolo[4,3-e]purine-6,8(7H)-dione 10b. Yield 55%; colorless crystals, mp 215 °C; IR (KBr) ν 3440 (OH), 3242 (NH), 1682, 1650 (C=O) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆) δ 1.25 (t, 3H, C-CH₃), 3.0 (m, 2H, alditolyl 2H), 3.18-3.45 (m, 8H, alditolyl 2H, 2N-CH₃), 3.65-3.90 (m, 2H, alditolyl 2H), 4.20-4.55 (m, 4H, 2OH, N-CH₂, exchangeable), 4.95 (t, 1H,OH, exchangeable), 5.24, 5.78 (2d, 2H, 2OH, exchangeable); ¹³C NMR (75 MHz, DMSO-d₆) δ 23.0 (C-9 CH₂), 35.5 (C5), 37.5 (C9), 62.0-77.5 (C-alditol), 101.0 (C8a), 119.0 (C4a), 144.0 (C9a), 153.5 (C3), 155.5 (C6), 158.5 (C8); MS: m/z (%) = 398 (M+1⁺, 25), 384 (M⁺, 30), 300 (10), 285 (45), 275 (10), 265 (13),
233 (17), 220 (50), 214 (10). Anal. calcd. for C_{15}H_{22}N_{6}O_{7} (398): C, 45.22; H, 5.57; N, 21.10. Found: C, 45.00; H, 5.22; N, 21.35%.

9-Ethyl-5,7-dimethyl-3-(D-manno-pentitol-1-yl)-5,9-dihydro-6H-[1,2,4]triazolo[4,3-e]purine-6,8(7H)-dione 10c. Yield 52%; buff crystals, mp 222 °C; IR (KBr) v 3440 (OH), 3242 (NH), 1680, 1650 (C=O) cm⁻¹; ¹H NMR (300 MHz, DMSO-d6) δ 1.25 (t, 3H, C-CH₃), 3.1 (m, 2H, alditolyl 2H), 3.20-3.45 (m, 8H, alditolyl 2H, 2N-CH₃), 3.70-3.90 (2m, 2H, alditolyl 2H), 4.25-4.55 (m, 4H, 2OH, N-CH₂, exchangeable), 4.95 (t, 1H,OH, exchangeable), 5.24, 5.78 (2d, 2H, 2OH, exchangeable); ¹³C NMR (75 MHz, DMSO-d6) δ 23.0 (C-9 CH₂), 25.5 (C7), 35.5 (C5), 37.0 (C9), 61.5-77.5 (C-alditol), 102.0 (C8a), 119.0 (C4a), 145.0 (C9a), 153.5 (C3), 156.5 (C6), 158.0 (C8); MS: m/z (%) = 398 (M+1⁺, 15), 384 (M+, 32), 300 (8), 285 (35), 275 (10), 265 (11), 233 (20), 220 (45), 214 (7). Anal. calcd. for C_{15}H_{22}N_{6}O_{7} (398): C, 45.22; H, 5.57; N, 21.10. Found: C, 45.15; H, 5.45; N, 21.00%.

9-Ethyl-5,7-dimethyl-3-(D-ribo-tetritol-1-yl)-5,9-dihydro-6H-[1,2,4]triazolo[4,3-e]purine-6,8(7H)-dione 10d. Yield 40%; pale yellow crystals, mp 195 °C; IR (KBr) v 3356 (OH), 3235 (NH), 1690, 1635 (C=O ) cm⁻¹; ¹H NMR (300 MHz, DMSO-d6) δ 1.3 (t, 3H, C-CH₃), 3.31-3.61 (m, 10H, alditolyl 4H, 2 N-CH₃), 4.20-4.34 (m, 4H, alditolyl H, OH, N-CH₂, exchangeable), 4.50, 4.85, 5.15 (3d, 3H, 3OH, exchangeable); ¹³C NMR (75 MHz, DMSO-d6) δ 22.5 (C-9 CH₂), 26.0 (C7), 35.0 (C5), 38.0 (C9), 61.0-77.0 (C-alditol), 102.0 (C8a), 118.0 (C4a), 146.0 (C9a), 153.0 (C3), 156.0 (C6), 158.5 (C8); MS: m/z (%) = 369 (M+1⁺, 15), 368 (M+, 30), 320 (8), 280 (35), 255 (10), 235 (11), 220 (45). Anal. calcd. for C_{14}H_{20}N_{6}O_{6} (368): C, 45.65; H, 5.47; N, 22.82. Found: C, 45.90; H, 5.35; N, 22.58%.
9-Ethyl-5,7-dimethyl-3-(D-arabino-tetritol-1-yl)-5,9-dihydro-6H-[1,2,4]triazolo[4,3-e]purine-6,8(7H)-dione 10e. Yield 35%; pale yellow crystals, mp 183 °C; IR (KBr) v 3355 (OH), 3235 (NH), 1695, 1630 (C=O) cm\(^{-1}\); \(^1\)H NMR (300 MHz, DMSO-d6) \(\delta\) 1.5 (t, 3H, C-CH\(_3\)), 3.30-3.60 (m, 10H, alditolyl 4H, 2 N-CH\(_3\)), 4.20-4.35 (m, 4H, alditolyl H, OH, N-CH\(_2\), exchangeable), 4.50, 4.85, 5.16 (3d, 3H, 3OH, exchangeable); \(^{13}\)C NMR (75 MHz, DMSO-d6) \(\delta\) 23.5 (C-9 CH\(_2\)), 26.5 (C7), 35.5 (C5), 38.0 (C9), 61.0-77.5 (C-alditol), 101.0 (C8a), 118.0 (C4a), 145.0 (C9a), 154.0 (C3), 155.0 (C6), 157.5 (C8); MS: m/z (%) = 369 (M+1\(^+\), 20), 368 (M+, 30), 320 (8), 280 (35), 255 (10), 235 (25). Anal. calcd. for C\(_{14}\)H\(_{20}\)N\(_6\)O\(_6\) (368): C, 45.65; H, 5.47; N, 22.82. Found: C, 45.50; H, 5.20; N, 22.49%.

III.4. Antimicrobial assay. Cultures of three bacteria species, namely *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) as well as three fungal species, namely *Aspergillus fumigatus* (AF), *Aspergillus niger* (AN) and *Candida albicans* (CA) were used to investigate the antimicrobial activity of ten products, 9a-e and 10a-e. The antibacterial and antifungal activity assays were carried out in the Microbiology Division of Microanalytical Center of Cairo university, using the diffusion plate method\(^{25,26}\). The latter technique was carried out by pouring a spore suspension of the fungal species (1 cm\(^3\) of sterile water contains approximately 108 conidia) or spreading bacterial suspension over a solidified malt agar medium. The layer was allowed to set for 30 min. A solution of the test compounds (1.0 g = cm\(^3\)) in DMSO was placed onto sterile 5 mm filter paper discs and allowed to dry, then the discs were placed on the center of the malt agar plate and incubated at the optimum incubation temperature, 28±2°C. The
bactericide tetracycline and the fungicide amphotericin B were used as standards under the same conditions. Measurements were performed after 72 h incubation for fungi and 24 h, for bacteria. The results are summarized in the table 2.

IV. CONCLUSION

New purines and their N-alkyl derivatives such as, sugar 7-alkyl-8-theophyllinyl-hydrazones and their acyclo C-nucleosides (5,7,9-trimethyl-3-(D-pentitol and tetritol-1-yl)-5,9-dihydro-6H-[1,2,4]triazolo[4,3-e]purine-6,8(7H)-dione) have been synthesized using both conventional methods and microwave assisted conditions. The microwave methods proved very efficient in reducing reaction times as well as increasing the overall yield of the reactions. Some compounds showed moderate, whereas other compounds showed weak antimicrobial activity. So we recommend further studies on microwave techniques in Taif University to establish the green chemistry.

V. ACKNOWLEDGMENT

The authors are very grateful to Taif University, Taif, KSA, because this work was financial supported by Taif University, Taif, KSA, under project number 1/434/2560.

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