IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 2 Issue 7, July 2015.
www.ijiset.com

ISSN 2348 – 7968

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

Mahmoud A. Amin[a,b], Mosselhi A. M. Mohamed*[a,c] and Mohamed R. E. Aly[a,d]

[a]Department of Chemistry, Faculty of Science, Taif University, 21974-Taif/haweiah, Saudi Arabia, E-
[b]Department of Chemistry, Faculty of Science, Suez Canal University, Ismailia, Egypt, E-mail:
[c]Department of Chemistry, Faculty of Science, Cairo University, 12613-Giza, Egypt,
[d]Department of Chemistry, Faculty of Applied Science, Port Said University, 42522-Port Said, Egypt,

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 **(7,8a-e)** and their acyclo *C*-nucleosides (5,7,9-trimethyl-3-(D-pentitol and tetritol-1-yl)-5,9-dihydro-6*H*-[1,2,4]triazolo[4,3-*e*]purine-6,8(7*H*)-dione) **(9,10a-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 *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) and antifungal activities against three fungal species, namely *Aspergillus fumigatus* (AF), *Aspergillus niger* (AN) and *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.

ISSN 2348 – 7968

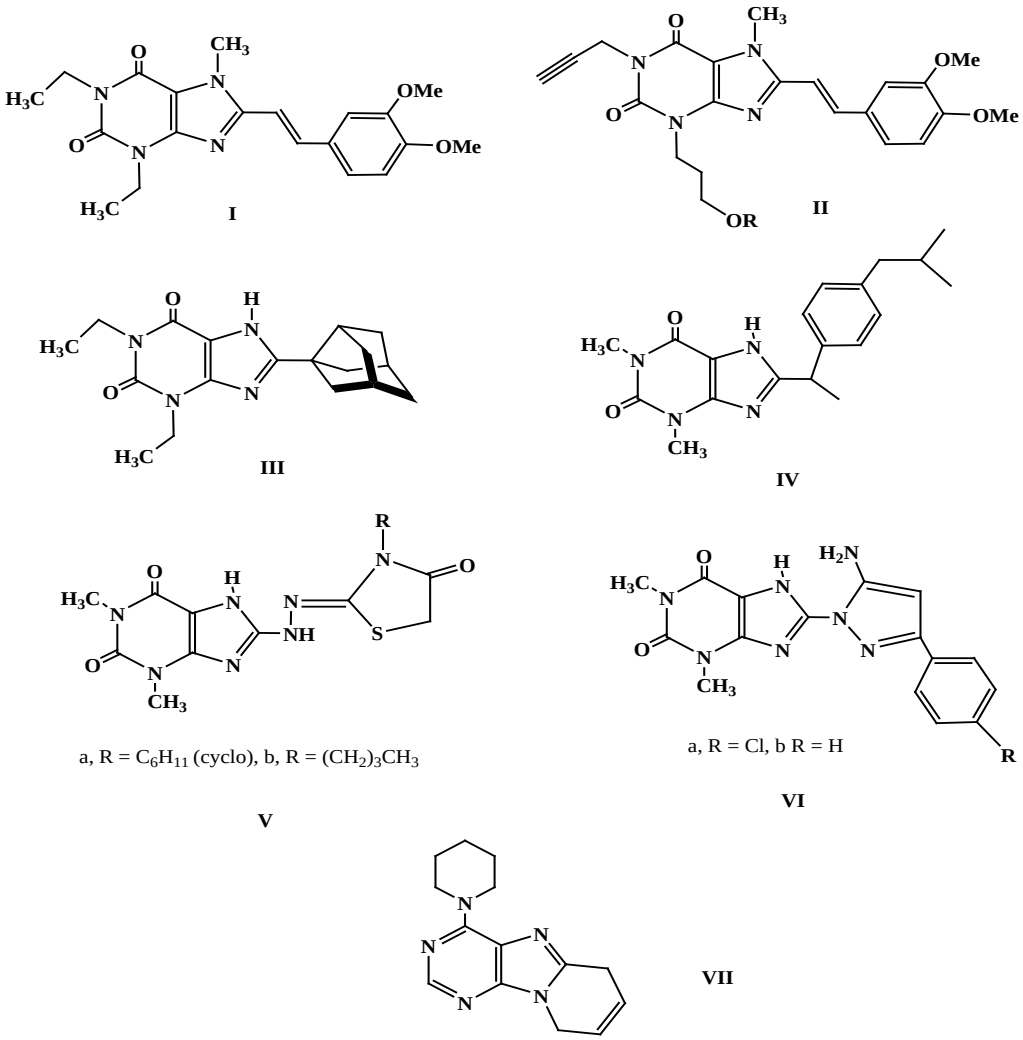
Microwaves are defined as electromagnetic waves with vacuum wavelengths ranging between 0.1 to 100 cm or, equivalently, with frequencies between 0.3- 300 GHz. Microwave dielectric heating uses the ability of some liquids and solids to transform electromagnetic radiation into heat to drive chemical reactions. 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].

ISSN 2348 – 7968

It has been reported that 8-substituted xanthine derivatives and fused purines as examples shown in Scheme, which known as A_{2A} and A_{2B}-selective antagonists and anticancer agents are synthesized by microwave technique and discovered[10-13].



Scheme: Purines as A_{2A} and A_{2B}-selective antagonists and anticancer agents

II. RESULTS AND DISCUSION

II.1. CHEMISTRY

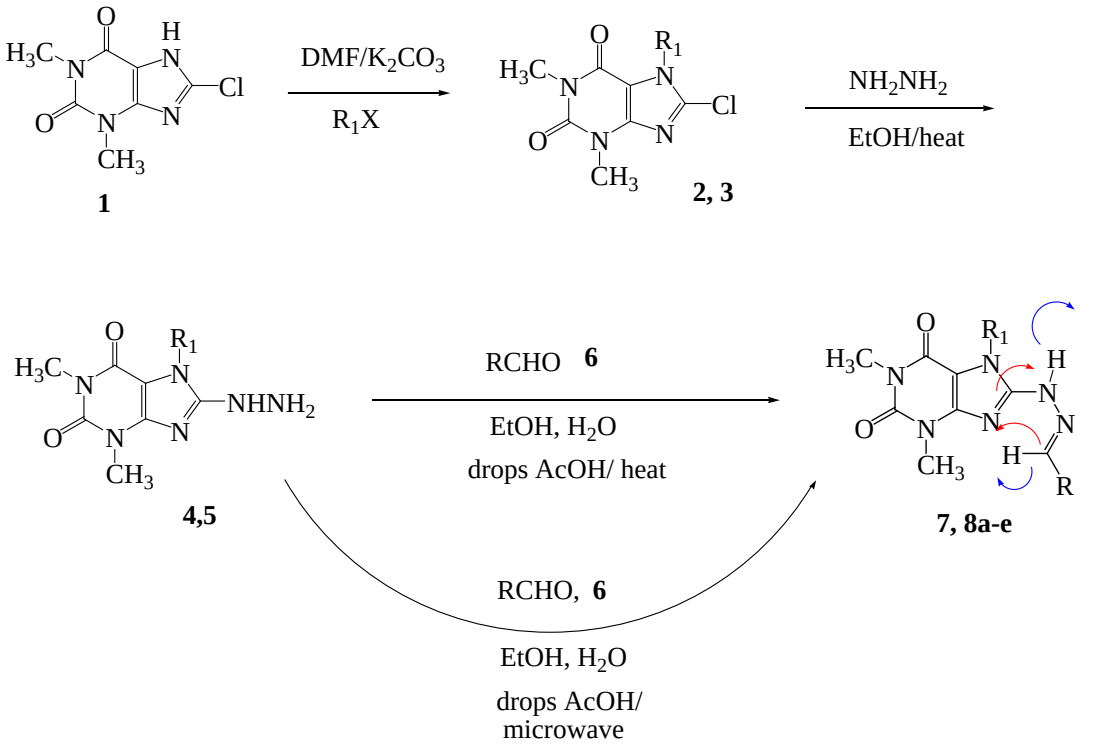
The required starting 8-hydrazinocaffeine **4** and 7-ethyl-8-hydrazinotheophyllines **5** have been obtained according to the reported literature[14]. They were prepared by alkylation of 8-chlorothephylline **1** with iodomethane in DMF containing potassium carbonate to afford 8-

ISSN 2348 – 7968

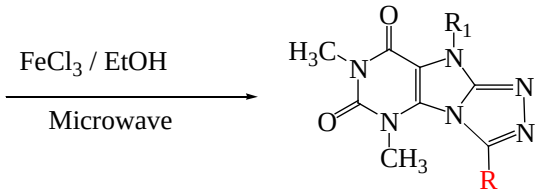
chlorocaffeine **2** and 8-chloro-7-ethylheophylline **3**[15], respectively, followed by treatment of **2** and **3** with hydrazine hydrate in boiling ethanol. In our previous paper[16], it was reported that the condensation of equimolar amounts of 7-ethyl-8-hydrazinotheophylline **5** with aldohexoses such as, D-glucose **6a**, D-galactose **6b**; D-mannose **6c**, and aldopentoses, such as D-ribose **6d**, D-arabinose **6e,** 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) **8a-e** (Scheme 1). By the same manner, the hydrazone derivatives **7a-e** were synthesized by reaction of 8-hydrazinocaffeine **4** with aldohexosesand aldopentoses **6**. The structures of obtained hydrazones **7a-e** and **8a-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 **7a-e** and **8a–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.

ISSN 2348 – 7968



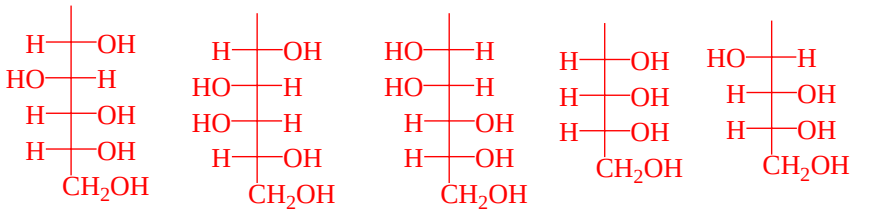
1 **2, 3** **4,5** **7, 8a-e**



9,10a-e

	R_1
2,4,7,9	CH_3
3,5,8,10	CH_2CH_3

6,7-10a-e, R =



D-Glucose (a) D-Galactose (b) D-Mannose (c) D-Ribose (d) D-Arabinose (e)

Scheme 1: Microwave assisted synthesis of purine hydrazones and their C-Nucleosides

ISSN 2348 – 7968

Table 1: The benefits of using microwave conditions for the synthesis of the above-mentioned compounds **7,8a-e**.

Compound no.	Reaction Yield %		Reaction Time	
	Microwave	Conventional Method	Microwave	Conventional Method
7a	78	60	15 min	3 h
7b	80	63	15 min	3 h
7c	80	70	15 min	3 h
7d	60	40	15 min	5 h
7e	65	50	15 min	6 h
8a	80	70	10 min	4 h
8b	85	73	10 min	4 h
8c	86	78	10 min	4 h
8d	70	55	10 min	6 h
8e	65	55	10 min	5 h

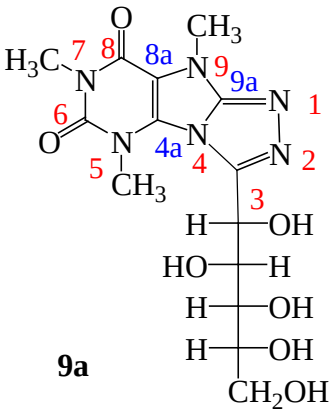
Thermal attempt of the dehydrogenative cyclization of the formed sugar hydrazones **7a-e** and **8a-e** with bromine in acetic acid/sodium acetate[17], ferric chloride in ethanol[18,19], copper dichloride in dimethylformamide[20], bromine in water[21] or in acetic acid[22], bromine in acetic acid, sodium 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

ISSN 2348 – 7968

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 δ = 3.17, 3.31, 3.42 for **9a-e**, and an ethyl group signals at δ = 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 δ = 3.20-4.06 (CH protons) and 4.20-5.79 (OH protons).

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



9a

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

ISSN 2348 – 7968

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*.

ISSN 2348 – 7968

Table 2. Antibacterial and Antifungal Activities of some of the Synthesized Compounds.

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

Compound No.	(EC) G^-	(PA) G^-	(SA) G^+	(AF) Fungus	(AN) Fungus	(CA) Fungus
Control: DMSO	0.0		0.0	0.0		0.0
Tetracycline Antibacterial agent	28 +++	28 +++	26 +++	-	-	-
Amphotericin B Antifungal agent	-	-	-	16 ++	15 ++	15 ++
9a	11 ++	13 ++	12 ++	0.0 -	0.0 -	0.0 -
9b	11 ++	15 ++	16 ++	0.0 -	0.0	11 ++
9c	11 ++	15 ++	15 ++	0.0 -	0.0	11 ++
9d	9 +	14 ++	14 ++	0.0 -	0.0 -	0.0 -
9e	10 +	9 +	9 +	0.0 -	0.0 -	0.0 -
10a	9 +	10 +	11 ++	0.0 -	0.0 -	0.0 -
10b	12 ++	11 ++	12 ++	0.0 -	0.0 -	0.0 -
10c	11 ++	10 +	11 ++	0.0 -	0.0 -	10 +
10d	11 ++	15 ++	11 ++	0.0 -	0.0 -	10 +
10e	10 +	9 +	11 ++	0.0 -	0.0 -	0.0 -

*IZD = 2-10 mm beyond control = + (low activity).

IZD = 11-24 mm beyond control = ++ (moderate activity).

IZD = 25-35 mm beyond control = +++ (high activity)

ISSN 2348 – 7968

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-d6 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-hydrazinotheophylline **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

ISSN 2348 – 7968

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**, **9 a-e, 10a-e** are listed below:

D-Glucose-7-mehyl-8-theophylinylhydrazone 7a (D-Glucose-8-caffeinylhydrazone 7a). Yield 60%; white crystals, mp 228-230 ^{o}C; IR (KBr) v 3446 (OH), 3248 (NH), 1697, 1655 (C=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d6) δ 3.02 (m, 2H, alditolyl 2H), 3.17-3.48 (m, 11H, alditolyl 2H, 3N-CH_3), 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); ^{13}C 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 $C_{14}H_{22}N_6O_7$ (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-caffeinylhydrazone 7b). Yield 63%; white crystals, mp 190-193 ^{o}C; IR (KBr) v 3462 (OH), 3235 (NH), 1696, 1629 (C=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-*d*6) δ 3.18 (s, 3H, N-CH_3), 3.24-3.41 (m, 5H, alditolyl 2H, N-CH3), 3.45 (s, 3H, N7-CH_3), 3.53, 3.83 (2m., 4H, alditolyl 4H), 4.23-4.28 (m, 1H, OH, exchangeable), 4.47 (t, 1H, OH, exchangeable), 4.69,

ISSN 2348 – 7968

5.21, 5.74 (3d, 3H, 3OH, exchangeable), 7.54 (d, 1H, N=CH), 8.46 (s,1H, exchangeable, =N-NH-); ^{13}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 $C_{14}H_{22}N_6O_7$ (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-caffeinylhydrazone 7c). Yield 70%; yellowish white crystals, mp 216-218^{o}C; IR (KBr) v 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}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 $C_{14}H_{22}N_6O_7$ (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-caffeinylhydrazone 7d). Yield 40%; white crystals, mp 115-116 ^{o}C; IR (KBr) v 3356 (OH), 3235 (NH), 1699, 1638 (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), 7.46 (d, 1H, N=CH), 10.90 (s, 1H, exchangeable, =N-NH-); ^{13}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

ISSN 2348 – 7968

(%) = 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 $C_{13}H_{20}N_6O_6$ (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 ^oC; IR (KBr) v 3356 (OH), 3208 (NH), 1688, 1655 (C=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d6) δ 3.19 (s, 3H, N-CH3), 3.31-3.58 (m, 10H, alditolyl 4H, 2N-CH3), 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, =N-NH-); ^{13}C NMR (75 MHz, DMSO-d6) δ 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 $C_{13}H_{20}N_6O_6$ (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:

ISSN 2348 – 7968

5,7,9-trimethyl-3-(D-gluco-pentitol-1-yl)-5,9-dihydro-6*H*-[1,2,4]triazolo[4,3-*e*]purine-6,8(7*H*)-dione 9a. Yield 60%; pale yellow crystals, mp 185 ^{o}C; IR (KBr) v 3440 (OH), 3240 (NH), 1680, 1650 (C=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d6) δ 3.02 (m, 2H, alditolyl 2H), 3.17-3.48 (m, 11H, alditolyl 2H, 3N-CH_3), 3.60-3.90 (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); ^{13}C NMR (75 MHz, DMSO-d6) δ 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 $C_{14}H_{20}N_6O_7$ (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-6*H*-[1,2,4]triazolo[4,3-*e*]purine-6,8(7*H*)-dione 9b. Yield 49%; buff crystals, mp 224-226 ^{o}C; IR (KBr) IR (KBr) v 3442 (OH), 3245 (NH), 1685, 1653 (C=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d6) δ 3.05 (m, 2H, alditolyl 2H), 3.19-3.50 (m, 11H, alditolyl 2H, 3N-CH_3), 3.60-3.90 (2m, 2H, alditolyl 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) δ 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 $C_{14}H_{20}N_6O_7$ (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-6*H*-[1,2,4]triazolo[4,3-*e*]purine-6,8(7*H*)-dione 9c. Yield 50%; buff crystals, mp 218-219 ^{o}C; IR (KBr) IR (KBr) v 3441(OH), 3245 (NH), 1688, 1655

ISSN 2348 – 7968

(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 $C_{14}H_{20}N_6O_7$ (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-6*H*-[1,2,4]triazolo[4,3-*e*]purine-6,8(7*H*)-dione 9d. Yield 40%; pale yellow crystals, mp 202-203 ^{o}C; 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 $C_{13}H_{18}N_6O_6$ (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-6*H*-[1,2,4]triazolo[4,3-*e*]purine-6,8(7*H*)-dione 9e. Yield 40%; buff crystals, mp 190-191 ^{o}C; 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);

ISSN 2348 – 7968

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 $C_{13}H_{18}N_6O_6$ (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-6*H*-[1,2,4]triazolo[4,3-*e*]purine-6,8(7*H*)-dione 10a. Yield 45%; colorless crystals, mp 204 ^oC; IR (KBr) v 3440 (OH), 3242 (NH), 1682, 1650 (C=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d6) δ 1.3 (t, 3H, C-CH_3), 3.10 (m, 2H, alditolyl 2H), 3.15-3.45 (m, 8H, alditolyl 2H, 2N-CH_3), 3.60-3.90 (2m, 2H, alditolyl 2H), 4.22-4.55 (m, 4H, 2OH, N-CH_2, exchangeable), 4.90 (t, 1H,OH, exchangeable), 5.20, 5.75 (2d, 2H, 2OH, exchangeable); ^{13}C NMR (75 MHz, DMSO-d6) δ 22.5 (C-9 CH_2), 25.5 (C7), 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 $C_{15}H_{22}N_6O_7$ (398): C, 45.22; H, 5.57; N, 21.10. Found: C, 44.90; H, 5.20; N, 21.50%.

9-Ethyl-5,7-dimethyl-3-(D-galacto-pentitol-1-yl)-5,9-dihydro-6*H*-[1,2,4]triazolo[4,3-*e*]purine-6,8(7*H*)-dione 10b. Yield 55%; colorless crystals, mp 215 ^oC; IR (KBr) v 3440 (OH), 3242 (NH), 1682, 1650 (C=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d6) δ 1.25 (t, 3H, C-CH_3), 3.0 (m, 2H, alditolyl 2H), 3.18-3.45 (m, 8H, alditolyl 2H, 2N-CH_3), 3.65-3.90 (m, 2H, alditolyl 2H), 4.20-4.55 (m, 4H, 2OH, N-CH_2, exchangeable), 4.95 (t, 1H,OH, exchangeable), 5.24, 5.78 (2d, 2H, 2OH, exchangeable); ^{13}C NMR (75 MHz, DMSO-d6) δ 23.0 (C-9 CH_2), 25.0 (C7), 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),

ISSN 2348 – 7968

233 (17), 220 (50), 214 (10). Anal. calcd. for $C_{15}H_{22}N_6O_7$ (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-6*H*-[1,2,4]triazolo[4,3-*e*]purine-6,8(7*H*)-dione 10c. Yield 52%; buff crystals, mp 222 ^{o}C; IR (KBr) v 3440 (OH), 3242 (NH), 1680, 1650 (C=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d6) δ 1.25 (t, 3H, C-CH_3), 3.1 (m, 2H, alditolyl 2H), 3.20-3.45 (m, 8H, alditolyl 2H, 2N-CH_3), 3.70-3.90 (2m, 2H, alditolyl 2H), 4.25-4.55 (m, 4H, 2OH, N-CH_2, exchangeable), 4.95 (t, 1H,OH, exchangeable), 5.24, 5.78 (2d, 2H, 2OH, exchangeable); ^{13}C NMR (75 MHz, DMSO-d6) δ 23.0 (C-9 CH_2), 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 $C_{15}H_{22}N_6O_7$ (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-6*H*-[1,2,4]triazolo[4,3-*e*]purine-6,8(7*H*)-dione 10d. Yield 40%; pale yellow crystals, mp 195 ^{o}C; IR (KBr) v 3356 (OH), 3235 (NH), 1690, 1635 (C=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d6) δ 1.3 (t, 3H, C-CH_3), 3.31-3.61 (m, 10H, alditolyl 4H, 2 N-CH_3), 4.20-4.34 (m, 4H, alditolyl H, OH, N-CH_2, exchangeable), 4.50, 4.85, 5.15 (3d, 3H, 3OH, exchangeable); ^{13}C NMR (75 MHz, DMSO-d6) δ 22.5 (C-9 CH_2), 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 $C_{14}H_{20}N_6O_6$ (368): C, 45.65; H, 5.47; N, 22.82. Found: C, 45.90; H, 5.35; N, 22.58%.

ISSN 2348 – 7968

9-Ethyl-5,7-dimethyl-3-(D-arabino-tetritol-1-yl)-5,9-dihydro-6*H*-[1,2,4]triazolo[4,3-*e*]purine-6,8(7*H*)-dione 10e. Yield 35%; pale yellow crystals, mp 183 ^{o}C; IR (KBr) v 3355 (OH), 3235 (NH), 1695, 1630 (C=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d6) δ 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) δ 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 $C_{14}H_{20}N_6O_6$ (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 cm3 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

ISSN 2348 – 7968

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-6*H*-[1,2,4]triazolo[4,3-*e*]purine-6,8(7*H*)-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.

VI. REFERENCES

[1] Sekhon, B. S.,"'Microwave-Assisted Pharmaceutical Synthesis: An Overview", Int. J. Pharm Tech Res., vol. 2, pp. 827- 833, 2010.

[2] Kappe, C. O.. “Controlled microwave heating in modern organic synthesis” Angew. Chem. Int. Ed., vol. 43, pp. 6250- 6284, 2004.

[3] Kappe, C. O.; Stadler, A. "Microwaves in organic and medicinal chemistry", Wiley-VCH, 2005.

ISSN 2348 – 7968

[4] Gregg, B.; Golden, K. and Quinn, J.; Indium(III) Trifluoromethanesulfonate as an Efficient Catalyst for the Deprotection of Acetals and Ketals, J. Org. Chem., vol. 72, pp. 5890 – 5893, 2007.

[5] Lerebours, R.; Wolf, C., alladium(II)-Catalyzed Conjugate Addition of Arylsiloxanes in Water, Org. Lett., vol. 9, pp. 2737-2740, 2007.

[6] Marion, N.; Gealageas, R. and Nolan, S.; [(NHC)Au^{I}]-Catalyzed Rearrangement of Allylic Acetates, Org. Lett., vol. 9, pp. 2653-2656, 2007.

[7] Palle, S., M. Sc. Thesis, Studies directed towards synthesis of azoles and its derivatives, *Jawaharlal Nehru Technological* University, Indian, pp.57-117, 2012.

[8] Zhong, Q.-F.; Li-Ping Sun, Li.-P; An efficient synthesisof 6,9-disubstitutedpurin-8-onesviacopper-catalyzed coupling/ cyclization, Tetrahedron, vol. 66, pp. 5107- 5111, 2010.

[9] Fhid , O.; Zeglam, T.; Masoud Alag, A.; Almog, T.; Tri- and Tetra- Cyclic Theophylline Derivatives as a Potential Agents Acting on CNS and CVS: An Overview, Int.l J. Pharm. & Biol. Arch., vol. 3, pp. 507-512, 2012.

[10] Burbiel, J. C.; Hockemeyer, J.; and Müller, C. E.; Microwave-assisted ring closure reactions: Synthesis of 8-substituted xanthine derivatives and related pyrimido- and diazepinopurinediones, Bei. J. Org. Chem., vol. 2, pp.1-7, 2006.

[11] Berk, B.; Akgün, H.; Erol, K.; Sırmagül, B.; Gao, Z. G.; and Jacobson, K. A.; New 8-substituted xanthine derivatives as potent bronchodilators, Il Farmaco, vol. 60, pp. 974-980, 2005.

[12] Rida, S. M.; Ashour, F. A.; El-Hawash, S. A. M.; El-Semary, M. M.; and Badr, M. H.; Synthesis of Some Novel

ISSN 2348 – 7968

Substituted Purine Derivatives As Potential Anticancer, Anti-HIV-1 and Antimicrobial Agents, Arch. Pharm. Chem. Life Sci., vol. 340, pp.185-194, 2007.

[13] Marzouk, V. H. R.; Hennum, M.; Gundersen, L.-L.; Efficient synthesis of cytotoxic pyrido[1,2-*e*]purines from purines employing direct C-allylation and RCM–oxidation as key steps, Tetrahedron Lett., vol. 54, pp. 3437–3439, 2013.

[14] Damiens, R.; Delaby, R., Sur quelques propriétés de la chloro-8 théophylline et de ses derives, Bull. Soc. Chim. Fr., pp. 888-895, 1955.

[15] Mosselhi, M. A. N.; Abbass, I. M,. Synthesis and properties of 8-nitro-7-alkylated theophylline derivatives, Bull. Polish Acad. Sci, vol. 41, pp.179-185, 1993.

[16] Mosselhi, M. A.N.; Abdallah, M. A.; Metwaly, N. H.; Ibrahim A. Eldesouky, I. A.; and Break, L. B.; Synthesis and Antimicrobial Activity of Sugar hydrazones of 7- ethyl-8-hydrazinotheophylline, Arkivoc, vol. xiv, pp. 53-63, 2009.

[17] Shaban, M. A.; Taha, M. A. M.; Morgaan, A. E. A., Sterically Controlled Regiospecific Cyclization of Aldose-5-ethyl-1,2,4-triazino[5,6-*b*]indol-3-ylhydrazones to Linearly Annelated 3-Polyhydroxyalkyl-10-ethyl-1,2,4-triazolo-[4′,3′:2,3]-1,2,4-triazino-[5,6-*b*]indoles, Monatsh. Chem., vol. 131, pp. 487- 500, 2000.

[18] Rashed, N.; Ibrahim, E. I.; El-Ashry, E. S. H.;, Sugar (lepidin-2-yl)hydrazones and synthesis of 1-(alditol-1-yl)-5-methyl[1,2,4]triazolo[4,3-*a*]quinoline, Carbohydrate Res., vol. 254, pp. 295- 300, 1994.

[19] Shawali, A. S.; Hassaneen, H. M.; Shurrab, N. K.; A new strategy for the synthesis of pyrazolo[4,3-*e*][1,2,4]triazolo[4,3-

c]pyrimidines and pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines, Tetrahedron, vol, 64, pp. 10339-10343, 2008.

[20] Ciesielski, M.; Pufky, D.; Doering, M.; A convenient new synthesis of fused 1,2,4-triazoles: the oxidation of heterocyclic hydrazones using copper dichloride, Tetrahedron, vol. 61, pp. 5942-5947, 2005.

[21] Shaban, M. A.; Taha, M. A. M.; Nasr, A. Z.; Morgaan, A. E. A.; Synthesis of acyclo C-nucleosides: 3-Alditol-1-yl)-5-methyl-7-oxo-1,2,4-triazolo[4,3-*a*]pyrimidines, Pharmazie, vol. 50, pp. 784-788, 1995.

[22] Shaban, M. A.; Taha, M. A. M.; Nasr, A. Z.; Synthesis of acyclo C-nucleosides: 3-(Alditol-1-yl)-6,7-diphenyl-1,2,4-triazolo[4,3-*b*][1,2,4]triazines, Pharmazie, vol. 51, pp. 707- 710, 1996.

[23] Taha, M. A. M.; El-Badry, S. M.; Synthesis of 1,3,4-Oxadiazole Acyclo *C*-Nucleosides Bearing 5-Methylthio{7-substituted-1,2,4-triazolo[1,5-*d*]tetrazol-6-yl}moieties‡ J. Chin. Chem. Soc., vol. 53, pp. 1181- 1188, 2006.

[24] Mosselhi, M. A. N.; Tawfik, N. M.; Shawali, A. S.; New [e]-Fused Caffeines: A Simple Synthesis of 3-Substituted [1,2,4]Triazolo[4,3-e]purines, Monatsh. Chem, vol. 134, pp. 565-571, 2003.

[25] Pfaller, M. A.; Burmeister, L.; Bartlett, M. A.; and Rinaldi, M. G.; "Multicenter evaluation of four methods of yeast inoulum preparation", J. Clin. Microbiol., vol. 26, pp. 1437-1441, 1988.

[26] Matar, M. J.; Ostrosky-Zeichner, L.; Paetznick, V. L.; Rodriguez, J. R.; Chen, E.; and Rex, J. H.; "Correlation between E-test, disk diffusion andmicrodilution methods for antifungal susceptibility testing of fluconazoleand voriconazole",

ISSN 2348 – 7968

Antimicrob. Agents Chemother., vol. 47, pp. 1647-1651, 2003.