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Synthesis and Antimicrobial Studying of Some New Formazan Derivatives from (8-Chlorotheophylline)

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Abstract

Some novel derivatives of 8-Chlorotheophylline (8-CTh) synthesized by Schiff base reaction (2-5). Synthesized by react 8-Chlorotheophylline with hydrazine to give 8-hydrazinyl-1,3-dimethyl-*1H*-purine-2,6(*3H,7H*)-dione then react with some substituted aldehyde to give imine compound. In other hand this compound reacts with azo compound give Formazan compound. The reaction was monitored by thin-layer chromatography (TLC) technique. All new compounds were characterized by melting points, elemental analysis, FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy. The antibacterial activity of these derivatives was also determined.

Keyword: 8-Chlorotheophylline, Formazan, Schiff base, antibacterial, Xanthine ,azo compound

INTRODUCTION

Xanthine a purine base produces in most human body tissues and fluids and in other organisms [1] it is produced by purine metabolism. Caffeine and Theophylline. They naturally occurring in methyl xanthine, are the classical adenosine receptor antagonists[2] Caffeine is widely consumed in beverages, theophylline is used as a drug in the treatment of bronchial asthma and several other xanthine's derived from caffeine and theophylline are therapeutically used as analeptics[3], antiasthmatics [4], vasodilators[5], antihypertensive[6] and diuretics[7]. Theophylline and its salts are used in the therapy of bronchial asthma and chronic obstructive pulmonary disease (COPD)[9], 8-CTh is a purine derivative which is obtained from theophylline. In therapy, 8-CTh is used in combination with antihistamines in order to enhance their action against travel sickness and other hearing labyrinth diseases[10].In other hand Formazans have been found to possess important medical applications which contain the typical chain of atoms N=N–C=N–NH, have been found to possess important medical applications due to their various activities [11] such as antimicrobial [12], analgesic [13], antifungal [14], anticancer, anti-HIV [15], etc. Several formazans showed promising anti-fertility[16], anti-parkinsonian [16] and anticonvulsant activities. In this work prepared some derivatives of formazan by reacting azo compound with 8-CTh.

EXPERIMENTAL

All materials were of highest purity and supplied by Merck, Sigma Aldrich and Fluka- company. Melting points were measured on a Buchi melting point apparatus B-545 (Buchi Labortechnik AG, Switzerland). Microanalytical data were obtained with a Vario, Elementar apparatus (Shimadzu, Japan). The IR spectra were recorded on Schimadzu Fourier Transform Infra-red spectrophotometer (Model 270), using KBr discs. NMR spectra were recorded on 600 MHz (^{1}H) and at 100 MHz and (^{13}C) spectrometers (Bruker, Germany) with TMS as the internal standard and on δ scale in ppm. (TLC) was performed on silica gel for (TLC) and spots were visualized by Iodine vapors. The reagents used were of analytical grade while the solvents were purified before use.

Preparation of 8-hydrazinyl-1,3-dimethyl-*1H*-purine-2,6(*3H,7H*)-dione In Round flask of 100 ml it was dissolve (0.001 mol / 0.21 g) of 8-Cth in 10 ml of hydrazine and stir the mixture in the ice bath (0-5°C). For 3 hours and then remains stirring for 7 hours at room temperature, after that add 1 ml of tri ethyl amine and reflux for 9 hours. Monitored reaction by TLC using the solution (methanol: dichloromethane) (4.5: 0.5) after which the solution was neutralized by 10% HCl solution and then washing the precipitate with distilled water, ether and then returned crystallized with ethanol. Yield: (81%) as a white solid. ***m.p*** = 176-178 °C. ***Rf*** =0.44 **.FT-IR (KBr, cm^{-1})**: (3244) NH-NH_2, (3383) N-H, (2887, 2980, 3140) C-$H_{ali.}$, (1716) C=O, (1637) C=C (1530) C=N.**^{1}H NMR (DMSO-*d*6)**:□□ = 3.17 (s, 3H, N_1-CH_3), 3.33 (s, 3H, N_2-CH_3), 7.52 (br., s ,1H, N_9H), 10.43 (s, 1H, N_{10} NH), 9.68, 9.71 (d, 2H, NH_2, *J* = 5.6 Hz). **^{13}C NMR(DMSO-*d*6):** □= 27.7 (N_1-CH_3), 29.9 (N_3-CH_3), 114.1 (C_5), 148.0 (C_4),150.9 (C=O $_2$), 153.1 (C=O $_6$), 164.1 (C_8). **Anal. calc**. for $C_7H_{10}N_6O_2.H_2O$ (228.10): C 36.48, H 5.30, N 36.83. Found: C 36.68, H 5.21, N 36.70.

General method to preper Schiff bases derivatives drevatives (2-6)

In Round flask of 100 ml it was dissolved 1 (0.0035) in 10 ml of ethanol then refluxed with various substituted aromatic aldehydes (0.0035 mol) then add drops of glacial acetic acid for two hours and monitored reaction by TLC using the solution (methanol: dichloromethane) (9:1) after the reaction is neutralized by tri ethyl amine. the precipitate washing with distilled water, ether and then returned crystallized with ethanol. (18)

Preparation of 8-(2-(4-bromobenzylidene) hydrazinyl)-1,3-dimethyl-*1H*-purine-2,6(3H,7H)-dione (2)

was prepared using the same method described for the preparation of compounds (2-5) by Mix (210 mg) from 1 with p-Bromo benzaldehyde (185 mg): Yield (74%) as a yellow solid. **m.p** = 165-166 °C R_f = 0.57 **.FT-IR (KBr, ν, cm^{-1})** : (3407) N-H, (1604) C=C_{arom}. (3050) C-H_{arom}. (1500) C=N, (1008) C-Br.**^{1}H NMR (DMSO-*d*6)**: □ = 3.24 (s, 3H, N_1-CH_3), 3.40 (s, 3H, N_2-CH_3), 9.24 (s ,1H, N_9H), 14.50 (s, br. 1H, N_{10}H), 7.73-7.74 (d, 1H, H2´+H6´ *J* = 8.1 Hz), 7.83-7.85 (d, 1H, H3´+H5´ *J* = 8.1 Hz), 8.71 (s, 1H, H_{12}). **^{13}C NMR (DMSO-*d*6):**□□ = 28.2 (N_1-CH_3), 30.3 (N_3-CH_3), 111.2 (C5), 124.7 (C4´), 130.7 (C2´+C6´), 132.5 (C3´+C5´), 133.4 (C1´), 138.5 (C12), 146.9 (C4), 151.1 (C=O 2), 153.7 (C=O 6), 160.9 (C8).**Anal. calc.** for $C_{14}H_{13}BrN_6O_2$ (377.20): C 44.58, H 3.47, N 22.28. Found: C 44.32, H 3.32, N 22.09.

Preparation8-((E)-2-(4-chlorobenzylidene) hydrazinyl)-1,3-dimethyl-*1H*-purine-2,6(*3H,7H*)-dione (3)

was prepared using the same method described for the preparation of compounds (2-5) by mix (210 mg) from 1 with p-Chloro benzaldehyde (140 mg). Yield: (77%) as a yellow solid **m.p**= 208-209 °C, **R_f** = 0.62.**FT-IR (KBr, ν, cm^{-1})** : (3410) N-H, (1620) C=C_{arom}. (3100) C-H_{arom}. (1520) C=N, (750) C-Cl.**^{1}H NMR (DMSO-*d*6):** □ = 3.13 (s, 3H, N_1-CH_3), 3.63 (s, 3H, N_2-CH_3), 9.22 (s ,1H, N_9H), 10.00 (s, br. 1H, N_{10} NH), 7.90-7.92 (d, 1H, H2´+H6´ *J* = 8.5 Hz), 7.58-7.61 (d, 1H, H3´+H5´ *J* = 8.5 Hz), 8.72 (s, 1H, H_{12}). **^{13}C NMR (DMSO-*d*6):**□□=28.5 (N_1-CH_3), 29.7 (N_3-CH_3), 111.0 (C5), 129.6 (C3´+C5´), 130.5 (C2´+C6´),

134.7 (C1´), 136.5 (C4´), 138.2 (C12), 147.2 (C4), 152.1 (C=O 2), 153.2 (C=O 6), 161.0 (C8). **Anal. calc.** for $C_{14}H_{13}ClN_6O_2$ (332.75): C 50.53, H 3.94, N 25.26. Found: C 50.31, H 3.83, N 25.07.

Preparation 8-2-(4-dimethylaminbenzylidene) hydrazinyl)-1,3-dimethyl-*1H*-purine-2,6(*3H,7H*)-dione (4)

was prepared using the same method described for the preparation of compounds (2-5) by mix (210 mg) from 1 with dimethylamino benzaldehyde (149 mg). Yield: (80%) as a greensh yellow solid **m.p** = 162-163 ^oC , $\mathbf{R_f}$ = 0.38.**FT-IR (KBr, ν, cm^{-1})** : (3413) N-H, (1640) C=C_{arom}. (3018) C-H_{arom}. (1505) C=N.**^{1}H NMR (DMSO-*d6*)**: □ = 3.08 (NMe_2), 3.22 (N1-CH_3), 3.36 (N3-CH_3), 6.93-6.96 (H3'+H5'), 7.42-7.45 (H2'+H6'), 8.02 (N=CH), 8.44 (N_9H),13.69 (N_{10}H).**^{13}C NMR (DMSO-*d6*)**□□□ 27.3 (N1-CH3), 29.4 (N3-CH3), 41.4 (C4-N-(CH_3)$_2$), 112.8 (C3'+5'),116.6 (C5), 123.4 (C1'), 128.73-128.78 (C2'+6'), 138.1 (Ca), 147.0 (C4), 150.7 (C=O 6), 152.4 (C=O 2), 155.3 (C4'), 167.8 (C8).**Anal. calc.** for $C_{16}H_{19}N_7O_2$ (341.37): C 56.29, H 5.61, N 28.72. Found: C 56.06, H 5.53, N 28.

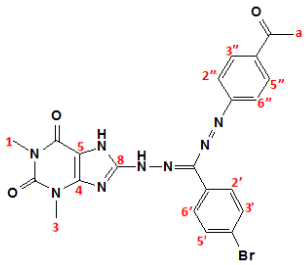
Preparation8-((E)-2-(4-hydroxylbenzylidene) hydrazinyl)-1,3-dimethyl-*1H*-purine-2,6(*3H,7H*)-dione (5).

was prepared using the same method described for the preparation of compounds (2-5) by Mix (210 mg) from 1 with P-hydroxybenzaldehyde (122 mg). Yield: (69 %) a Greenish yellow solid. **m.p** = 168-169 ^oC, $\mathbf{R_f}$ = 0.57.**FT-IR (KBr, ν, cm^{-1})** : (3420) N-H, (1633) C=C_{arom}. (3059) C-H_{arom}. (1544) C=N, (3300) O-H.**^{1}H NMR(DMSO-*d6*)** □ = 3.19 (s, 3H, N_1-CH_3), 3.52 (s, 3H, N_2-CH_3), 9.25 (s ,1H, N_9H), 10.10 (s, 1H, N_{10} NH), 7.69-7.70 (d, 1H, H2´+H6´), 6.87-6.88 (d, 1H, H3´+H5´), 8.56 (s, 1H, H_{12}), 9.67 (s,1H, OH). **^{13}C-NMR□(DMSO-*d6*)**□□ = 27.4 (N_1-CH_3), 29.8 (N_3-CH_3), 109.6 (C5), 115.7-115.8 (C3´+C5´), 125.1 (C1´), 129.6-130.0 (C2´+C6´), 137.7 (C12), 147.7 (C4) 151.7 (C=O 2), 154.3 (C=O 6), 160.3 (C8), 163.3 (C4´). **Anal. calc.** for $C_{14}H_{14}N_6O_3$ (314.11): C 53.50, H 4.49, N 26.74. Found: C 53.31, H 4.35, N 26.52.

General method to prepare drevatives

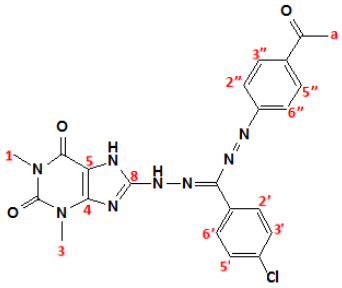
In Round flask of 100 ml it was dissolved from imine compound **(2-6)** (10) mmol and 10 mmol prepared diazinum salt and add 20 ml of pyridine in the presence of sodium acetate in ice bath (0-5 °C) and stirring for 3 hrs. then add 25ml of cold water on the mixture with continue of stirring, the reaction was monitored by TLC using the solution (methanol: dichloromethane) (9:1) the precipitate washing with distilled water, ether and then returned crystallized with ethanol.(19)

Preparation of (4-acetylphenyl)-3-(4-bromophenyl)-1-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-*1H*-purin-8-yl) formazan.



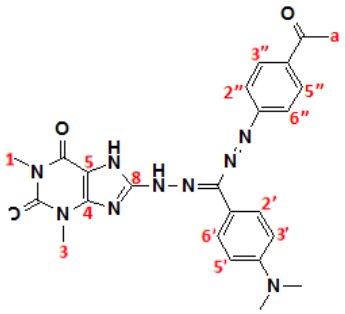
This compound was prepared by using the same method described for the preparation of compounds (7-12) by Mixing (377mg) from 1 with 4-acetylphenyl diazanium chloride (182 mg). Yield: (89 %) a brown yellow solid. **m.p** = 170-172 ^oC $\mathbf{R_f}$ = 0.29.**FT-IR (KBr, ν, cm^{-1}):** (3325**)** N-H, (3001,2927,2823) C-H_{ali}., (3047) C-$H_{arom,}$ (1581) C=N, (1604) C=C_{arom}, (1201) C-O-C, (1395,1437) N=N, (1006) C-Br.**^{1}H-NMR(DMSO-*d6*)** □□ = 2.65 (s, br. Ha), 3.20 (s, 3H, N_1-CH_3), 3.34 (s, 3H, N_3-CH_3), 7.22-7.27 (d, 2H, H2"+H6"), 7.33-7.34 (d, 2H, H3´+H5´), 7.91-7.93 (d, 2H, H3"+H5"), 10.07 (s, 1H, N_9H). 13.02 (s, 1H, N_{10}H). **^{13}C-NMR(DMSO-*d6*)** □□= 26.5 (Cc), 27.4 (N1-CH3), 29.4 (N3-CH3), 112.7 (C5), 125.1 (C4'), 127.2 (C2'+6'), 128.7-128.73 (C2"+C6"), 128.92-128.97 (C3"+5"), 131.8 (C3'+5'), 133.6 (C1'),137.0 (C4"), 149.9 ((C4), 151.002 (C=O6), 151.006 (C=O 2), 153.5 (C1"), 156.01 (Ca), 167.8 (C8), 197.4 (Cb). **Anal. calc.** for $C_{22}H_{19}BrN_8O_3$ (523.34): C 50.49, H 3.66, N 21.41. Found: C 50.38, H 3.52, N 21.30.

Preparation of 5-(4-acetylphenyl)-3-(4-chlorophenyl)-1-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-*1H*-purin-8-yl) formazan.



This compound was prepared by using the same method described for the preparation of compounds (7-12) by Mixing (332 mg) from 1 with 4-acetylphenyl diazanium chloride (182 mg). Yield: (77 %) a brown yellow solid. **m.p** = 173-175 ^oC $\mathbf{R_f}$ = 0.25 .**FT-IR (KBr, ν, cm^{-1})**: (3355**)** N-H,(1688) C=O, (2993,2877) C-H_{ali}, (3055) C-$H_{arom.,}$ (1555) C=N, (1635) C=$C_{arom.}$ (1202) C-O-C, (1373,1450) N=N. **^{1}H-NMR (DMSO-*d6*)** □□= 2.63 (s, 3H, Ha), 3.20 (s, 3H, N_1-CH_3), 3.37 (s, 3H, N_3-CH_3), 7.50-7.57 (d, 2H, H3´+H5´+H2"+H6"), 8.02-8.14 (d, 2H, H3"+H5"), 7.85-7.87 (d, 1H, H6´+H2´), 10.20 (s, 1H, N_9H). 13.15 (s, 1H, N_{10}H). **^{13}C-NMR(DMSO-*d6*)** □□= 26.5 (Cc), 27.4 (N1-CH_3), 29.4 (N3-CH_3), 113.8 (C5), 127.2-128.8 (C6"+5"+3"+2"+5'+3'), 130.8 (C2'+6'), 132.7 (C1'), 135.9-136.1 (C4'+4"), 149.8 (C4), 151.09 (C=O 6), 153.4 (C=O 2), 154.5 (C1"), 157.8 (Ca), 165.4 (C8), 197.3 (Cb). **Anal. calc.** for $C_{22}H_{19}ClN_8O_3$ (478.89): C 55.18, H 4.00, N 23.40. Found: C 55.37, H 4.29, N 23.26.

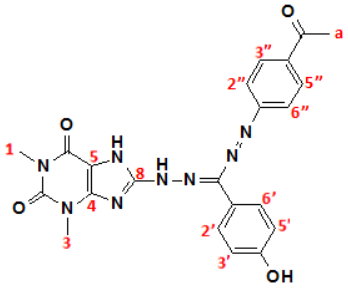
Preparation of 5-(4-acetylphenyl)-1-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-*1H*-purin-8-yl)-3-(4-(dimethylamino)phenyl) formazan



This compound was prepared by using the same method described for the preparation of compounds (7-12) by Mixing (341 mg) from 1 with 4-acetylphenyl diazanium chloride (182 mg). Yield: (85 %) a brown solid. **m.p** = 158-160 ^oC $\mathbf{R_f}$ = 0.62 .**FT-IR (KBr, ν, cm^{-1})** : (3303**)** N-H, (2993,2897) C-H_{ali}, (3066) C-$H_{arom.,}$ (1565) C=N, (1604) C=C $_{arom}$, (1202) C-O-C, (1434,1342) N=N.**^{1}H-NMR(DMSO-*d6*)** □□=3.14 (s, 3H, CH_3), 3.15 (s, 3H, NMe_2), 3.20 (s, 3H, N_1-CH_3), 3.37 (s, 3H, N_3-CH_3), 7.85-7.87 (d, 2H, H2"+H6"), 8.02-8.14 (d, 2H, H3"+H5"), 7.94-7.96 (d, 1H, H6´+H2´), 6.83-6.85 (d, 1H, H3´+H5´) 10.52 (s, 1H, N_9H). 13.76 (s, 1H, N_{10} H).**^{13}C-NMR(DMSO-*d6*):**□□= 25.4 (Cc), 27.2 (N1-

CH_3), 29.3 (N3-CH_3), 112.8 (C3'+5'), 116.6 (C5), 124.9 (C1'), 128.7 (C2"+6"), 128.9 (C3"+5"), 130.4 (C2'+6'), 135.3 (C4"), 149.2 (C4), 150.3 (C=O6), 151.8 (C=O2), 153.3-153.5 (C1"+4'), 155.8 (Ca), 167.4 (C8), 193.8 (Cb). **Anal. calc.** for $C_{24}H_{25}N_9O_3$ (487.51): C 59.13, H 5.17, N 25.86. Found: C 59.35, H 5.30, N 26.07.

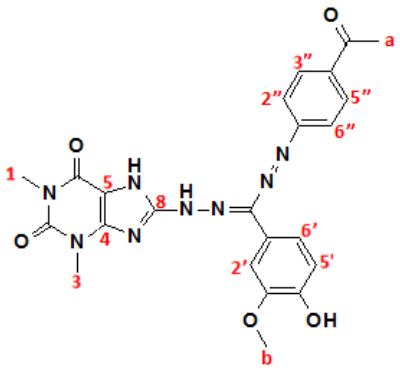
Preparation of 5-(4-acetylphenyl)-1-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-*1H*-purin-8-yl)-3-(4-hydroxyphenyl) formazan.



This compound was prepared by using the same method described for the preparation of compounds (7-12) by Mixing (314 mg) from 1 with 4-acetylphenyl diazanium chloride (182 mg). Yield: (85 %) a brown solid. **m.p** = 161-163 °C **R_f** = 0.31.**FT-IR (KBr, ν, cm^{-1}):** (N-H), 2993,2877 (C-H)$_{ali}$, 3055 (C-H)$_{arom}$, 1672 (C=N), 1604 (C=C) $_{arom}$, 1112 (C-O-C), 3439, (O-H) 1473,1435 (N=N). **^{1}H-NMR (DMSO-*d*6)** □□= 2.69 (s, 3H, H_a), 3.26 (s, 3H, N_1-CH_3), 3.42 (s, 3H, N_3-CH_3), 7.25-7.28 (d, 2H, H2"+H6"), 6.96-6.99 (d, 2H, H3´+H5´), 8.00-8.01 (d, 2H, H3"+H5"), 7.51-7.54 (d, 2H, H2´+H6´), 10.65 (s, 1H, N_9H), 13.53 (s, 1H, N_{10}H), 10.00 (s, 1H, OH). **^{13}C-NMR(DMSO-*d*6)** □□□= 25.25 (Cc), 27.3 (N1-CH_3), 29.4 (N3-CH_3), 115.9 (C3'+5'), 116.6 (C5), 127.1 (C1'), 127.5 (C2'+6'), 128.5 (C2"+6"), 128.7 (C3"+5"), 137.7 (C4"), 149.9 (C4), 150.2 (C=O6), 152.8 (C1"), 153.1 (C=O2), 157.3 (Ca), 163.7 (C4'), 169.3 (C8), 194.9 (Cb). **Anal. calc.** for $C_{22}H_{20}N_8O_4$ (460.45): C 57.39, H 4.38, N 24.34. Found: C 57.61, H 4.54, N 24.23.

Preparation of 5-(4-acetylphenyl)-1-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-*1H*-purin-8-yl)-3-(4-hydroxy-3-methoxyphenyl) formazan

This compound was prepared by using the same method described for the preparation of compounds (7-12) by Mixing (344 mg) from 1 with 4-acetylphenyl diazanium chloride (182 mg). Yield: (87%) a brown solid. **m.p** = 185-188 °C **R_f** = 0.38.**FT-IR (KBr, ν, cm^{-1})** : (3313**)** N-H, (2960,2806) C-H$_{ali}$, (3066) C-H$_{arom,}$ (1672) C=N, (1597) C=C$_{arom}$, (1117) C-O-C, (3340) O-H (1450) N=N. **^{1}H-NMR (DMSO-*d*6) :**□□= 2.65 (s, 3H, Ha), 3.21 (s, 3H, N_1-CH_3), 3.38 (s, 3H, N_3-CH_3), 3.68 (s, 3H, Hb), 6.52 (d, br.,1H, H5´), 7.42-7.45 (d, br.,1H, H6´), 7.65-7.68 (d, br.,1H, H2´), 7.92-7.94 (d, 2H, H2"+H6"), 8.12-8.17 (d, 2H, H3"+H5"),10.67 (s, 1H, N_9H). 13.72 (s, 1H, N_{10}H), 10.02 (s, 1H, OH). **^{13}C-NMR(DMSO-*d*6)** □□= 25.25 (Cc), 27.3 (N1-CH_3),29.4 (N3-CH_3),55.2 (C3'-O-*CH_3*),115.5-116.6 (C5+2'+5'), 120.4 (C6'), 128.3 (C1'), 128.5 (C2"+6"), 128.7 (C3"+5"), 148.7-148.9 (C3'+4), 150.1 (C=O6), 151.3 (C4'), 151.8 (C=O2), 152.3 (C1"),155.2 (Ca), 166.8 (C8), 196.2 (Cb). **Anal. calc.** for $C_{23}H_{22}N_8O_5$ (490.47): C 56.32, H 4.52, N 22.85. Found: C 56.38, H 4.61, N 22.99.



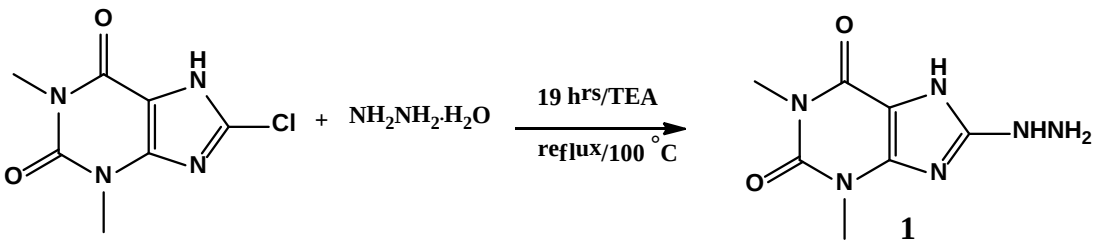
Antimicrobial Evolution

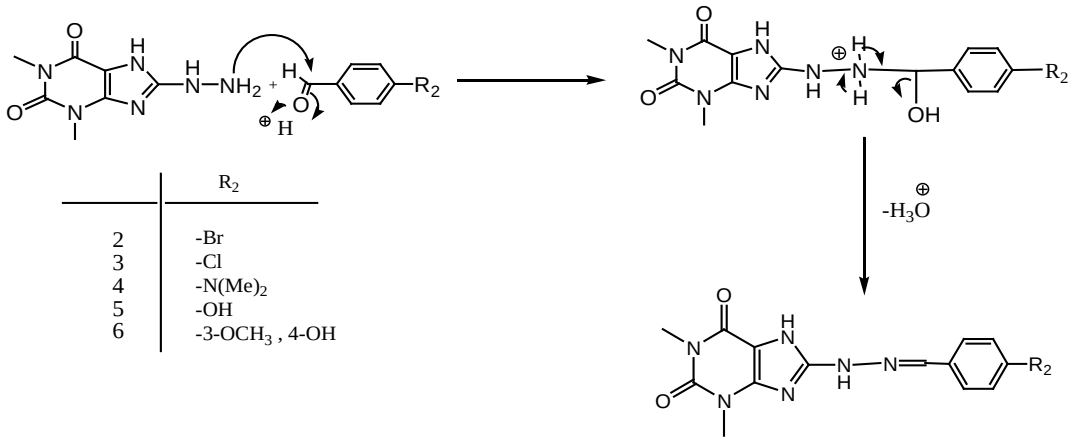
The newly synthesized compounds were selected for their antimicrobial activities against different bacteria and fungi. The microorganisms used were *Staphylococcus aureus* (Gram positive),and *Escherichia coli*, (Gram negative) by using the agar diffusion method [20] to select the most potent compounds. 5 mg of each compound was dissolved in dimethyl sulfoxide (DMSO, 1 mL) then complete up to 10 mL with distal water to give a concentration of 500 µg/mL. The bacteria were maintained on Muller hentone agar media, the dishes incubated at 37 °C for 24 hr for bacteria while 72 hr for fungal. [21,22]

Results And Discussion

8-CTh was a starting material for the synthesis of new Formazan compound over a reaction hydrazine hydrate to formation hydrazine derivatives .This derivatives react with some aromatic aldehyde to formation Schiff base .

^{1}H-NMR spectrum for N-H 14.54 (s, br,1H, NH), are attributed to the formation amine compound [23] ^{13}C-NMR spectrum for 24.6 (Me), 113.9 (C2´+6´), 127.3 (C4´), 131 (C3´+5´), 143.8 (C1´) [24]. The reaction of 1 compound with different aromatic aldehydes in the presence of glacial acetic acid the mechanism of formation Schiff base can be show in scheme 2 and the appearance of stretching band can be show in the table 1 Some of prepared compounds studding



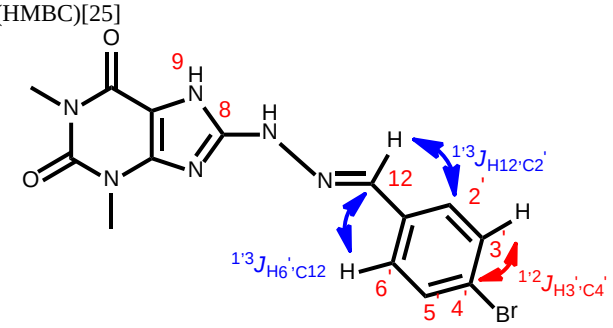


	R2
2	-Br
3	-Cl
4	-N(Me)2
5	-OH
6	-3-OCH3 , 4-OH

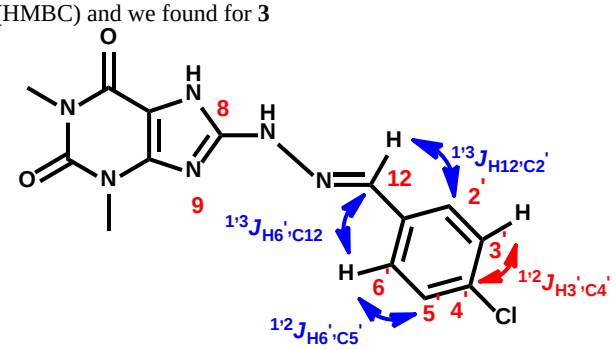
Table 1: The appearance of stretching band , ^{1}HNMR and ^{13}C-NMR can be show in the table 1

No. of Comp	FT-IR (KBr, ν, cm^{-1})						H-NMR		^{13}C-NMR	
	ν(C=N)	ν(C=C) arom	ν(C-Br).	ν(C-Cl).	ν(C-H) arom	ν(O-H)	H12	H arom	C12	Carom
2	1568 1546	1625	1009	-	3047	-	8.71	7.73-7.85	138.5	130.7-132.5
3							8.71	7.92-7.58	138.2	-129.6.5130
4	1591	1604	-	-	3091	-	8.02	6.93-7.45	138.1	128.7 ,112.8
5	1591	1608	-	-	3061	3342	8.56	7.70-6.87	137.7	130.0-129.6
6	1591	1604	-	-	3000	3436	8.71	6.83-7.92	136.9	-113.04149.

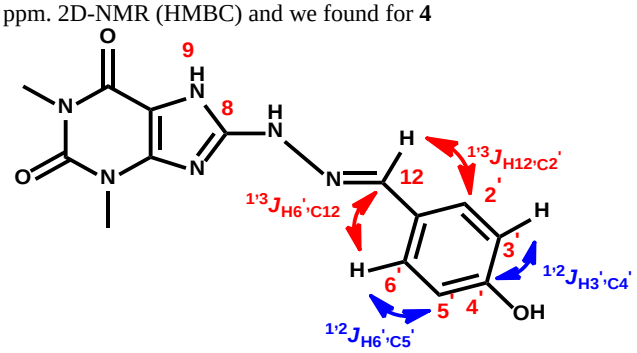
Some of prepared compounds studding the 2D-NMR (HMBC)[25]



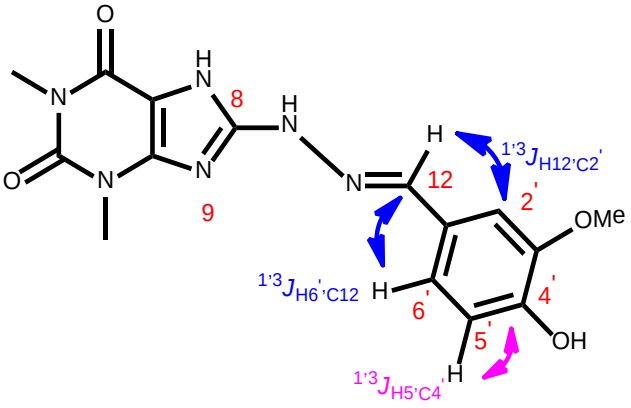
2D-NMR (HMBC) and we found for **2** three coupling for type ^{1,3} $J_{H,\,C}$ the first; between C2′ to be the distance □=124.0 ppm and proton H12 □=8.8 ppm, and the second between C12 for the distance □□=135 ppm and proton H6′ □ =7.7 ppm. Third of coupling for type ^{1,2} $J_{H,\,C}$ between C4′ for the distance □= 122 ppm and proton H3′ for the distance □ = 7.8 ppm. 2D-NMR (HMBC) and we found for **3**

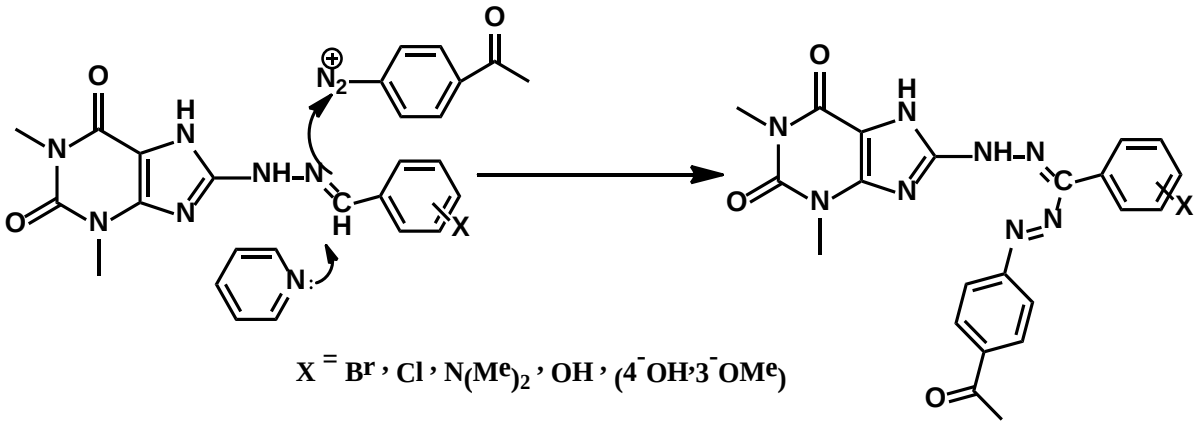


two coupling for type ^{1,3} $J_{H,\,C}$ the first; between C2′ to be the distance □=135.0 ppm and proton H12 □=8.4 ppm, and the second between C12 for the distance □□=139 ppm and proton H6′ □ =7.8 ppm. Third and four of coupling for type ^{1,2} $J_{H,\,C}$ between C4′ for the distance □= 139 ppm and proton H3′ for the distance □ = 7.7 ppm and the second between C5′ for the distance □ = 108 ppm and proton H6′ for the distance □ = 7.8 ppm. 2D-NMR (HMBC) and we found for **4**



two coupling for type ^{1,3} $J_{H,\,C}$ the first; between C2′ to be the distance □=130.0 ppm and proton H12 □=8.5 ppm, and the second between C12 for the distance □□=137.7 ppm and proton H6′ □ =7.8 ppm. Third and four of coupling for type ^{1,2} $J_{H,\,C}$ between C4′ for the distance □= 160 ppm and proton H3′ for the distance □ = 7.0 ppm and the second between C5′ for the distance □ = 115 ppm and proton H6′ for the distance □ = 6.8 ppm. **2D-NMR (HMBC)** and we found for **5**





Scheme 1: Mechanism of formazan derivatives

Table 2: The appearance of stretching band , ^{1}HNMR and ^{13}C-NMR can be show in the table 1

No. of Comp	FT-IR (KBr, ν, cm^{-1})						H-NMR			^{13}C-NMR	
	ν(C=N)	ν(C=C) arom	ν(N=N).	ν(C=O) Ketone	ν(C-H) arom	ν(O-H)	Ha	H	H b	Ca	Cb
7	1581	1604	1395 1437	1681	3047	-			-	156.0	197.4
8	1555	1635	1373 1450	1655	3055	-	2.63		-	157.8	197.3
9	1565	1604	1342 1434	1704	3066	-	2.63		-	157.8	197.3
10	1550	1604	1373 1435	1704	3055	3395	2.96		-	157.3	194.9
11	1596	1550	1373 1450	1650	3055	3436	3.68		2.65	155.2	196.2

three coupling for type ^{1,3} $J_{H,C}$ the first; between C2′ to be the distance □=118.0 ppm and proton H12 □=8.7 ppm, and the second between C12 for the distance □□=138 ppm and proton H6′ □=6.8 ppm. Third of coupling for type ^{1,3} $J_{H,C}$ between C4′ for the distance □= 144 ppm and proton H5′ for the distance □ = 7.4 ppm. In other hand react this Schiff base derivatives with daizinum salt to in the presence of pyridine as a solvent in ice bath and stirring for 3 hrs. to formation formazan derivatives the mechanism of this reaction can be show as below .

Biological part

Control of microbial population is necessary to prevent show of disease, infection, decomposition, spoilage and contamination and caused by them. The newly synthesized compounds were screened for their antimicrobial activity invitro against bacteria (*Staphylococcus aureues, Escherichia coli)*

The antimicrobial activity results discovered that most of the tested compounds have moderate to strong activity. The most effective compounds are **5**, **8** and **10** When these compounds were compared with the reference compounds (DMSO, distill water) we found that they have an antimicrobial activity higher or almost equal to them.

No. of Comp.	Gram-positive	Gram-negative
	S. Aureus	*E-coli*
1	12	18
2	14	22
3	17	23
4	14	25
5	13	20
6	19	23
7	15	17
8	16	24
9	13	16
10	15	18
11	12	16
Control/DMSO	0	0
Distill water	0	0

Zone of inhibition measured in mm: no activity (0.0), very weak activity (< 7 mm), weak activity (7–10), moderate activity (11–15 mm), strong activity (> 15 mm).

CONCLUSION

In this study we are reported synthesis of many Formazan derivatives from 8-CTh. The work included preparation of diazinum salt compounds and some of Schiff base then prepared formazan. These derivatives were found active agnist bacterial and show some of these derivatives active agnast *S. Aureus* and *Ecoli*. These derivatives confirmed from spectral data analysis; FT-IR, H^{1}-NMR, C^{13}-NMR and 2D-NMR.

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