Synthesis and evaluation of anti-microbial of some new Biotin carrying sugar moieties

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ABSTRACT: The current research involves preparing biotin sugar derivatives and evaluating their vital activity against some types of microbial, through a set of interactions, where the biotin was initially converted into an ester derivative according to the Fischer method. Then the ester derivative was treated with aqueous hydrazine to prepare the derivative 3. After that, the derivative 3 was condensed with a number of monosaccharides using the Schiff-Base reaction. The prepared compounds were spectrophotometrically characterized via modern methods. Results of the biological tests showed that the derivatives 4-8 possess antibacterial vital activity as well as anti-fungi.

KEYWORDS: Biotin, Biotin derivatives, Sugars, biological activity, Hydrazones

INTRODUCTION

Biotin is a derivative of Imidazole and it is available in natural foods as an essential ingredient and in some foods as a dietary supplement[1]. Also, a large portion of biotin is manufactured by gut bacteria that human cells, as well as many organisms, cannot produce [2]. In the year 1953, Donald B. Melville [3] was synthesized biotin sulfoxide that showed good biological activity, and as well as the great importance of biotin derivatives in various areas of life led to an interest in the synthesis of more new derivatives for it. Either in the year 2018, Heather Nesbitt was concluded that the biotin derivatives which contain the sugar molecules in their composition were used as medicinal compounds against cancer cells in the pancreas[4]. As sugars contribute to the importance of the drug and its penetration and dissolution inside the body[5]. Biotin derivatives were used as an anti-inflammatory for certain types of bacteria and good breast cancer[6,7]. As They can also be used as a new drug for cancer and vaccine therapy, as well as the use of ligands that inhibit the action of TLR receptors, enzyme inhibiting agents, making them resistant to enzymatic and chemical decomposition sometimes because they do not have oxygenic or glycoside binding [8]. They are many sugars that have been studied with various biological activities, including it is immune system[9], antioxidant[10], anti-inflammatory[11]. In this work, we prepared a series of biotin derivatives combined with a number of monosaccharides and were evaluated for their bioactivity.

EXPERIMENTAL SECTION

Melting point were measured using steuart andare uncorrected. FT-IR Spectra using KBr disk were recorded on shimadzu-FT-IR-8400 S Fourier transform, ¹³C-NMR, ¹H-NMR Spectra were recorded on a Bruker Avance II spectrometer at 400 MHz using DMSO-d⁶ with TMS reference university Ghazi Osman Pasha in Turkey. all materials used are pure and processed by companies Fluka, Aldrich, GCC.

Chemical synthesis

Synthesis of Ethyl 5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate 2[12]

In round potom flask 100 ml with three neck put on heater with magnetic stirrer, condenser and thermometer with a balloon filled with inert nitrogen gas, was taken (1g,4.1 mmol) of biotin1 and dissolved in 20ml of absolute ethanol with the addition 3ml of concentrated sulfuric acid and the
reaction mixture was reflux for 13h. The progress of reaction was monitored by TLC in A mixture of (MeOH:CH2Cl2) with a ratio of (2:8)(v:v)as a mobile phase, after that the solvent residual was evaporated under the vacuum pressure. It was neutralized by adding drops of 10% Na2SO4 with stirring then poured on ice crush, then the product precipitated and filtered through a Buckner funnel and wash with distilled water, dried and recrystallized with ethanol to give white precipitate.

Yield: 75%, m.p=151-153°C, $R_f=0.42$

**Synthesis of 5-(2-Oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanehydrazide**

In round bottom flask 100 ml ,was taken (1.7g ,0.0062 mmol) of derivative 2 biotin ethyl ester dissolved in 50ml of absolute ethanol with the addition of 6ml of hydrazine hydrate and the mixture was reflux for 14h at a temperature 78°C the reaction was monitored by TLC in a mixture of (CHCl3:MeOH) in the ratio(9:1)(v:v) As a mobile phase, the reaction mixture was left to cool at room temperature. After that, the precipitate was filtered and washed with ether. The resulting product was purified as a white solid by recrystallizing it from ethanol.

Yield: 51% , m.p =242-243°C , $R_f=0.2$

**General Method of the Preparation**

Sugar derivatives of biotin 4-8

In round bottom flask 100 ml ,was taken (0.1163g ,0.45mmol) of derivative 3 biotin hydrazide with (0.5mmol) of monosaccharides dissolved in 20ml of absolute ethanol and the mixture was reflux for(4-6)h. the reaction was monitored by TLC in a mixture of (CHCl3:MeOH) at a ratio (8:2)(v:v) As a mobile phase, after the completion of the reaction, the reaction mixture was left for cooling at room temperature. After that, the resulting product was collected and recrystallized to obtain the sugars derivatives of biotin.

**Synthesis of 5-(2-Oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-N’-(2S,3S,4R,Z)-2,3,4,5-tetrahydroxypentylidene)pentanehydrazide**

The derivative 4 was prepared through the reaction of derivative 3 (0.1163g ,0.45mmol) with (0.075g ,0.5mmol) from the ribose sugar and the mixture was reflux for 6h according to the general method to obtain the compound in the form of a yellow powder, the technique was recrystallized with ethanol. Yield: 80% , M.P : 71-73°C , $R_f=0.8$

**Synthesis of 5-(2-Oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-N’-(2S,3R,4R,Z)-2,3,4,5-tetrahydroxypentylidene)pentanehydrazide**

The derivative 5 was prepared through the reaction of derivative 3 (0.1163g ,0.45mmol) with (0.075g ,0.5mmol) from the xylose sugar and the mixture was reflux for 6h according to the general method to obtain the compound in the form of a light brown powder, the technique was recrystallized with ethanol. Yield : 79% , M.P : 70-71°C , $R_f=0.85$

**Synthesis of 5-(2-Oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-N’-(2S,3R,4S,5R,Z)-2,3,4,5,6-pentahydroxyhexylidene)pentanehydrazide**

The derivative 6 was prepared through the reaction of derivative 3 (0.1163g ,0.45mmol) with (0.09g ,0.5mmol) from galactose sugar and the mixture was reflux for 5h according to the general method to obtain the compound in the form of a grey powder, the technique was recrystallized with ethanol. Yield : 79% , M.P : 79-80°C , $R_f=0.74$

**Synthesis of 5-(2-Oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-N’-(2S,3R,4R,5R,Z)-2,3,4,5,6-pentahydroxyhexylidene)pentanehydrazide**

The derivative 7 was prepared through the reaction of derivative 3 (0.1163g ,0.45mmol) with (0.09g ,0.5mmol) from glucose sugar and the mixture was reflux for 6h according to the general method to obtain the compound in the form of a brown powder, the technique was recrystallized with ethanol. Yield : 85% , M.P : 80-82°C , $R_f=0.62$

**Synthesis of 5-(2-Oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-N’-(3R,4S,5R,Z)-1,3,4,5,6-pentahydroxyhexan-2-ylidene)pentanehydrazide**

The derivative 8 was prepared through the reaction of derivative 3 (0.1163g ,0.45mmol) with (0.09g ,0.5mmol) from fructose sugar and the mixture was reflux for 5:30 h according to the general method to obtain the compound in the form yellow powder, the technique was recrystallized with ethanol. Yield : 66% , M.P : 75-77°C , $R_f=0.5$

**Biological activities**

We are conducting evaluation experiment for antimicrobial activity of the synthesized compounds were investigated in vitro against both Gram positive bacteria (Staphylococcus aureus)
and Gram negative bacteria (Escherichia coil) as well as Fungi (Candida albicans, Aspergillus niger) using the broth dilution method [14] preparation of nutrient broth, subculture, base layer medium, agar medium was according to the standard procedure. The standard and test compounds were dissolved in DMSO to obtained a concentration of 50 Mg/ml and 100Mg/ml. The samples were incubated at 37°C for 24h (bacteria), at 25°C for 72h (fungi), zone of inhibition was measured in mm. Cephalexin and Fluconazole were used as standard antibiotics to compare the results.

RESULT AND DISCUSSION

Chemistry
Ester derivative of biotin2 were prepared via reaction of biotin1 with ethanol in acidic medium. Next, ester derivative 2 was condensed with hydrazine hydrate under reflux to get the derivative 3. Later, the derivative 3 was condensed with a number of monosaccharides using the Schiff-Base reaction. The synthesis methods to the novel compounds were shown in the following scheme 1.

Scheme 1: The experimental steps for synthesis of benzo[f]coumarin derivatives (2-8).

Spectrally

Compound 2 : ethyl 5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate

FTIR (KBr, V_{max}, cm^{-1}) of derivative (2) offer band stretching 1118 (C-O), 1743 (C=O)_{ester}, 1712 (C=O)_{amide}, 3247 (N-H)_{sec} and 2931, 2862 (C-H)_{aliph}. H-NMR(DMSO, ppm) of derivative (2) offer 6.36 (d, H, NH_lacta), 4.31 (m, H, CH_1), 2.83 (d, 2H, CH_6), 3.10 (q, H, CH_8), 2.28 (t, 2H, CH_12), 3.75 (q, 2H, CH_18), 1.15 (t, 3H, CH_17), 1.55 (m, 2H, CH_11, 10)
Compound 3: 5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanehydrazide

FTIR (KBr, νmax, cm⁻¹) spectra of derivative 3 offer two bands stretching 3301, 3250 (NH₂), 3224 (NH) sec and 1697 (C=O) lact+amid. ¹H-NMR (DMSO, ppm) spectra of derivative 3 offer 6.42 (s, H, NH amide), 1.49 (s, 2H, NH₂), 8.92 (d, H, NH lact), 4.31 (m, H, CH₁), 4.13 (d, 2H, CH₂), 3.74 (q, 2H, CH₆), 3.10 (q, 2H, CH₈,₉), 1.54 (m, 2H, CH₁₁), 1.34 (m, 2H, CH₁₀), 2.01 (t, 2H, CH₁₂).
Compound 4: 5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-N’-((2S,3S,4R,Z)-2,3,4,5-tetrahydroxypentyldene)pentanehydrazide

FTIR(KBr, νmax, cm⁻¹) spectra of derivative 4 offer band stretching 3332 (O-H) alcoh, 3209 for (N-H), 2931, 2869 (C-H) aliph, 1681 (C=O) lact+amid and 1666 cm⁻¹ for (C=N). ¹H-NMR(DMSO, ppm) spectra of derivative 4 offer 1.09 (d, H, NH lact), 4.32 (m, H, CH₂), 4.15 (q, H, CH₂), 2.60 (d, 2H, CH₆), 3.35 (q, H, CH₈), 1.09 (m, 2H, CH₉, 11), 1.53 (m, 2H, CH₁₀), 1.63 ppm(t, 2H, CH₁₃), 6.30 (s, H, NH amid), 6.35 (s, H, CH), 3.9-2.85(t, 3XOH +CH₂OH).
Figure 5 : IR spectra of derivative 4

Figure 6 : ¹H-NMR spectra of derivative 4

Compound 5 : 5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-N'-(2S,3R,4R,Z)-2,3,4,5-tetrahydroxypentylidene)pentanehydrazide

FTIR(KBr, V_max, cm⁻¹) spectra of derivative(5) offer band stretching 3301 (O-H), 3232(N-H) sec, 1704 (C=O) amid, 1681(C=O) lact, and 2931, 2862 (C-H) aliph and 1650 cm⁻¹ for (C=N). ¹H-NMR(DMSO, ppm) spectra of derivative (5) offer 6.43(s,H,NH amid ,18), 4.31(m,H,CH₁,2,2₃,2₉), (6.37-3.13)(m,3xOH sugar),1.6(m,H ,CH₉,11,) ,1.08(m,H,CH₁₀) ,2.23(m,H,CH₁₃), 2.58(d,2H,CH₆).
Compound 6: 5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-N’-((2S,3R,4S,5R,Z)-2,3,4,5,6-pentahydroxyhexylidene)pentanehydrazide

FTIR (KBr, V_max/cm⁻¹) spectra of derivative (6) offer band stretching 3363 (O-H), 3224 (N-H)_sec, 2931, 2869 (C-H), 1697 (C=O)_amid, 1681 (C=O)_lacta and 1666 (C=N). ¹H-NMR (DMSO, ppm) spectra of derivative (6) offer 2.60 (d, H, NH_lact), 4.23 (m, H, CH₁,₂) 2.84 (t, 2H, CH₆), 4.23 (q, H, CH₈), 1.32 (m, 2H, CH₉), 1.89 (m, 2H, CH₁₀), 1.54 (m, 2H, CH₁₁), 2.18 (t, 2H, CH₁₃), 4.23 (s, H, NH_amid), 4.30 (m, H, N=CH), (3.46-4.23) (m, 5xOH_sugar).
Figure 9: IR spectra of derivative 6

Figure 10: 1H-NMR spectra of derivative 6

Compound 7: 5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-N’-(2S,3R,4R,5R,Z)-2,3,4,5,6-pentahydroxyhexylidene)pentanehydrazide

FTIR(KBr, VMax, Cm⁻¹) spectra of derivative(7) offer band stretching 3348 (O-H), 3232 (N-H), 2931, 2869 (C-H) aliph., 1681 (C=O) lacta, 1697 (C=O) amid and 1658 (C=N). 1H-NMR spectra of derivative(7) offer 4.32 (d, H, NH lact), 4.15 (m, H, CH1), 3.66 (m, H, CH2), 2.60 (d, 2H, CH6), 3.10 (m, H, CH8), 1.11 (m, 2H, CH9,11), 1.54 (m, 2H, CH10), 2.87 (t, 2H, CH13), 1.37 (s, H, NH amid), 4.31 (m, N=CH), 3.10-4.32 (m, 5xOH sugar).
Figure 11: IR spectra of derivative 7

Figure 12: 1H-NMR spectra of derivative 7

Compound 8: 5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-N’-((3R,4S,5R,Z)-1,3,4,5,6-pentahydroxyhexan-2-ylidene)pentanehydrazide

FTIR(KBr, V_max, Cm⁻¹) spectra of derivative(8) offer band stretching at 3301 (O-H), 3255 (N-H), 1650 (C=O) Lacta, 1697 (C=O) Amid, and 2931, 2854. 1H-NMR(DMSO, ppm) spectra of derivative(8) offer 2.58 (d, 2H, CH₆), 6.35 (s, H, NH Amid), 4.52 (m, H, CH₉, CH₁₁), 4.1 (t, CCH₂OH), 3.33-4.52 (5xOH Sugar).
Biological study

The compound's preparation can be evaluated against bacteria [E.Coli, S.aureus] as well as fungi [C.albicans, A.niger] through using well agar the broth dilution method, all results were summaries in the table (1). The compound activity against [E.coli] and fungi type [C.albicans] showed that derivative 4 have high activity, the same derivative have mid activity for fungi [A.niger] and slightly for [S.aureus], while derivative 5 showed no activity toward bacterial against [E.coli, S.aureus] and fungi [C.albicans] and slightly activity for fungi [A.niger]. Either the derivative 7 showed mid activity toward bacterial against [E.coli, S.aureus] and fungi [C.albicans] and slightly activity for fungi [A.niger], finally the bacteria [E.coli] show mid activity for derivative 6 and high activity for derivative 8, otherwise bacteria [S.aureus] show slightly for derivative 6 and no activity for derivative 8, also fungi [A.niger] show slightly activity for derivative 8 and mid activity for derivative 6, otherwise fungi [C.albicans] show high activity for derivative 8 and slightly activity for derivative 6. Depending on the measurement unit 100Mg/ml.

Table -1: Antimicrobial activity of compounds 4-8

| Compound | Inhibition zone diameter in mm |
|----------|-------------------------------|

Figure 13: IR spectra of derivative 8

Fig 14: $^1$H-NMR spectra of derivative 8
CONCLUSION

In conclusion, a series of new biotin derivatives were successfully synthesized and were conjugated with a number of monosaccharides using the Schiff-Base reaction, and obtained yields were good. The synthesized compounds were screened for antimicrobial activities. The in-vitro results presented on the above tables reveal that the biotin derivatives with substituted groups [-OMe, -OH and -NH] within the structure of the compound were showed good antibacterial and antifungal activities compared to stander drugs.

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