Synthesis of some saccharin derivatives containing 1,2,3-triazoline *ring*

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Abstract

Some of 1,2,3-triazoline containing compounds were prepared starting from sodium saccharin. The later was reacted with allyl chloride forming allyl saccharin, then reacted with n-heptyl azide to give compound (1) named as (N-((1-n-heptyl-1,2,3-triazolin-4-yl)methyl) saccharin. Compound (1) was hydrolyzed under strong basic conditions to form the carboxylic acid derivative (2). Compound (2) reacted with ethanol to produce the ester derivative (3), the ester derivative (3) reacted with hydrazine to form the hydrazide derivative (4). Compound (4) was reacted with propargyl chloride to form (5). Compound (5) finally reacted again with n-heptyl azide to form (6). The compounds were identified using the analytical and spectral methods shown in the work. The compounds were tested for their biological and antioxidant activity; some compounds were active and other was not.

الكلمات المفتاحية: السكرين, 3,2,1-ترايازولين, مضادات البكتيريا, مضادات الأكسدة.

الخلاصة:-

تم تحضير بعض مشتقات من 3,2,1-ترايازولين وذلك من خلال مفاعلة سكرين الصوديوم مع كلوريد الأليل ينتج الايل اسكرين والذي نفاعل مع ن-هبتيل أزيد يكون المركب رقم (1) والمسمى (N-((1-n-heptyl-1,2,3-triazolin-4-yl)methyl) والأخير تحلل مائياً بوسط قاعدي قوي ينتج مشتق الحامض الكاربوكسيلي (2) والذي تفاعل مع الأيثانول المطلق ينتج مشتق الأستر (3) ومشتق الأستر (3) تفاعل نع 80% هايدرازين مكوناً مشتق الهيدرازايد (4) ومشتق الهيدرازايد (4) تفاعل مع كلوريد الأليل يكون المركب رقم (5) والمركب رقم (5) تفاعل مع ن-هبتيل أزيد ينتج المركب رقم (6). المركبات المحضرة تم تشخيصها بأستخدام الطرق الطيفية الموضحة في البحث. وكذلك تم إختبار الفعالية البايلوجية وفعالي مضاد الأكسدة وكانت بعض المركبات فعالة في حين البعض لم يكن ذا فعالية.

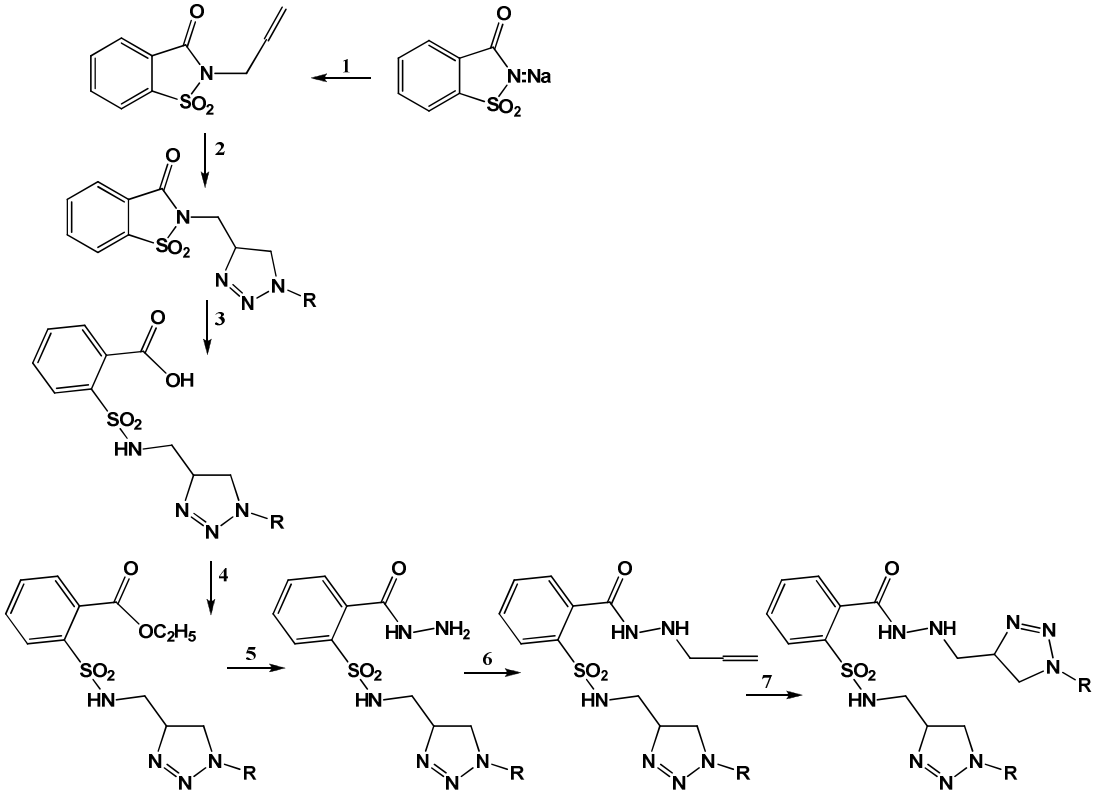
Introduction

Triazoline and its derivatives belong to the heterocyclic class of compounds and possess interesting biological characteristics. These compounds can be used in medicine as antibacterial, antiviral, anticancerous, antiasthmatic, analgesic and anti-inflammatory drugs because of their pharmaceutical properties [1]. Furthermore, triazolines. Interest in 1,3-dipolar cycloadditions involving olefins as dipolarophiles and azides as 1,3dipoles originates from the synthetic potential of these reactions which lead to the formation of five membered nitrogen containing heterocycles like 1,2,3-triazolines. The The first involves the isomerisation of arylazoaziridines [3]. The second synthetic route to triazolines is the 1,3-dipolar cycloaddition of diazoalkenes to Schiff bases (imines) [4]. A third route to 1,2,3-triazoline is the 1,3-dipolar cycloaddition of azides to ethylenic compounds [5]. 1,2,3-triazolines have been used as versatile precursors of *N*-containing heterocycles, E. Erba and D. Sporchia [6] synthesized 4-aminoquinazolines and 6-aminopurines from their

corresponding triazolines. Triazoline can be converted into thiadiazole ring under acidic conditions [7], also they used in the synthesis of 1,2,3triazoles [8]. In this work we synthesized six new 1,2,3-triazoline rings starting from allyl saccharin and and heptyl azide.

Experimental and Methods

The synthesis of the target molecules (1-6) are shown in the sequences of reactions depicted in the scheme below. The F.T.IR spectral data were recorded on F.T.IR-8300 Fourier Transform Infrared Spectrophotometer *SHIMADZU* using potassium bromide disc, Double-beam UV-VISIBLE spectrophotometer (UV 1700 CP), *SHIMADZU*, ^{1}H-NMR and ^{13}C-NMR was recorder on Bruker Ultra Shield, 400MHz, using DMSO as solvent and TMS as internal standard, Melting points (^{o}C) were recorded on hot stage Gallen Kamp melting point apparatus and were uncorrected and Thin-layer chromatography was performed glass plats coated with 0.25 mm layer of silica-gel (Fluka). Chemical names follow the IUPAC nomenclature. Some starting materials were purchased from EMD Millipore Corporation, Germany Tel. +49(0)615172-440 and were used without purification.



R : n-heptyl
1: allyl chloride, 2: n-heptyl azide, 3: hydrolysis (strong base),
4: ester formation (abs EtOH), 5: hydrazide formation(80% hydrazine hydrate),
6: allyl chloride, 7: n-heptyl azide.

2.3.1. Preparation of N-allyl saccharin:

Sodium saccharin 0.02 mol was dissolved in DMF 50 mL, allyl chloride 0.02 mol was added and then the mixture was refluxed for 4 hours, after cooling 50 mL of distilled water was added to the mixture forming a precipitate, the precipitate was filtered, washed with distilled water. The melting point is (121^{o}C) with 90% percentage yield and R_f (0.88). F.T-IR (KBr) cm^{-1}) 3186 cm-^{1}, 3095 cm^{-1}, (2933-2856) cm^{-1}.

2.3.2. Preparation of n-heptyl azide:

Sodium azide 0.03 mol was added slowly to refluxed DMF 50 mL; the reflux was continued until all the sodium azide dissolved then the n-heptyl chloride 0.015 mol was slowly added; the mixture then was refluxed overnight (the temperature was fixed at 75 ^{o}C), after cooling 50 mL of distilled water was added, 50 mL of diethyl ether was also added to the mixture and the organic layer was extracted (the addition of diethyl ether was repeated three times), then the combined organic layers were dried using magnesium sulfate, and evaporated under reduced pressure. The residue was chromatographed (silica gel, light petroleum ether) to give the n-heptyl azide with 88% percentage yield and R_f (0.72). F.T-IR (KBr) cm^{-1}) 2096 cm^{-1}, (2929-2858) cm^{-1}.

2.3.3. Synthesis of N-((1-n-heptyl-1,2,3-triazolin-4-yl) methyl) saccharin (1):

Allyl saccharin 0.01 mol was slowly added with stirring to a solution containing n-heptyl azide 0.01 mol dissolved in 50mL DMF; the mixture then refluxed overnight; after cooling 50 mL of distilled water was added, 50 mL of diethyl ether was also added to the mixture and the organic layer was extracted (the addition of diethyl ether was repeated three times), then the combined organic layers were dried using magnesium sulfate, and evaporated under reduced pressure. The residue was chromatographed (silica gel, light petroleum ether) to give the titled compound. The melting point is (139^{o}C) with 79% percentage yield and R_f (0.73). F.T-IR (KBr) cm^{-1}) 3095 cm^{-1}, (2931-2858) cm^{-1},1732 cm^{-1}, 1625 cm^{-1}, 1593cm^{-1}. ^{1}H-NMR (DMSO) δ: 0.83(m,3H), 1.1-3.7(s,m,15H), 4.1(m,2H), 7.4-7.9(m,5H), ^{13}C-NMR (DMSO) δ: 13, 38-78, 116-142, 168.

2.3.4. Synthesis of 2-(N-((1-n-heptyl-1,2,3-triazolin-4-yl)methyl) sulfamoyl) benzoic acid (2):

Compound (1) 0.01 mol was refluxed with equivalent amount of sodium hydroxide solution, the reflux was continued until the solution becomes clear; a white precipitate was formed after the acidification by hydrochloric acid, the precipitate was filtered and washed with distilled water to give the desired product. The melting point is (190^{o}C) with 90% percentage yield and R_f (0.70). F.T-IR (KBr) cm^{-1} (3100-3600) cm^{-1}, 3014 cm^{-1}, (2927-2852) cm^{-1}, 1718 cm^{-1}, 1625 cm^{-1}, 1575 cm^{-1}. ^{1}H-NMR (DMSO) δ: 0.90(m,3H), 1.7-3.6(s,m), 4.2(m,2H), 7.9-8.0 (m,5H), 8.2(m, 1H), 12.9(w, 1H). ^{13}C-NMR (DMSO) δ: 20, 38-74, 116-136, 158.

2.3.5. Synthesis of ethyl 2-(N-((1-n-heptyl-1,2,3-triazolin-4-yl)methyl) sulfamoyl) benzoate (3):

Compound (2) 0.01 mol was dissolved in refluxed absolute ethanol 50 mL, then few drops of concentrated sulfuric acid were added; the mixture was then refluxed for 8 hours, after cooling the mixture was neutralized with sodium hydrogen carbonate. The titled product was achieved by evaporating the solution under reduced pressure. The melting point is about (150^{o}C) with 66% percentage yield and R_f (0.68). F.T-IR (KBr) cm^{-1}, 3101 cm^{-1}, (2924-2852) cm^{-1}, 1730 cm^{-1}, 1625 cm^{-1}, 1560 cm^{-1}. ^{1}H-NMR (DMSO) δ: 1.9(m,6H), 2.4-4.0(s,m,17H), 4.1(m,2H), 7.1-7.5 (m,5H), 7.8(m, 1H). ^{13}C-NMR (DMSO) δ: 17, 38-74, 116-137, 163.

2.3.6. Synthesis of 2-(N-((1-n-heptyl-1,2,3-triazolin-4-yl)methyl) sulfamoyl) benzoic hydrazide (4):

Compound (3) 0.01 mol was dissolved in refluxed ethanol 20 mL, hydrazine hydrate (0.06 mol) was slowly added to the mixture. The solution was refluxed for 24 hours. The ethanol was removed by evaporating under reduced pressure; the residue was cooled in an ice bath forming a precipitate. The product was recrystallized from abs ethanol and ethyl acetate to give the titled compound. The melting point is

(198ºC) with 72% percentage yield and R_f (0.70). F.T-IR (KBr) cm^{-1}, (3471-3352) cm^{-1}, 3209 cm^{-1}, 3125 cm^{-1}, (2958-2889) cm^{-1}, 1681 cm^{-1}, 1625 cm^{-1}, 1595 cm^{-1}, ^{1}H-NMR (DMSO) δ: 1.3(m,3H), 2.4-4.0(s,m,15H), 4.2(m,2H), 7.1-7.9(m,5H), 8.1-8.4(m,3H). ^{13}C-NMR (DMSO) δ: 20, 38-74, 116-140, 158.

Synthesis of 2-(N-((1-n-heptyl-1,2,3-triazolin-4-yl)methyl)sulfamoyl)-N'-allyl benzoic hydrazide (5):

Compound (4) 0.02 mol was dissolved in DMF 50 mL, 0.02 mol allyl chloride was added, and then the mixture was refluxed for 4 hours, after cooling 50 mL of distilled water was added to the mixture forming a precipitate, the precipitate was filtered, washed with distilled water and recrystallized from benzene and absolute ethanol. The melting point is (185ºC) with 84% percentage yield and R_f (0.71). F.T-IR (KBr) cm^{-1}, 3319 cm^{-1}, 3124 cm^{-1}, 3014 cm^{-1}, (2929-2810) cm^{-1}, 1654 cm^{-1}, 1624 cm^{-1}, 1580 cm^{-1}. ^{1}H-NMR (DMSO) δ: 1.6(m,3H), 1.8-3.3(s,m,15H), 3.9-40(m,4H), 7.1-7.2(m,7H), 7.30(m,2H), 7.37(m,1H). ^{13}C-NMR (DMSO) δ: 17, 25-43, 70, 78, 121-136, 163.

Synthesis of 2-(N-((1-n-heptyl-1,2,3-triazolin-4-yl) methyl) sulfamoyl)-N'-((1-n-heptyl-1,2,3-triazolin-4-yl) methyl) benzoic hydrazide (6):

Compound (5) 0.01 mol was slowly added with stirring to a solution containing n-heptyl azide 0.01 mol dissolved in DMF; the mixture then refluxed overnight, after cooling 50 mL of distilled water was added, 50 mL of diethyl ether was also added to the mixture and the organic layer was extracted (the addition of diethyl ether was repeated three times), then the combined organic layers were dried using magnesium sulfate, and evaporated under reduced pressure. The residue was chromatographed (silica gel, light petroleum ether) to give the titled compound. The melting point is (207ºC) with 75% percentage yield and R_f (0.70). F.T-IR (KBr) cm^{-1}, 3243 cm^{-1}, 3207 cm^{-1}, 3034 cm^{-1}, (2995-2810) cm^{-1}, 1633 cm^{-1}, 1620 cm^{-1}, 1530 cm^{-1}, ^{1}H-NMR (DMSO) δ: 0.8(m,6H), 1.3-3.3(m,s,30H), 4.5(s,4H), 7.9-8.0(s,4H), 8.03-8.06(s,4H), 8.10-8.11(s,2H), 8.30-8.31(s,2H). ^{13}C-NMR (DMSO) δ: 20,22,31,36,42-43,49.1-49.4,58,62,123,130-136,162.

Biological activity:

The test was performed according to the disk diffusion method. The prepared compounds were tested against one strain of Gram positive bacteria (Staphylococcus Aureus), and two Gram negative bacteria (Escherichia coli) and (Enterobacter). Prepared agar and Petridishes were sterilized by autoclaving for (15min) at 121ºC. The agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In the solidified medium suitably spaced apart holes were made all (6mm) in diameter, were filled with 100µl of the prepared compounds (1mg of the compound dissolved in 1ml of DMSO solvent). These plates were incubated at (37ºC) for (24hours). The inhibition zones caused by the various compounds on the bacteria were examined. The results of the preliminary screening test are listed in Table (1).

Table (1): Biological activity of compounds (1-6) against selected bacteria.

compound	Staphylococcus Aureus	Escherichia coli	Enterobacter

1	++	++	++
2	++	+	++
3	+	++	+
4	++	+	+
5	+	++	++
6	+	+	+

Key to symbols:
Moderately active = ++ (inhibition zone 11-20mm).
Slightly active = + (inhibition zone 5-10mm).
Inactive = - (inhibition zone <5mm).

Antioxidant Activity:

1) Ferric ion (Fe^{+3}) antioxidant properties (reducing activity):

The antioxidant properties of the prepared compounds containing 1,2,3-triazoline ring to reduce (Fe^{+3} to Fe^{+2}) were measured by using ferrozine [9]. The reduction of (Fe^{+3}) by 1,3-oxzoline was studied at pH 5.5, due to low solubility of iron at physiological pH, the reaction mixture contained 50 mM sodium acetate buffer (pH 5.5). 1 mM ferrozine, 50, 100 µM of tested compounds and 100 µM of $Fe(NO_3)_3$. The reaction was started by the addition of $Fe(NO_3)_3$ and the increase of absorbance at 562 nm after 3 minutes was recorded, Fe^{+2} concentration was determined by using an extinction coefficient for $Fe(ferrozine)_3^{+2}$ complex which is equal to $27.9 \times 103\ M^{-1}.cm^{-1}$ [10].

2) Copper ion (Cu^{+2}) antioxidant properties (reducing activity):

The antioxidant properties of the prepared compounds containing 1,2,3-triazoline ring to reduce (Cu^{+2} to Cu^{+1}) were measured by using 2,9-dimethyl-1,10-phenanthroline (neocuproine) [11], an indicator molecule that binds specifically to the reduced form of copper (Cu^{+1} but no the oxidized form Cu^{+2}) [11]. The reaction mixture contained (20 mM) KH_2PO_4/KOH buffer (pH 7.4), 200 µM $Cu(NO_3)_2$, 600 µM 2,9-dimethyl-1,10-phenanthroline, 100 µM of the tested compounds. The mixtures were incubated at room temperature for 120 minutes and then the absorbances were recorded at 455 nm. The copper concentration was determined by using an extinction coefficient for $Cu(neocuproine)_2^{+2}$ complex which is $7.2 \times 10^3\ mM^{-1}.cm^{-1}$, that was determined by reducing Cu^{+2} with ascorbate [12].

Table (2): Antioxidant activity of compounds (1-6) against Fe^{+2}.

Compound	100 µM
1	0.0190
2	0.0049
3	0.0062
4	0.0054
5	0.0044
6	0.0052

Table (3): Antioxidant activity of compounds (1-6) against Cu^{+1}.

Compound	100 μM
0	0.82
1	0.29
2	0.53
3	0.43
4	0.58
5	0.47
6	0.54

Results and Discussion

Triazolines synthesized in this work were identified using F.T-IR, ^{1}NMR and ^{13}C-NMR and tested for their biological activity (anti-bacterial and anti-oxidant) the synthesized Triazolines were achieved by starting from sodium saccharin the well-known compound, the later was reacted with allyl chloride in DMF and gave allyl saccharin, in the other hand sodium azide was reacted with n-heptyl bromide in DMF which formed n-heptyl azide, the later was reacted with allyl saccharin to form compound (1).

Compound (1) was hydrolyzed under strong basic conditions gave compound (2) and after several steps involving the formation of ester isomer, hydrazide isomer, allyl isomer (5) again then to give compound (6).

The anti-bacterial activity for the synthesized compounds shows that the compound (1) has higher anti-bacterial activity than the other compounds and generally the compounds have higher activity in Staphylococcus Aureus and Enterobacter while have lower activity in Escherichia coli.

Triazolines (1-6) studied show higher reducing capacity for copper ions than for iron ions, this can be attributed to the standard reduction and oxidation potentials of the metals, the standard reduction potential of the Cu^{+2}/Cu^{+1} (0.15 V) which is much lower than that for Fe^{+3}/Fe^{+2} (0.77 V). Compound (1) exhibits higher anti-oxidant activity than the other compounds against both Cu and Fe.

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