DOI: 10.47743/achi-2024-1-0003 *ACTA CHEMICA IASI*, **32_1**, 33-54 (2024)

DOCKING, ANTI-BREAST CANCER AND ANTIOXIDANT STUDY OF SOME NEW SULFISOXAZOLE BASED ON CARBOHYDRATE

Mohammed Ridha A. Alhaideri* and Ezzat H. Zimam

Chemistry Department, Faculty of Sciences, University of Kufa, Iraq

Abstract

New amide **1** synthesized by reaction sulfisoxazole with acetic anhydride, is reacted with different aldehydes (p-N,N-dimethylaminobenzaldehyde and thiophen-2-carboxaldehyde to yield new chalcone **4** and **5** derivatives. Glucosepentaacetate was reacted using two different method to prepare compound **2A** and **2B**. New triazoline derivatives **6** and **7** were obtained in good yield using [3+2] strategy reaction of compound **2** with different chalcones (**4**,**5**), and study of the antioxidant activities (inhibition) for compounds **4-7** by using DPPH assay showed all selected compounds have antioxidants activity. Compound **7** is very active with an antioxidant inhibition of 82.037. Docking of compound **6** showed very good interaction with receptors THR 347 (C) and LEU 346 (C), LYS 346 (C) with S = -11.10 and rmsd =1.683 and compound **7** LYS 416 (A) with S = -10.60, rmsd = 1.687 in breast cancer (protein 6WOK).

***Keywords*:** sulfa-drugs; sulfisoxazole; DPPH; antioxidant; anti-breast cancer.

Introduction

Sulfa drugs are the first chemicals systematically used to prevent bacterial infections in humans.[1,2] They possess a wide range of biological properties such as: antifungal,[3] anticancer agent,[4] antiprotozoal,[5] anti-inflammatory,[6] antidiabetic[7] and anti-tuberculosis.[8] Sulfisoxazole

* Mohammed Ridha A. Alhaideri: *e-mail:* redhaabod@gmail.com

(sulfafurazole) is an isoxazole derivatives having at 3-position (4-aminobenzenesulfonamide) group and methyl substituted at the 4 and 5-position, its used to treat or prevent many different types of infection caused by bacteria (gram-positive and negative) such as air and bladder infection or meningitis by acting as an inhibitor to 4-aminobenzoic acid (PABA) substrate for the active site of dihydropteroate synthase enzyme (DPHS) and thus inhibits generation of dihydrofolic acid.[8-10] Synthesis of carbohydrates with triazole derivatives were synthesized by click chemistry with high yield product,[9] and these derivatives have large-skill biological activities such as antibacterial,[10] anti-fungal, antioxidant,[11] antitumor[12] and anti-diabetic agents.[13] Click reaction have several advantages. Among these, chemo selectivity of products, high stability, and good yield are offered by 1,2,3-triazoline which are synthesized by CuAAC click approach technique (10). Increase of conjugation system lead to protection of body different disease caused by free radical and antioxidants are compounds in food and drugs that scavenge and neutralize free radicals.[14-18] The graphic diagram of antioxidant shows in (Figure 1).

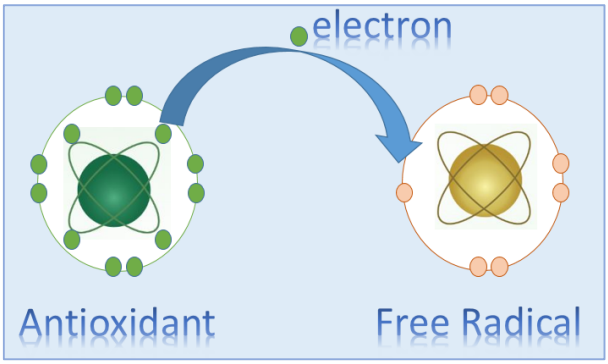
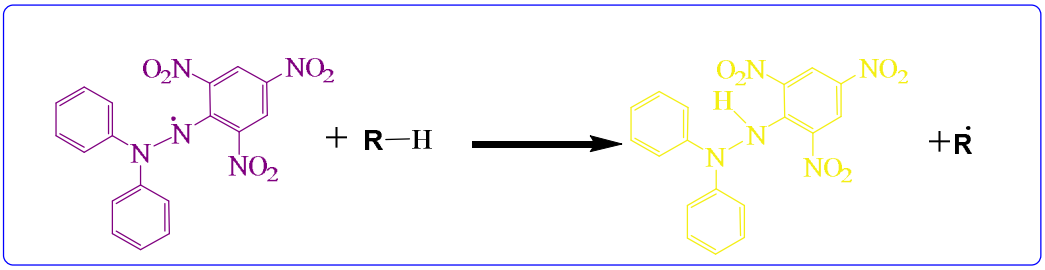


Figure 1. Graphic diagram of antioxidant.

There are many techniques for antioxidant assay spectroscopy (such as TRAP,[15] CUPRAC,[16] PFRAP,[17] and DPPH,[18,19] fluorimetric analysis,[20] electrothermal technique[21] (such as voltammetry), and chromatography[22,23] (such as gas chromatography and HPLC). DPPH is a stable free radical used for measuring the radical scavenging activity of antioxidants because its fast method, didn't have interference and used for a wide range of chemicals such as food, nano and micro-compounds[18,24] and the mechanism action of DPPH is presented in (**Scheme 1**).[25]



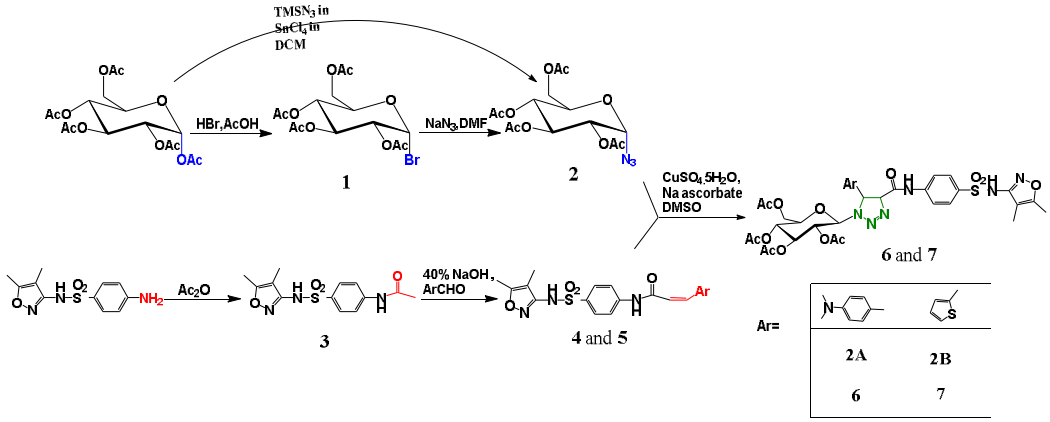
Scheme 1. The mechanism action of DPPH.

Breast cancer is the largest incident woman cancers, in 2020 near 2 million new cases expected with about 600,000 deaths, the new chemotherapeutic drugs developments is necessary because not all type of breast cancer treated with the anti-hormonal therapy.[28,29] Molecular docking is widely used to assume the binding orientation of small molecule healing applicants to their protein targets on the way to are expecting the small molecule's affinity and interest.[30,31] There are many study uses this application for study of biological activities different compounds.[12,14,15,32-35] The sterically block antagonist conformation of Helix 12 by different set of attraction in protein (6WOK) by addition different ligands.[36-38]

Results and discussion

Synthesis and identification of carbohydrate derivatives

Acetobromo-*α*-D-glucose **1** has been synthesize by substitution of acetate group in anomeric position of starting material with bromo by using HBr in acetic acid. The S_N2 reaction with sodium azide in dry DMF results a high yield of azide carbohydrate from compound **2**. In another route (GPA) is directly converted to compound **2** by adding $TMSN_3$ in the presence of $SnCl_4$ in DCM. The general synthesis pathway for triazoline derivatives is showed in Scheme 2.



Scheme 2. The general synthesis for triazoline derivatives.

Synthesis and identification of chalcone derivatives

Sulfisoxazole by primary amine reacts with acetic anhydride in the presence of glacial acetic acid to prepare amide derivatives. These compounds can be identified by the appearance of a new band at 1650 cm^{-1} in FTIR spectrum and a new signal at 1.73 ppm for CH for new methyl group for amide in ^{1}H-NMR and a new signal at 169 ppm for carbonyl $COCH_3$ and $CO\mathbf{C}H_3$ at 24 ppm ^{13}C-NMR respectively. This amide can react with a different aldehyde to prepare new chalcone derivatives.

A new chalcone can be identified by the appearance of a new signal for CH= at 6.81 and 6.66 ppm in ^{1}H-NMR and appearance of a new signal

at 112 and 118 ppm for C=C chalcone in ^{13}C-NMR spectrum for compounds **4** and **5** respectively.

Synthesis and identification of triazoline derivatives

New triazoline derivatives **6** and **7** synthesis by reaction chalcones **4** and **5** with azido sugar **2** in presence catalyst (cupric and ascorbic acid) and identified by disappearance of azido group in **2** and C=C in **4** and **5** with appearance new signals at 2.7 ppm for C**H**-Ar and 4.20 ppm for C**H**-CO with new signal at 77 ppm for new C-C $_{triazoline}$ group in ^{13}C-NMR spectrum for compound **6** . Compound **7** identified by appearance new signals at 3.05 ppm for C**H**-Ar and 4.20 ppm for C**H**-CO in ^{1}H-NMR spectrum with new signal at 70 ppm for new C-C $_{triazoline}$ group in ^{13}C-NMR spectrum.

Anti-Oxidant activates[39-43]

The most used approach for determining anti-oxidant activity was the **DPPH Radical Scavenging Method** because it's fast, simple, and sensitive to estimate the anti-oxidant activity of substances. The absorbance of the DPPH solution after added **4-7** at different times (5, 10, and 15 min.) decrease and the inhibition value shows in (**Table 1**). And in the (**Table 1**) and (**Figure 2**) shows the compound 7 have larger value of inhibition from other prepared compounds.

Table 1. The inhibition % of antioxidant activity for some sulfa drugs derivatives at different times.

Control Absorbance = 1.119 λ = 517 nm						
Comp.	After Time 5 min		After Time 10 min		After Time 15 min	
	Sample	% Inhibition	Sample Abs.	% Inhibition	Sample Abs.	% Inhibition
4	0.748	33.1546	0.742	33.6908	0.737	34.1376229
5	0.754	32.61841	0.742	33.6908	0.73	34.7631814
6	0.495	55.76408	0.47	57.99821	0.455	59.3386953
7	0.372	66.75603	0.28	74.97766	0.201	82.0375335

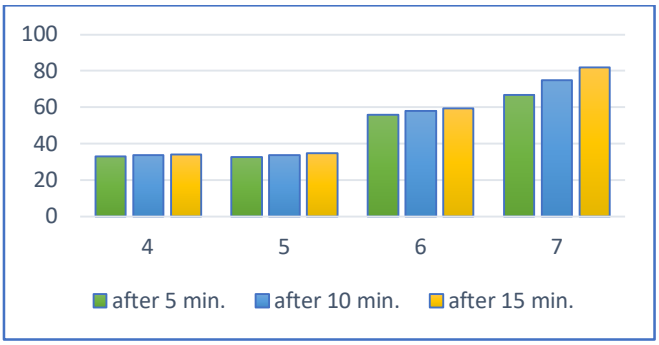


Figure 2. DPPH assay for sulfisoxazole derivatives at 5, 10, and 15 min.

All chosen derivatives **4-6** have been demonstrated antioxidants with high inhibition and compound **7** showed the best result 82.0375, and inhibition increases by increasing the time of mixing.

Docking study (Anti-Breast cancer)

The molecular Operating Environment used to docking simulations for compounds **6** and **7** shows the highest binding affinity with breast cancer (6WOK),in compound **6** heterocyclic moiety observed to play a vital role in binding through H-donor with LEU 346 and H-acceptor interaction with LYS 531 while oxygen of carbonyl of acetate group interaction with LYS 416 by H-acceptor with S = -11.10 and rmsd = 1.683, aromatic part in compound **7** attraction with amino acid (THR 347) by Pi-H interaction S = -10.60, rmsd = 1.687. And the (Figures 3 and 5) shows the 2D and (Figures 4 and 6) shows the 3D picture for interaction between ligands and receptors. And (Table 2 and 3) shows the molecular docking data for compounds 6 and 7 respectively.

Table 2. Molecular docking data of compound **6**.

Comp.	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
6	N34 52 O	LEU 346 (C)	H-donor	3.25	-0.5
	N5 7 CA	LYS 531 (C)	H-acceptor	3.49	-0.5
	O2 95 NZ	LYS 416 (A)	H-acceptor	3.03	-7.8

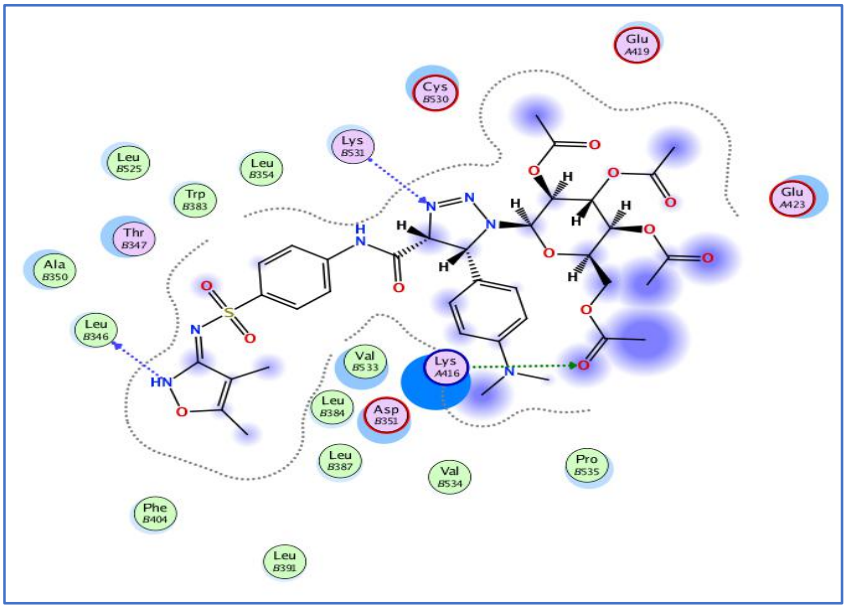


Figure 3. (2D) Molecular docking of compound **6**.

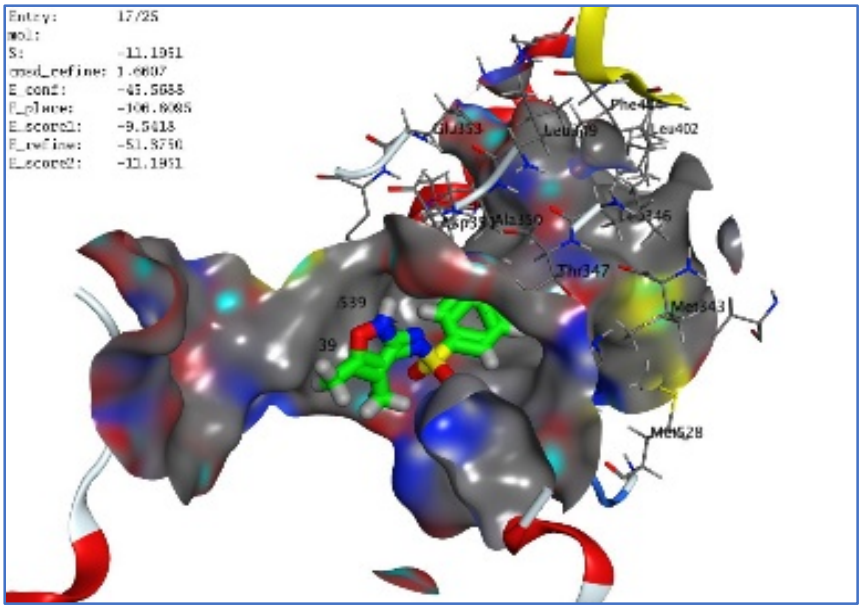


Figure 4. (3D) Molecular docking of compound **6**.

Table 3. Molecular docking data of compound **7**.

Comp.	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
7	6-ring CG2	THR 347 (C)	pi-H	4.31	-0.5

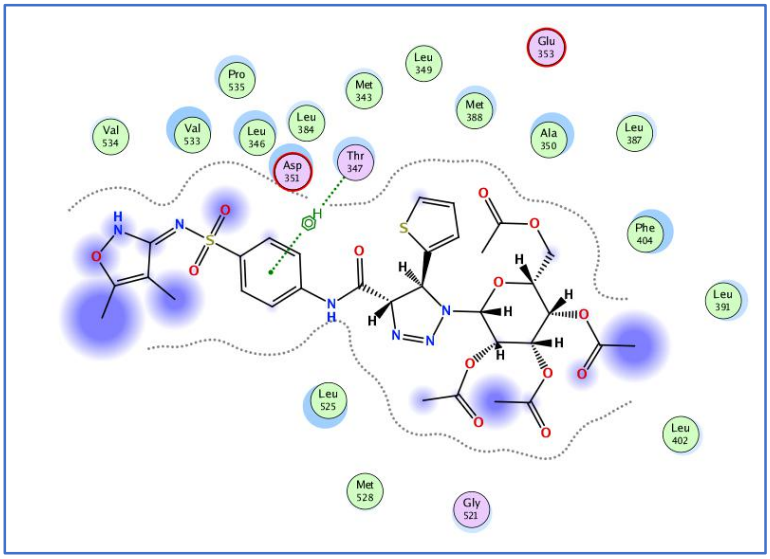


Figure 5. (2D) Molecular docking of compound **7**.

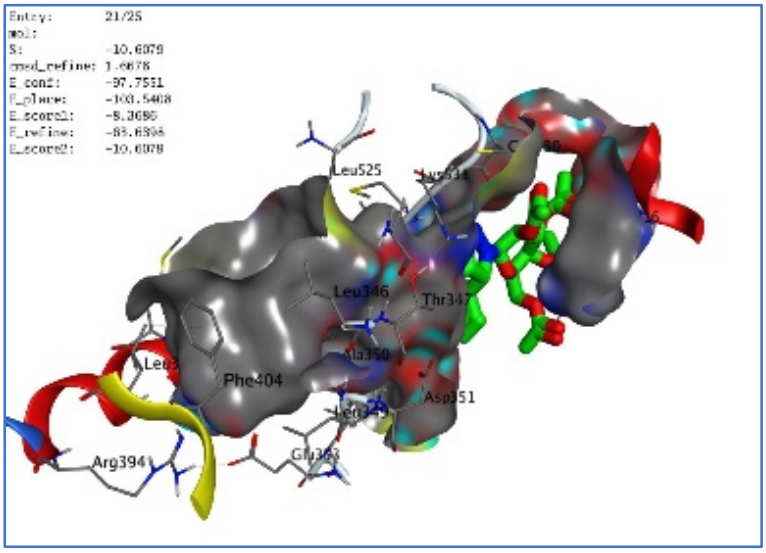


Figure 6. (3D) Molecular docking of compound **7**.

Experimental

The melting points were recorded by the Reichert Thermovar apparatus and are uncorrected. The infrared spectra are a Perkin-Elmer (model 1720) FTIR spectrometer. Nuclear magnetic resonance investigations were performed on a Bruker AC-300 or DPX-300 spectrometer (400 MHz for ^{1}H-NMR) (100 MHz for ^{13}C-NMR). The values of ppm (chemical shifts) are reported relative to TMS as a standard reference. The progress of the reaction was checked and guided by TLC using aluminum silica gel 60 F245. IR, ^{1}H-NMR, and ^{13}C-NMR.

General procedure for the synthesis of 2,3,4,6-tetra-*O*-acetyl-*α*-D-glucopyranosyl bromide **1.**[26] 1,2,3,4,6-Penta-*O*-acetyl-*β*-D-glucopyranose (24 mmol) was added portion wise (0.5 g at a time) to a stirred solution of HBr (33%) in glacial acetic acid (25 mL) at 0 ℃. After the sugar has been added, the reaction mixture was stirred at room temperature for 45 min, TLC analysis (hexane: ethyl acetate, 1:1). The reaction was quenched with ice water (50 mL), extracted with DCM (3 x 40 mL), the combined organic extracts were washed with a saturated solution of $NaHCO_3$ (2 x 50 mL), dried with anhydrous sodium sulfate (Na_2SO_4), filtered and then concentrated in vacuo.

$C_{14}H_{19}BrO_9$; Yield=90%, White ppt., m.p. = 88-90 ℃, R_f = 0.36 (1:1 ethyl acetate: n-hexane). **FTIR (KBr) cm^{-1}:** ($\upsilon_{C=O}$) 1737, ($\upsilon_{CH\ alph.}$) 2947,
(υ_{C-Br}) 667.

General procedure for the synthesis of azide sugars 2[27]

Synthesis **2A**: After dissolving (0.01mol) the compound 1 in (15 ml) DMF sodium azide (0.01 mol) was added of and the obtained mixture was

refluxed at 120 °C. The reaction course was followed by TLC ethyl acetate: hexane (2:1) as eluent. The crude material was purified by column chromatography ethyl acetate: hexane and concentrated under a rotary evaporator to give the desired compound.

$C_{14}H_{19}N_3O_9$; Yield=84%, White ppt., m.p. = 120-121 °C, R_f = 0.48 (1:1 ethyl acetate: *n*-hexane).

FTIR (KBr) cm^{-1} 2A: (υ_{N3}) 2115, ($\upsilon_{CH\ alph.}$) 2951, ($\upsilon_{C=O}$) 1738.

Synthesis **2B**: Trimethylsilyl azide (190 mmol, 15.5 mL) was added to a GPA (82 mmol, 31 g) in dry DCM (450 mL) with $SnCl_4$ and stirred for 3 h, added (300 mL DCM), washed three times with 10% K_2CO_3 and twice with brine and crystallized from EtOAc/hexane. Yield = 92%.

FTIR (KBr) cm^{-1} 2B: (υ_{N3}) 2117, ($\upsilon_{CH\ alph.}$) 2956, ($\upsilon_{C=O}$) 1737. MS: 373 (M^+).

*Synthesis of N-(4-(N-(4,5-dimethylisoxazol-3-yl)sulfamoyl)phenyl)acetamide **3***

Sulfisoxazole (1 mmol) was added to acetic anhydride (1 mmol) with glacial acetic acid (few drops) and stirred for 72 h. The reaction course was followed by TLC ethyl acetate: hexane (3:2) as eluent. The precipitate was filtered and washed few times by distilled water and crystallized by abs. ethanol.

$C_{13}H_{15}N_3O_4S$; Yield=70%, White solid, m.p. = 142-146 °C. R_f=0.40 (3:2 ethyl acetate: *n*-hexane). **FTIR (KBr) cm^{-1}:** (υ_{NH}) 3336, ($\upsilon_{C=O}$) 1705, ($\upsilon_{CHar.}$) 3054, ($\upsilon_{CH\ alph.}$) 2931. **^{1}H-NMR (400 MHz, DMSO-d^6)** δ: 1.61 (s, 3H, (CO-C<u>H</u>$_3$)), 2.07 and 2.11 (s, 6H, 2(-C<u>H</u>$_3$)), 6.6-7.9 (m,4H(-C<u>Har</u>)), 10.53 (s, 1H(-SO$_2$<u>NH</u>)), 10.39 (s, 1H,<u>NH$_{amide}$</u>), 2.62 (s (DMSO)). **^{13}C-NMR (101 MHz, DMSO-d^6) δ:** 24 (CO-<u>C</u>H$_3$), 169 (<u>C</u>O-CH$_3$), 144 (<u>C1</u>ar.), 118 (<u>C2,5</u>ar.), 127 (<u>C3,5</u>ar.), 130 (<u>C4</u>ar.), 133 (<u>C1</u>oxa.ring),113 (<u>C2</u>oxa.ring),162 (<u>C3</u>oxa. ring) , 6 (-<u>C</u>H$_3$) , 10 (<u>C</u>H$_3$).

*General procedure for the synthesis of chalcone derivatives **4** and **5***[28] Sulfisoxazole amide (0.01mol) was stirred with 40% NaOH. After 10 min. an equimolar (0.01 mol) aromatic aldehyde derivatives (*N*,*N*-dimethylaminobenzaldehyde, and thiophen-2-carboxaldehyde) in ethanol (20 mL) was added and stirred until the reaction was completed. The reaction course was monitored by TLC using (ethyl acetate: *n*-hexane 4:1). The obtained precipitate was washed well with cold D.W. and recrystallized from abs. ethanol.

((Z)-3-(4-(dimethylamino)phenyl)-N-(4-(N-(4,5-dimethylisoxazol-3-yl)sulfamoyl)phenyl)acrylamide) 4: $C_{22}H_{24}N_4O_4S$; Yield = 70 % , Yellow solid, m.p. = 180-182 °C, R_f=0.32 (4:1 ethyl acetate: n-hexane), time of reaction= 10 h. **FTIR (KBr) cm^{-1}:** (υ NHCOR) 3314, (υCH alph.) 2906, (υCH ar.) 3045, (υC=O) 1663, (υC=C) 1591. **^{1}H-NMR (400 MHz, DMSO-*d*^{6})** δ: 3.23 (s, 6H, 2(-C<u>H</u>$_3$)), 3.35 (s, 6H, 2(NC<u>H</u>$_3$)), 6.81 (d, J = 3 MHz, 2H, 2(-C<u>H</u>=)), 6.80-7.72 (m,4H(-C<u>H</u>ar)), 10.07(s, 1H(-SO$_2$<u>NH</u>)), 9.68 (s, 1H(<u>NH</u>)), 2.62 (s (DMSO)). **^{13}C-NMR (101 MHz, DMSO-*d*^{6}) δ:** 24 (CO-<u>C</u>H$_3$), 166 (<u>C</u>O-CH$_3$), 148 (<u>C1</u>ar.), 124 (<u>C2,5</u>ar.), 129 (<u>C3,5</u>ar.), 131 (<u>C4</u>ar.), 144 (<u>C1</u>oxa.ring), 111 (<u>C2</u>oxa.ring), 156 (<u>C3</u>oxa. ring), 6 (-<u>C</u>H$_3$), 10 (<u>C</u>H$_3$).

((*Z*)-*N*-(4-(*N*-(4,5-dimethylisoxazol-3-yl)sulfamoyl)phenyl)-3-(thiophen-2-yl)acrylamide) 5: $C_{18}H_{17}N_3O_4S_2$; Yield = 80 %, orange ppt., m.p. = 126 °C, R_f = 0.38 (4:1 ethyl acetate: *n*-hexane), time of reaction = 7 h. **FTIR (KBr) cm^{-1}:** (υ NHCOR) 3312, (υCH alph.) 2846, (υCH ar.) 3043, (υC=O) 1676, (υC=C) 1536. **^{1}H-NMR (400 MHz, DMSO-*d*^{6})** δ: 2.5 (s, 6H, 2(-C<u>H</u>$_3$)), 6.66 (d, J = 8.2 Hz, 2H, (-C<u>H</u>=)), 7.41 (d, J=8.2 Hz, 2H, (-C<u>H</u>=)), 7.10, 7.63 (m,4H(-C<u>H</u>ar)), 10.53 (s, 1H(-SO$_2$<u>NH</u>)), 10.40 (s, 1H(<u>NH</u>)), 7.80 (<u>CH$_1$</u>thiazol), 7.70 (<u>CH$_2$</u> thiazol),7.68 (<u>CH$_3$</u> thiazol). **^{13}C-NMR (101 MHz, DMSO-*d*^{6})** δ: 24 (CO-<u>C</u>H$_3$), 169 (<u>C</u>O-CH$_3$),133 (<u>C1</u>ar.), 125 (<u>C2,5</u>ar.), 128

(C3,5ar.), 130 (C4ar.), 155 (C1oxa.ring),113 (C2oxa.ring), 161 (C3oxa. ring), 6 (-CH_3), 10 (CH_3).

General procedure for synthesis of 1,2,3-triazoline derivatives ***6*** *and* ***7***[29]

To a solution of azido sugar **2** (0.01 mol) in *t*-butanol: D.W. (1:1), the chalcone derivatives **4** and **5** (0.01 mol), cupric sulfate pentahydrate (1.78 mol, 0.444 g) and sodium ascorbate (1.78 mol, 0.35 g) were added and the mixture was refluxed at 70 °C for 68-72 hrs. The reaction was controlled by TLC (ethyl acetate: hexane 2:1). The solvent was reduced by a rotary evaporator, extracted by $CHCl_3$, the organic layer dried by anhydrous sodium sulfate (Na_2SO_4), filtered, and the solvent was removed under vacuum. The crude material was purified by flash column chromatography (ethyl acetate: hexane 2:1).

(2R,3R,5R,6R)-2-(acetoxymethyl)-6-(5-(4-(dimethylamino)phenyl)-4-((4-(N-(4,5-dimethylisoxazol-3-yl) sulfamoyl)phenyl)carbamoyl)-4,5-dihydro-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate 6: $C_{36}H_{43}N_7O_{13}S$; Yield = 66 %, pale yellow oil, R_f= 0.32 (2:1 ethyl acetate: n-hexane), time of reaction = 72 hrs. **FTIR (KBr) cm^{-1}:** (υ_{NH}) 3333, ($\upsilon_{CH\ ar.}$) abs., ($\upsilon_{CH\ alph.}$) 2921, ($\upsilon_{C=C}$) 1678, ($\upsilon_{CO.}$) 1678. **^{1}H-NMR (400 MHz, DMSO-*d*^{6})** δ: 2.72 (t, 2H triazoline), 4.2 (t, 2H triazoline), 1.35 (s, 6H, 2(-CH_3)), 2.57 (s, 6H, 2(NCH_3)), 1.55 (s, 12H, COCH_3)), 7.5-7.9 (m, 4H(-CHar)), 11.8 (s, 1H(-SO_2NH)), 5.78 (m, 1H), 4.95 – 4.90 (m, 1H), 4.87 – 4.85 (m, 1H), 4.16 (dd, *J* = 12.4, 6.7 Hz, 2H), 4.15 – 4.00 (m, 1H) of CH tetrahydropyran, 3.72-3.38 (ddd, *J* = 11.3, 8.7, 3.0 Hz, 1H), 7.19 (s ($CDCl_3$)). **^{13}C-NMR (101 MHz, DMSO-*d*^{6})** δ: 163 (CO-CH_3), 148 (C1ar.), 124 (C2,5ar.), 129 (C3,5ar.), 131 (C4ar.), 139 (C1oxa.ring), 114 (C2oxa.ring), 156 (C3oxa. ring), 77 (CH_2CO-triazoline ring), (72, 68, 64,

55, 53) C of tetrahydropyran, 48 ($\underline{C}H_2OAc$), 29 (CO-$\underline{C}H_3$), 14 (-$\underline{C}H_3$), 10 ($\underline{C}H_3$).

(2R,3R,5R,6R)-2-(acetoxymethyl)-6-(4-((4-(N-(4,5-dimethylisoxazol-3-yl)sulfamoyl)phenyl) carbamoyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate 7: $\mathbf{C_{32}H_{36}N_6O_{13}S_2}$; Yield = 70 %, white oil, R_f = 0.28 (2:1 ethyl acetate: *n*-hexane), time of reaction = 68 hrs. **FTIR (KBr) cm^{-1}:** (υ_{NH}) 3164, ($\upsilon_{CH\ ar.}$) 3014, ($\upsilon_{CH\ alph.}$) 2978, ($\upsilon_{C=C}$) 1673, ($\upsilon_{CO.}$) 1673. **^{1}H-NMR (400 MHz, DMSO-*d*^{6})** δ: 2.56 (t, 2H triazoline), 4.2 (t, 2H triazoline), 1.18 (s, 6H, 2(-C$\underline{H_3}$)), 1.54-2.04 (s, 12H, COC$\underline{H_3}$)), 7.1-7.7 (m, 4H(-C$\underline{H}$ar)), 9.83 (s, 1H ($\underline{NH}$)), 4.67 – 4.75 (t, 2H of methylene), 5.20 (t, *J* = 9.5 Hz, 1H), 5.05 – 4.87 (m, 1H), 4.23 – 4.20 (m, 1H), 4.18 (dd, *J* = 11.2, 6.9 Hz, 2H), 4.17–3.70 (m, 1H) of CH tetrahydropyran, 3.69 (ddd, *J* = 10.2, 4.8, 2.3 Hz, 1H), 3.67 – 3.52 (m, 1H), 7.19 (s ($CDCl_3$)). **^{13}C-NMR (101 MHz, DMSO-*d*^{6})** δ: 171, 169 ($\underline{C}O$-CH_3), 77 ($\underline{C}H_2CO$-triazoline ring), 72 ($\underline{C}H_2$-triazoline ring), 147 ($\underline{C1}$ar.), 120 ($\underline{C2,5}$ar.), 130 ($\underline{C3,5}$ar.), 134 ($\underline{C4}$ar.), 138 ($\underline{C1}$oxa.ring), 110 ($\underline{C2}$oxa.ring), 127 ($\underline{C3}$oxa. ring), (77, 67, 73,69,76) $\underline{C}$ of tetrahydropyran, 61 ($\underline{C}H_2OAc$), 29 (CO-$\underline{C}H_3$), 20 (-2$\underline{C}H_3$).

Antioxidant assay: (42,43)

Preparation of DPPH (3 mg/100mL) solution

3 mg of DPPH dissolve in (100 mL) beaker with about 20 mL of methanol, and transfer into (100 mL) volumetric flask and complete with methanol.

Preparation solution (50 µ/mL) of sample solution

5 mg of compounds (**4**, **5**, **6**, and **7**) were dissolved in about 20 mL of methanol, then transferred into 100 mL volumetric flask and filled with methanol.

Determination of inhibition

Mixing sample solution (50 µg/mL) and DPPH solution tested intervals of 5, 10, and 15 min, the absorbance measured at λ_{max} = 517 nm. The % inhibition was calculated by apply equation below.

$$\%\ inhibition\ of\ activity\ of\ DPPH = \frac{A - B}{A} * X\ 100$$

where A is the control (DPPH and methanol) absorbed density and B is the sample absorbed density.

Molecular docking studies

The Glide module of the Schrödinger program installed in Intel (R) Core(TM) i5-8350U CPU @ 1.70GHz, 1.90 GHz, and docking experiments with moderately and highly active were conducted. The Chemdraw 22.0.0 program was used to design the specific ligands, the protein data repository RCSB was the chosen source and provided the protein structure.

Ligand and protein preparation

Reduce energy as ligands were introduced to the workstation, the Optimized Potentials for Liquid Simulations (OPLS3e) force field was implemented in Ligprep (Version 2019 - 1, Schrodinger). The best ligand conformations output file was used in docking studies. The Schrodinger protein preparation wizard was the primary protein preparation and minimization tool, a hydrogen atom added to it, and charges were also assigned. Generated Het states were done with Epik at a pH of 7.0 2.0. The protein is preprocessed, refined and modified by evaluating the water molecules and other factors in the working space. The most important water molecules were unaltered, removed all other molecules save those

containing heteroatoms. The OPLS3 force field was used to achieve maximum protein minimization. Using the ligand that was co-crystallized with the protein of interest (PDB-4GQR), a grid was constructed that represents the active site of the chosen target molecules. Root mean square deviation (RMSD) was used to verify the protein after the last docking step with the co-crystal ligand in XP mode, and the value was found to be in the region of 0.46. By selecting inhibitory ligands, a receptor grid (X-ray posture of the ligand in the protein; PDB:4GQR) is created around the protein. The Vander Waal radii of the receptor atoms were scaled to 1.00 if a partial atomic charge is 0.25, and a box was created around the centroid of the ligand.

Docking and analysis

The described protein and ligands were used as input for molecular docking experiments. XP (Version 2019-1, Schrodinger) was used to evaluate the results of the docking studies. The compounds' SMILES format is created using OSIRIS Datawarrior. Schrödinger's Glide module was used to perform docking experiments on planned and produced compounds. We used XP mode (ultra-precision) for all docking calculations. The atomic weight of the protein is reduced by a factor of 0.8 and the partial atomic atomic charge of the atoms is less than 0.15. Selected the best-docked conformation based on Glide docking score. Deeper analysis of the interactions between these docked conformations was done using the XP-visualizer.

Conclusion

A series of carbohydrate based new chalcone, triazoline of sulfisoxazole were synthesized and characterized using IR, ^{1}H-NMR and

^{13}C-NMR. DPPH antioxidant activity was performed on all compounds and the results indicated compound **7** as the best candidate in terms of percent inhibition. Anticancer activity corroborated with molecular docking was employed and results were promising, with compounds **6** and **7** being the most active from the tested series.

References

1. El-ghamry, H.; Takroni, K.; AL-Rashidi, D.; Alfear, E.; Alsaedi, R. Design, spectral, thermal decomposition, antimicrobial, docking simulation and DNA binding tendency of sulfisoxazole azo dye derivative and its metal chelates with Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+}. *Appl. Organomet. Chem.* **2022**, *36*, e6813.

2. Aminov, R. History of antimicrobial drug discovery: Major classes and health impact. *Biochemical Pharmacology*. **2017**, *133*, 4 – 19.

3. Nasr, T.; Bondock, S.; Eid, S. Design, synthesis, antimicrobial evaluation and molecular docking studies of some new thiophene, pyrazole and pyridone derivatives bearing sulfisoxazole moiety. *Eur. J. Med. Chem*. **2014**, *84*, 491 – 504.

4. Alyar, S.; Özmen, Ü.; Adem, Ş.; Alyar, H.; Bilen, E.; Kaya, K. Synthesis, spectroscopic characterizations, carbonic anhydrase II inhibitory activity, anticancer activity and docking studies of new Schiff bases of sulfa drugs. *J. Mol. Struct*. **2021**, *5*, 1223.

5. Ojuromi, O. T.; Ashafa, A. O. An overview of some medicinal plants and isolated active compounds with potential antiprotozoal activity. *Trop. J. Pharm. Res.* **2020**, *19*, 1551 – 1563.

6. Pallavi, H. M.; Al-Ostoot, F. H.; Vivek, H. K.; Khanum, S. A. Synthesis, characterization, DFT, docking studies and molecular dynamics of some 3-phenyl-5-furan isoxazole derivatives as anti-inflammatory and anti-ulcer agents. *J. Mol. Struct.* **2022**, *1250*, 131812.

7. Dayma, V.; Chopra, J.; Sharma, P.; Dwivedi, A.; Tripathi, I. P.; Bhargava, A. Synthesis, antidiabetic, antioxidant and anti-inflammatory

activities of novel hydroxytriazenes based on sulpha drugs. *Heliyon* **2020**, *6*, e04787.

8. Courtice, R.; Sniatynski, M.; Rubin, J. E. Characterization of antimicrobial-resistant *Escherichia coli* causing urinary tract infections in dogs: Passive surveillance in Saskatchewan, Canada 2014 to 2018. *J. Vet. Intern. Med*. **2021**, *35*, 1389 – 1396.

9. Sabry, E.; Mohamed, H. A.; Ewies, E. F.; Kariuki, B. M.; Darwesh, O. M.; Bekheit, M. S. Microwave-assisted synthesis of novel sulfonamide-based compounds bearing α-aminophosphonate and their antimicrobial properties. *J. Mol. Struct*. **2022**, *1266*, 133553.

10. Lolak, N.; Akocak, S.; Durgun, M.; Duran, H. E.; Necip, A.; Türkeş, C. Novel bis-ureido-substituted sulfaguanidines and sulfisoxazoles as carbonic anhydrase and acetylcholinesterase inhibitors. *Mol. Diversity* **2023**, *27*, 1735 – 1749.

11. Tiwari, V. K.; Mishra, B. B.; Mishra, K. B.; Mishra, N.; Singh, A. S.; Chen, X. Cu-catalyzed click reaction in carbohydrate chemistry. *Chem. Rev*. **2016**, *116*, 3086 – 240.

12. Tashkandi, N. Y.; Al-Amshany, Z. M.; Hassan, N. A. Design, synthesis, molecular docking and antimicrobial activities of novel triazole-ferulic acid ester hybrid carbohydrates. *J. Mol. Struct*. **2022**, *1269*, 133832.

13. Jenifer, V. R.; Muthuvel, P.; Das, T. M. Rational design of heterocyclic moieties incorporated in [1,2,3]sugar-triazole derivatives for antioxidant studies. *ChemistrySelect* **2021**, *6*, 9955 – 9959.

14. El Azab, I. H.; El-Sheshtawy, H.S.; Bakr, R. B.; Elkanzi, N. A. New 1,2,3-triazole-containing hybrids as antitumor candidates: design, click reaction synthesis, DFT calculations, and molecular docking study. *Molecules* **2021**, *26*, 708.

15. Fallah, Z.; Tajbakhsh, M.; Alikhani, M.; Larijani, B.; Faramarzi, M.; Hamedifar, H. A review on synthesis, mechanism of action, and structure-activity relationships of 1,2,3-triazole-based α-glucosidase

inhibitors as promising anti-diabetic agents. *J. Mol. Struct*. **2022**, *1255*, 132469.

16. Akbari, B.; Baghaei-Yazdi, N.; Bahmaie, M.; Mahdavi, F. The role of plant-derived natural antioxidants in reduction of oxidative stress. *BioFactors* **2022,** *48*, 611 – 633.

17. Altomare, A.; Baron, G.; Brioschi, M.; Longoni, M.; Butti, R.; Valvassori, E. N-acetyl-cysteine regenerates albumin cys34 by a thiol-disulfide breaking mechanism: An explanation of its extracellular antioxidant activity. *Antioxidants* **2020**, *9*, 367.

18. Dziurka, M.; Kubica, P.; Kwiecień, I.; Biesaga-Kościelniak, J.; Ekiert, H.; Abdelmohsen, S. In vitro cultures of some medicinal plant species (Cistus × incanus, verbena officinalis, scutellaria lateriflora, and scutellaria baicalensis) as a rich potential source of antioxidants-evaluation by cuprac and quencher-cuprac assays. *Plants* **2021**, *10*, 1 – 10.

19. Skroza, D.; Šimat, V.; Vrdoljak, L.; Jolić, N.; Skelin, A.; Čagalj, M. Investigation of antioxidant synergisms and antagonisms among phenolic acids in the model matrices using FRAP and ORAC methods. *Antioxidants* **2022**, *11*, 1784.

20. Munteanu, I. G.; Apetrei, C. Analytical methods used in determining antioxidant activity: A review. *Int. J. Mol. Sci*. **2021**, *22*, 3380.

21. Nopitasari, N.; Suhartati, T.; Suharso.; Herasari, D.; Pandiangan, K. D.; Hadi, S. Synthesis, characterization, and antioxidant activity of some organotin (IV) 2-nitrobenzoate using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method. *J. Phys.: Conf. Ser*. **2021**, *1751*, 12098.

22. Abeyrathne, E.; Nam, K.; Ahn, D. U. Analytical methods for lipid oxidation and antioxidant capacity in food systems. *Antioxidants* **2021**, *10*, 1587.

23. Rajauria, G.; Ravindran, R.; Garcia-Vaquero, M.; Rai, D. K.; Sweeney, T.; O'Doherty, J. Molecular characteristics and antioxidant activity of laminarin extracted from the seaweed species *Laminaria hyperborea*,

using hydrothermal-assisted extraction and a multi-step purification procedure. *Food Hydrocoll*. **2021**, *112*, 106332.

24. Dabetic, N.; Todorovic, V.; Malenovic, A.; Sobajic, S.; Markovic, B. Optimization of extraction and HPLC–MS/MS profiling of phenolic compounds from red grape seed extracts using conventional and deep eutectic solvents. *Antioxidants* **2022**, *11*, 1595.

25. Flieger, J.; Flieger, M. The [DPPH·/DPPH-H]-HPLC-DAD method on tracking the antioxidant activity of pure antioxidants and goutweed (*Aegopodium podagraria L.*) hydroalcoholic extracts. *Molecules* **2020**, *25*, 6005.

26. Marano, S.; Minnelli, C.; Ripani, L.; Marcaccio, M.; Laudadio, E.; Mobbili, G.; Amici A.; Armeni T.; Stipa P. Insights into the antioxidant mechanism of newly synthesized benzoxazinic nitrones: *In vitro* and *in silico* studies with DPPH model radical. *Antioxidants* **2021**, *10*, 1224.

27. Liang, N.; Kitts, D. D. Antioxidant property of coffee components: Assessment of methods that define mechanism of action. *Molecules* **2014**, *19*, 19180 – 19208.

28. Ottoni, F. M.; Gomes, E. R.; Pádua, R. M.; Oliveira, M. C.; Silva, I. T.; Alves, R. J. Synthesis and cytotoxicity evaluation of glycosidic derivatives of lawsone against breast cancer cell lines. *Bioorg. Med. Chem. Lett*. **2020**, *30*, 126817.

29. Ananth, A. H.; Manikandan, N.; Rajan, R. K.; Elancheran, R.; Lakshmithendral, K.; Ramanathan, M.; Bhattacharjee, A.; K. Senthamaraikannan. Design, synthesis, and biological evaluation of 2-(2-bromo-3-nitrophenyl)-5-phenyl-1,3,4-oxadiazole derivatives as possible anti-breast cancer agents. *Chem. Biodivers*. **2020**, *17*, e9122.

30. Rudrapal, M.; Egbuna, C. *Computer aided drug design (CADD): from ligand-based methods to structure-based approaches*. Elsevier, Amsterdam, 2022; pp 125 – 155.

31. Stanzione, F.; Giangreco, I.; Cole, J. C. Use of molecular docking computational tools in drug discovery. *Prog. Med. Chem*. **2021**, *60*, 273 – 343.

32. Ünlü, A.; Özmen, Ü. Ö.; Alyar, S.; Öztürk, A.; Alyar, H.; Gündüzalp, A.B. Biological evaluation of Schiff bases containing dopamine as antibacterial/antifungal and potential Anti COVID-19 agents: Design, synthesis, characterization, molecular docking studies, and ADME properties. *J. Mol. Struct*. **2023**, *1293*, 136318.

33. Benjamin, I.; Udoikono, A. D.; Louis, H.; Agwamba, E. C.; Unimuke, T.O.; Owen, A.E. Antimalarial potential of naphthalene-sulfonic acid derivatives: Molecular electronic properties, vibrational assignments, and in-silico molecular docking studies. *J. Mol. Struct*. **2022**, *1264*, 133298.

34. Dai, R.; Bao, X.; Zhang, Y.; Huang, Y.; Zhu, H.; Yang, K.; Wang, B.; Wen, H.; Li, W.; Liu, J. Hot-Spot Residue-Based Virtual Screening of Novel Selective Estrogen-Receptor Degraders for Breast Cancer Treatment. *J. Chem. Inf. Model*. **2023**, *63*, 7588-7602.

35. Bhat, M.; Ganavi, B. C.; Sagar, B. K.; Vijay, S. E. Sulfadiazine guanidyl derivatives: Synthesis, characterization and docking studies for potential antituberculosis agents. *Asian J. Chem*. **2018**, *30*, 2141 – 2144.

36. Liang, J.; Blake, R.; Chang, J.; Friedman, L. S.; Goodacre, S.; Hartman, S. Discovery of GNE-149 as a Full Antagonist and Efficient Degrader of Estrogen Receptor alpha for ER+ Breast Cancer. *ACS Med. Chem. Lett*. **2020**, *11*, 1342 – 1347.

37. Zbieg, J. R.; Liang, J.; Li, J.; Blake, R. A.; Chang, J.; Friedman, L.; Friedman L.; Goodacre, S.; Hartman, S. J.; Rei, I. E.; Kiefer, J. R.; Kleinheinz, T.; Labadie, S.; Lai, T.; Liao, J.; McLean, N.; Metcalfe, C.; Mody, V.; Nannini, M.; Ortwine, D. F.; Ran, Y.; Ray, N.; Roussel, F.; Sambrone, A.; Sampath, D.; Vinogradova, M.; Wai, J.; Wang, T.; Yeap, K.; Zhang, B.; Zheng, X.; Zhong, Y.; Wang, X. Discovery of GNE-502 as an orally bioavailable and potent degrader for estrogen receptor positive breast cancer. *Bioorg. Med. Chem. Lett*. **2021**, *50*, 128335.

38. Bevacqua, A.; Bakshi, S.; Xia, Y. Principal component analysis of alpha-helix deformations in transmembrane proteins. *PLoS One* **2021**, *16*, e0257318.

39. Abd Elkader, A. M.; Labib, S.; Taha, T. F.; Althobaiti, F.; Aldhahrani, A.; Salem, H. M. Saad, A.; Ibrahim, F. M. Phytogenic compounds from avocado (*Persea americana L.*) extracts; antioxidant activity, amylase inhibitory activity, therapeutic potential of type 2 diabetes. *Saudi J. Biol. Sci*. **2022**, *29*, 1428 – 1433.

40. Wairata, J.; Fadlan, A.; Purnomo, S., A.; Taher, M.; Ersam, T. Total phenolic and flavonoid contents, antioxidant, antidiabetic and antiplasmodial activities of Garcinia forbesii King: A correlation study. *Arab. J. Chem*. **2022**, *15*, 103541.

41. Arguelles, E. D. Preliminary studies on the potential antioxidant and antidiabetic activities of sargassum polycystum C. Agardh (Phaeophyceae, Ochrophyta). *Jordan J. Biol. Sci*. **2022**, *15*, 449 – 456.

42. Arraq, R. R.; Hadi, A. G. Enhanced the antioxidant activity of tri organotin (IV) complexes derived from cephalexin. *Azerb. Med. Assoc. J*. **2022**, *62*, 2197 – 2209.

43. Arraq, R. R.; Hadi, A. G. Synthesis, identification, and anti-oxidant activity of di-organotin (IV)-cephalexin complexes. *J. Med. Chem. Sci.* **2023***, 6*, 392 – 401.

44. Hao, S.; Cheng, X.; Wang, X.; An, R.; Xu, H.; Guo, M.; Li, C.; Wang, Y.; Hou, Z.; Guo, C. Design, synthesis and biological evaluation of novel carbohydrate-based sulfonamide derivatives as antitumor agents. *Bioorg. Chem*. **2020**, *104*, 104237.

45. Mohammed, M. J.; Kadhum, A. H.; Mohammed, A. I.; Al-Rekabi, S.H. Synthesis of one new sugar imine molecule. *J. Chem. Soc. Pakistan* **2020**, *42*, 103 – 108.

46. Mahmood, Y. H.; Wassim, A. R.; Noor, K. I.; Abduljabaar, J. A. Synthesis, characterization and antibacterial activity of chalcones

derivatives from 4-hydroxyacetophenone as a starting material. *Int. J. Adv. Sci. Eng. Technol*. **2020**, *8*, 10 – 14.

47. Adam, R. W.; Zimam, E. H. Design, synthesis, anticancer activity and molecular docking of new 1,2,3-triazole combined glucosides with coumarin. *J. Popul. Ther. Clin. Pharmacol*. **2023**, *30*, e345 – 356.