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Synthesis of Some New Thioglycosides Derived from Thieno[2,3-*d*]pyrimidine Derivatives and Their Anticancer and Antioxidant Activity.

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ABSTRACT

A series of new substituted 2-glucopyranosylthiothieno[2,3-*d*]pyrimidine derivatives have been synthesized. The products were elucidated by spectroscopic data. Some of the isolated products were tested for biologically activity as antioxidants and anticancer.

Keywords: Cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-one; 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide; *S*-Glycoside; Anticancer and Antioxidant Activity.

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INTRODUCTION

Derivatives of Thienopyrimidine possess construction parallel to biogenic purines. Derivatives of thienopyrimidine can be regard as tolerable nucleic acid antimetabolites and possess salient activity, e.g. antibacterial [**1–4**], antifungal agents [**5,6**], ant malarial [**7**], analgesics [**8–12**], anti-inflammatory [**9–14**], antipyretic [**10**], antihistaminic agents [**15**], antihypertensive agents [**16,17**], gastric ant secretory [**18**], potential spasmolytic agents [**19**], selective 5-HT3 receptor ligands CNS [**20**], hypnotics [**21**], herbicides [**22,23**], pesticides [**24**], plant growth regulators [**23**]. Nucleosides of thieno [2,3-*d*] pyrimidine were reported as anti-(Human Immunodeficiency Virus-1) HIV-1 [**25**] and antitumor [**26, 27**]. In continuation of our interest in the synthesis of bioactive heterocyclic compounds and nucleosides [**28-34**], we report here the synthesis of novel thioglycosides of thieno[2, 3-*d*]pyrimidine.

MATERIALS AND METHODS

All melting points were determined and measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan) and are uncorrected. Micro analytical data were performed by Vario El-Mentar apparatus (Shimadzu, Japan), National Research Centre (NRC), Cairo, Egypt. IR spectra (KBr) were recorded on a Perkin-Elmer 1650 spectrophotometer, NRC, Cairo, Egypt. ^{1}H NMR and ^{13}C NMR spectra were recorded using Jeol ECA 500 and EX-270 spectrometer, Central Labs at NRC, Cairo, Egypt. The chemical shift was expressed in ppm and the coupling constants *J* were reported in Hz. Mass spectra were recorded at 70 eV on EI Ms-QP 1000 EX (Shimadzu, Japan), NRC, and Cairo, Egypt. Measurement of UV carried out using instrument spectrum 2, model V-630, serial No.c321561148, NRC, Cairo, Egypt.

EXPERIMENTAL

Synthesis of 1

A mixture of cyclopentanone (0.84 g, 10 mmol) and ethyl cyanoacetate (1.13 g, 10 mmol) in presence of diethylamine (0.73 g, 10 mmol) was stirred for 15 minutes. To the resulting solution was added elemental sulfur (0.32 g, 10 mmol) portion-wise while heating it on a water bath till complete dissolution of sulfur and left to stand overnight in the refrigerator. The brownish red crystals formed were filtered off and washed with cold ethanol to give brown precipitate. The latter precipitate was collected by filtration and crystallized from ethanol to give the title compound **1**.

Ethyl 2-amino-5, 6-dihydro-4H-cyclopenta[b]thiophene-3-carboxylate (1)

Yield 85%; Mp 85-87 °C (Lit. Mp 90-91 °C). [**35**]

Synthesis of 2

To vigorously stirred solution of **1** (2.11 g; 10 mmol) in dimethyl sulfoxide (10 mL) at room temperature, carbon disulfide (0.60 mL, 10 mmol) and aqueous sodium hydroxide (2.50 g dissolved in 3 mL water) solution were added simultaneously over 30 minutes and the stirring was continued for further 30 min. Dimethyl sulfate (0.94 ml, 10 mmol) was added drop wise to the reaction mixture with stirring at 5-10 °C. It was further stirred for (3 h) and poured in into ice-water; the solid obtained was filtered off, dried and recrystallized from ethanol/dioxane (1: 1).

Methyl-N-(3-ethoxycarbonyl-5,6-dihydro-4H-cyclopenta[b]thien-2-yl)dithiocarbamate (2)

Pale brown powder in yield 60 %; Mp 115–117 °C. IR (KBr, υ, cm^{-1}): 3419 (NH), 1659 (C=O), 1054 (C=S). ^{1}H NMR ($CDCl_3$, δ ppm): 1.37 (t, 3H, *J* = 5.0 Hz, CH_3), 2.38-2.56 (m, 2H, CH_2), 2.68 (s, 3H, CH_3), 2.72 (t, 2H, *J* = 5.0 Hz, CH_2), 2.83 (t, 2H, *J* =5.0 Hz, CH_2), 4.35 (q, 2H, *J* = 5.0 Hz, CH_2), 12.7 (br s, 1H, NH exchangeable with D_2O). ^{13}C NMR ($CDCl_3$, δ, ppm): 14.37, 18.61, 27.90, 28.98, 30.37, 60.97, 110.05, 132.28, 141.34, 153.24, 166.47, 191.77; MS (m/z, %) (301, 68Calcd. for $C_{12}H_{15}NO_2S_3$ (301.44): C, 47.81; H, 5.02; N, 4.65; S, 31.91; Found: C, 47.70; H, 5.14; N, 4.60; S, 31.87 %.

Synthesis of 3a-e

Equimolar amounts of compound **2** (10 mmol) and the appropriate amine [methylamine, ethylamine, cyclohexylamine, benzylamine and hydrazine hydrate] (10 mmol) in dichloroethane (20 ml) [in case of hydrazine hydrate we used dioxane (20 ml) instead of dichloromethane] were refluxed for (6h). The precipitate formed was filtered off, washed with cold ethanol, dried and recrystallized from ethanol to afford **3a-e**.

Ethyl 2-(3-methylthioureido)-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carboxylate (3a)

Pale brown solid in yield 65 %; Mp 138-140 °C. IR (KBr, υ, cm^{-1}): 3219, 3089 (2 NH), 1642 (C=O) and 1054 (C=S). ^{1}H NMR ($CDCl_3$, δ ppm): 1.39 (t, 3H, *J* = 6.0 Hz, CH_3), 2.34-2.41 (m, 2H, CH_2), 2.64 (s, 3H, CH_3), 2.85 (t, 2H, *J* = 6.0 Hz, CH_2), 2.92 (t, 2H, *J* = 6.0 Hz, CH_2), 4.32 (q, 2H, *J* = 6.0 Hz, CH_2), 6.40 (br s, 1H, NH, D_2O exchangeable),11.9 (br s, 1H, NH, D_2O exchangeable). MS (m/z, %) (284, 48); Anal.Calcd for $C_{12}H_{16}N_2OS_2$ (284.39): C, 50.68; H. 5.67; N. 9.85; S. 22.55 %. found: C, 50.55; H; 5.69; N, 9.87; S,22.59 %.

Ethyl 2-(3-ethylthioureido)-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carboxylate (3b)

Pale brown solid in yield 68 %; Mp 116-118 °C. IR (KBr, υ, cm^{-1}): 3217, 3100 (NH), 1665 (C=O) and 1056 (C=S); ^{1}H NMR ($CDCl_3$, δ ppm): 1.37 (t, 3H, *J* = 5.0 Hz, CH_3), 1.39 (t, 3H, *J* = 5.0 Hz, CH_3), 2.37-2.38 (m, 2H, CH_2), 2.72 (t, 2H, *J* = 5.0 Hz, CH_2), 2.90 (t, 2H, *J* = 5.0 Hz, CH_2), 4.31 (q, 2H, *J* = 5.0 Hz, CH_2), 4.34 (q, 2H, *J* = 5.0 Hz, CH_2), 6.38 (br s, 1H, NH, D_2O exchangeable), 11.70 (br s, 1H, NH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm): 14.36, 18.59, 28.97, 30.52, 39.15, 60.97, 67.17, 132.36, 141.34, 141.39, 155.06, 167.09, 179.23. MS (m/z, %) (298, 60); Anal. Calcd $C_{13}H_{18}N_2O_2S_2$ (298.42): C, 52.32; H, 6.08; N; 9.39; S,21.49 %. Found: C,52.29; H, 5.98;N; 9.38; S. 21.50 %.

Ethyl 2-(3-cyclohexylthioureido)-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carboxylate (3c)

Pale gray solid in yield 65 %; Mp 122-124 °C. IR (KBr, υ, cm^{-1}): 3230, 3074 (NH), 1660 (C=O) and 1058 (C=S). ^{1}H NMR ($CDCl_3$, δ ppm): 1.30-1.31 (m, 4H, $2CH_2$), 1.33 (t, 3H, *J* =5.0 Hz, CH_3), 1.35-1.36 (m, 6H, $3CH_2$), 2.33-2.34 (m, 2H, CH_2), 2.81 (t, 2H, *J* =5.0 Hz, CH_2), 2.85 (t, 2H, *J* = 5.0 Hz, CH_2), 3.50 (m, 1H, CH), 4.30 (q, 2H, *J* = 5.0 Hz, CH_2), 6.32 (br s, 1H, NH, D_2O exchangeable),11.73 (br s, 1H, NH, D_2O exchangeable). MS (m/z, %) (532, 24); Anal. Calcd for $C_{17}H_{24}N_2O_2S_2$ (352.51): C. 57.92; H, 6.86; N,7.95; S. 18.19 %. Found:C,57.88; H. 6.89;N, 7.85; S. 18.20 %.

Ethyl 2-(3-benzylthioureido)-5,6-dihydro-4H-cyclopenta [b]thiophene-3-carboxylate (3d)

Gray solid in yield 69 %; Mp 120-122 °C. IR (KBr, υ, cm^{-1}): 3218, 3063 (NH), 1662 (C=O) and 1053 (C=S). ^{1}H NMR ($CDCl_3$, δ ppm): 1.39 (t, 3H, J = 6.0 Hz, CH_3), 2.33-2.41 (m, 2H, CH_2), 2.89 (t, 2H, J = 6.0 Hz, CH_2), 2.93 (t, 2H, *J* = 6.0 Hz, CH_2), 4.32 (q, 2H, *J* = 6.0 Hz, CH_2), 5.70 (s, 2H, N-CH_2), 6.70 (br s, 1H, NH, D_2O exchangeable), 7.25-7.54 (m, 5H, Ar-H), 11.90 (br s, 1H, NH, D_2O exchangeable). MS (m/z, %) (360, 12); Anal. Calcd for $C_{18}H_{20}N_2O_2S_2$ (360.49): C, 59.97; H, 5.59; N,7.77; S. 17.79 %. Found: C, 59.93; H, 5.61; N, 7.70; S, 17.78 %.

Ethyl 2-(hydrazinecarbothioamido)-5,6,dihydro-4H-cyclopenta[b]thiophene-3-carboxylate (3e)

Colorless crystals in yield 71 %; Mp 183-185 °C, IR (KBr, υ, cm-1): 3426, 3192 (NH2 + NH), 1623 (C=O), 1052 (C=S),1H NMR (DMSO-d_6, δ ppm): 1.31 (t, 3H, *J* =6.0 Hz, CH_3), 2.23-2.33 (m, 2H, CH_2), 2.76 (t, 2H, *J* = 6.0 Hz, CH_2), 2.83 (t, 2H, *J* = 6.0 Hz, CH_2), 3.29 (br s, 3H, NH and NH_2, exchangeable with D_2O), 4.23 (q, 2H, *J* = 6.00 Hz, CH_2), 9.65 (br s, 1H, NH, exchangeable with D_2O). MS: (m/z, %) (285, 40); Anal. Calcd for $C_{11}H_{15}N_3O_2S_2$ (285.38): C, 46.30; H, 5.30; N,14.72; S, 22.47 %. found: C, 46. 49; H, 5.19;N, 14.83; S, 22.41 %.

Synthesis of 4a-e

Thioureido derivatives **3a-e** (10 mmol) was added to aqueous solution of 30 % potassium hydroxide (50 mL) and the mixture was refluxed under stirring for (3 h). The reaction mixture was filtered off, cooled, neutralized with concentrated hydrochloric acid (37 %) and stirred at room temperature for 30 minutes. The solid product, so formed, was collected by filtration and washed with hot water then with cold ethanol. Recrystallization of the product from ethanol gave **4a-e**.

3-Methyl-2-thioxo-1,2,3,5,6,7-hexahydro-4H-cyclopenta[4,5]thieno[2,3-d]pyrimidin-4-one (4a)

Buff powder in yield 67 %; Mp 270-272 °C; IR (KBr, υ, cm^{-1}):3124 (NH), 1697 (C=O) and 1071 (C=S).^{1}H NMR ($CDCl_3$, δ ppm): 2.13-2.44 (m, 2H, CH_2), 2.91 (t, 2H, *J* = 5.0 Hz, CH_2), 3.03 (t, 2H, *J* = 5.0 Hz, CH_2), 3.76 (s, 3H, CH_3), 13.06 (br s, 1H, NH, D_2O exchangeable). MS (m/z, %) (238, 65); Anal. Calcd for $C_{10}H_{10}N_2OS_2$ (238.32): C, 50.40; H; 4.23; N, 11.75; S, 26.90 %. Found: C, 50.29; H, 4.28; N, 11.76; S, 26.93 %.

3-Ethyl2-thioxo-1,2,3,5,6,7-hexahydro-4H-cyclopenta[4,5]thieno[2,3-d]pyrimidin-4-one (4b)

Brown powder in yield 71 %; Mp 250-252 °C. IR (KBr, υ, cm^{-1}): 3189 (NH), 1642 (C=O) and 1076 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.20 (t, 3H, *J* = 3.0 Hz, CH_3), 2.32-2.39 (m, 2H, CH_2), 2.50 (t, 2H, *J* = 3.0 Hz, CH_2), 2.83 (t, 2H, *J* = 3.0 Hz, CH_2), 4.40 (q, 2H, *J* = 6.0 Hz, CH_2), 13.53 (br s, 1H, NH, D_2O exchangeable). MS (m/z, %) (252, 65); Anal. Calcd for $C_{11}H_{12}N_2OS_2$ (252.35): C, 52.36; H; 4.79; N11.10; S, 25.41%.Found: C,52.39; H,4.76;N, 11.21; S, 25.33 %.

3-Cyclohexyl-2-thioxo-1,2,3,5,6,7-hexahydro-4H-cyclopenta[4,5]thieno[2,3-d] pyrimidin-4-one (4c)

Gray powder in yield 65 %; Mp 197-199 °C,IR (KBr, υ, cm^{-1}): 3123 (NH), 1695 (C=O) and 1077 (C=S), ^{1}H NMR (DMSO-d_6, δ ppm): 1.11-1.21 (m, 4H, $2CH_2$), 1.58-1.89 (m, 6H, $3CH_2$), 2.33-2.44 (m, 2H, CH_2), 2.77 (t, 2H, *J* = 5.0 Hz, CH_2), 3.32 (t, 2H, *J* = 5.0 Hz, CH_2), 5.64 (m, 1H, CH), 13.49 (br s, 1H, NH, D_2O exchangeable). MS (m/z, %) (306, 8); Anal. Calcd for $C_{15}H_{18}N_2OS_2$ (306.44): C, 58.79; H, 5.92; N, 9.14; S, 20.92 %, Found: C, 58.70;H, 5.89; N, 9.19; S, 20.90 %.

3-Benzyl-2-thioxo-1,2,3,5,6,7-hexahydro-4H-cyclopenta [4,5]thieno[2,3-d]pyrimidin-4-one (4d)

Brown powder in yield 69 %; Mp 190-192 °C. IR (KBr, υ, cm-1):3282 (NH), 1649 (C=O) and 1073 (C=S).^{1}H-NMR (DMSO-d_6, δ ppm): 2.30-2.32 (m, 2H, CH_2), 2.33 (t, 2H, *J* =5.0 Hz, CH_2), 3.34 (t, 2H, *J* = 5.0 Hz, CH_2), 5.55 (s, 2H, N-CH_2), 7.19-7.25 (m, 5H, Ar-H), 13.71 (br s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ ppm): 28.21, 28.72, 29.09, 40.35, 113.43, 127.40, 127.41, 127.42, 127.61, 128.71, 134.52, 137.11, 140.54, 154.98, 156.81, 174.49. MS (m/z, %) (314, 100); Anal. Calcd for $C_{16}H_{14}N_2OS_2$ (314.42): C, 61.12; H, 4.49; N, 8.91; S,20.39 %. Found: C, 60.99; H, 4.47; N, 8.93; S, 20.21 %.

3-Amino-2-thioxo-1,2,3,5,6,7-hexahydro-4H-cyclopenta[4,5]thieno[2,3-d]pyrimidin-4-one (4e)

Pale yellow crystals in yield 65 %; Mp 253-255 °C. IR (KBr, υ, cm-1): 3416, 3306, 3211 (NH + NH_2), 1673 (C=O), 1107 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.30 (m, 2H, CH_2), 2.78 (m, 2H, CH_2), 3.51(m, 2H, CH_2),5.29 (br s, 2H, NH_2 exchangeable with D_2O), 8.24 (br s, 1H, NH exchangeable with D_2O). ^{13}C-NMR ((DMSO-d_6, δ ppm): 29.35, 39.53, 39.70, 116.96, 130.81, 139.28, 153.83, 157.78, 169.25. MS (m/z, %) (239, 100); Anal. Calcd. for $C_9H_9N_3OS_2$ (239.31): C, 45.17; H, 3.79; N, 17.56; S, 26.79; Found: C, 45.13; H, 3.83; N, 17.54; S, 26.73 %.

3-Amino-2-(methylthio)-3,5,6,7-tetrahydro-4H-cyclopenta[4,5]thieno[2,3-d]pyrimidin-4-one (6e)

To a warm ethanolic potassium hydroxide solution [prepared by dissolving (0.56 g, 10 mmol) of potassium hydroxide in ethanol (20 mL)] was added (2.39 g, 10 mmol) of **4e.** The mixture was heated under reflux for (2 h). After cooling to room temperature, a solution of methyl iodide (1.48 g, 10.50 mmol) in ethanol (10 mL) was added. The reaction mixture was refluxed for (6 h) then pour onto water. The solid product, so formed, was filtered off, washed well with water, cold ethanol, dried and recrystallized from ethanol. Colorless powder in yield70 % Mp 200–202 °C. UV λ max: 325 nm; IR spectrum (KBr, υ, cm^{-1}): 3307, 3206 (NH_2), 1664 (C=O). ^{1}H NMR ($CDCl_3$, δ ppm): 2.40-2.46 (m, 2H, CH_2), 2.49 (s, 3H, CH_3), 2.91 (t, 2H, *J* = 6.0 Hz, CH_2), 3.01 (t, 2H, *J* = 6.0 Hz, CH_2), 4.74 (br s, 2H, NH_2 exchangeable with D_2O). MS (m/z, %) (253, 100); Anal. Calcd. for $C_{10}H_{11}N_3OS_2$ (253.34): C, 47.41; H, 4.83; N, 16.59; S, 25.31 %; Found: C, 47.49; H, 4.80; N, 16.52; S, 25.34 %.

Synthesis of 7a-e

To a solution of **4a-e** (5 mmol) in aqueous potassium hydroxide (0.28 g, 5 mmol) in distilled water (4 mL) was added a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide 7 (2.14 g, 5.2 mmol) in acetone (20 mL). The reaction mixture was stirred at room temperature overnight (12 h) and judged to be complete by TLC. The solvent was evaporated under reduced pressure at 40 °C, the crude product was filtered off and washed with distilled water to remove potassium bromide formed. The crude product was purified by column chromatography on silica gel with ethyl acetate/petroleum ether (1 : 3); then the product was collected, dried to afford the pure glycosides **7a-e**.

3-Methyl-2-(2',3,'4',6'-tetra-O-acetyl-β-D-glucopyranosylthio)-3,5,6,7-tetrahydro-4H-cyclopenta[4,5]-thieno[2,3-d]pyrimidin-4-one (7a)

Pale grey crystals in yield 65 %; Mp. 147-149 °C. IR(KBr, υ, cm^{-1}): 1760 (OAc) and 1666 (C=O). ^{1}H NMR ($CDCl_3$, δ ppm): 1.96-2.31 (4s, 12H, $4CH_3$), 2.43-2.46 (m, 2H, CH_2), 2.76 (s, 3H, CH_3), 3.35 (t, 2H, *J* = 5.0 Hz, CH_2), 3.37 (t, 2H, *J* = 5.0 Hz, CH_2), 4.60-4.62 (m, 1H, H-5'), 4.86-4.88 (m, 2H, H-6', 6''), 4.89-4.93 (m, 2H, H-4' + H-2'), 5.63-5.67 (m, 1H, H-3'), 5.96 (d, 1H, *J*=10.50 Hz, H-1'); Anal. Calcd for $C_{24}H_{28}N_2O_{10}S_2$ (568.61): C, 50.70; H, 4.96; N, 4.93; S, 11.28 %; Found: C, 50.71; H, 4.97; N, 4.91; S, 11.27 %.

3-Ethyl-2-(2',3,'4',6'-tetra-O-acetyl-β-D-glucopyranosylthio)-3,5,6,7-tetrahydro-4H-cyclopenta [4,5]-thieno[2,3-d]pyrimidin-4-one (7b)

Pale brown crystals in yield 56 %; Mp 140-142 °C. IR (KBr, υ, cm^{-1}): 1753 (OAc) and 1678 (C=O). ^{1}H NMR ($CDCl_3$, δ, ppm): 1.25 (t, 3H, *J* = 5.0 Hz, CH_3), 2.04 (4s, 12H, $4CH_3$), 2.84-2.88 (m, 2H, CH_2), 2.91 (t, 2H, *J* = 5.0 Hz, CH_2), 2.96 (t, 2H, *J* = 5.0 Hz, CH_2), 4.08-4.10 (m, 1H, H-5'), 4.13 (q, 2H, *J* = 5.0 Hz, CH_2), 4.21-4.25 (m, 2H, H-6', 6''), 5.11-5.13 (m, 2H, H-4' + H-2'), 5.80-5.83 (m, 1H, H-3'), 6.32 (d, 1H, *J* = 10.50, H-1'); Anal. Calcd for $C_{25}H_{30}N_2O_{10}S_2$ (582.64): C, 51.54; H, 5.19; N, 4.81; S,11.01 %; Found: C, 51.57; H, 5.09; N, 4.92; S, 11.00 %.

3-Cyclohexyl-2-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosylthio)-3,5,6,7-tetrahydro-4H-cyclopenta [4,5]-thieno[2,3-d]pyrimidin-4-one (7c)

Brown crystals in yield 61 %; Mp 197-199 °C. IR (KBr, υ, cm^{-1}): 1749 ((OAc) and 1696 (C=O). ^{1}H NMR ($CDCl_3$, δ ppm): 1.88-1.92 (m, 4H, $2CH_2$), 1.96-2.33 (m, 6H, $3CH_2$), 2.46-2.78 (4s, 12H, $4CH_3$), 2.84-2.85 (m, 2H, CH_2), 3.36 (t, 2H, *J* = 5.0 Hz, CH_2), 3.38 (t, 2H, *J* = 5.0 Hz, CH_2), 3.53 (m, 1H, CH), 4.07-4.09 (m, 1H, H-5'), 4.21-4.22 (m, 2H, H-6', 6''), 4.86-4.92 (m, 2H, H-4' + H-2'), 5.09-5.13 (m, 1H, H-3'), 6.00 (d, 1H, *J*=10.5 , H-1'); Anal. Calcd for $C_{29}H_{36}N_2O_{10}S_2$ (636.73): C, 54.70; H, 5.70; N; 4.40; S,10.07 %; Found: C,54.68; H, 5.59; N,4.49; S,10.10 %.

3-Benzyl-2-(2',3,'4',6'-tetra-O-acetyl-β-D-glucopyranosylthio)-3,5,6,7-tetrahydro-4H-cyclopenta[4,5]-thieno[2,3-d]pyrimidin-4-one (7d)

Pale brown crystals in yield 65 %; mp 137-139 °C; UV: λ_{max}= 325 nm. IR (KBr, υ, cm^{-1}): 1745 (OAc) and 1680 (C=O).^{1}H NMR ($CDCl_3$, δ ppm): 1.82-2.03 (4s, 12H, $4CH_3$), 2.40-2.45 (m, 2H, CH_2), 2.93 (t, 2H, *J* = 5.0 Hz, CH_2), 3.05 (t, 2H, *J* = 5.0 Hz, CH_2), 3.91-4.12 (m, 1H, H-5'), 4.14-4. 21 (m, 2H, H-6', 6''), 5.11 (s, 2H, N-CH_2), 5.17-5.19 (m, 2H, H-4' + H-2'), 5.31-5.33 (m, 1H, H-3'), 5.74 (d, 1H, *J*=10.50, H-1'), 7.27-7.28 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$, δ ppm): 20.45, 23.72, 24.54, 25.17, 26.33, 27.91, 28.23, 29,56, 35.04, 60.37, 62.93, 76.48,76.90, 77.33, 119.191, 127.12, 127.71, 128.12, 133.89, 141.36, 144.43, 149.90, 154.27, 156.81, 158.81, 160.00, 169.40, 169.41, 170.11, 171.46; Anal. Calcd for $C_{30}H_{32}N_2O_{10}S_2$ (644.71): C, 55.89; H, 5.00; N, 4.35; S, 9.95 %; Found: C, 55.72; H, 4.98; N, 4.42; S, 9.92 %.

3-Amino-2-(2',3,'4',6'-tetra-O-acetyl-β-D-glucopyranosylthio)-3,5,6,7-tetrahydro-4H-cyclopenta[4,5]-thieno[2,3-d]pyrimidin-4-one (7e):

Brown crystals in yield 64 %; Mp 129-131 °C. IR (KBr, υ, cm^{-1}): 3211 (NH_2), 1747 (OAc), 1667 (C=O). ^{1}H NMR ($CDCl_3$, δ ppm): 2.00-2.20 (4s, 12H, $4CH_3$), 2.50 (m, 2H, CH_2), 2.90 (t, 2H, *J* = 5.0 Hz, CH_2), 3.10 (t, 2H, *J* = 5.0 Hz, CH_2), 3.90-4.01 (m, 1H, H-5'), 4.20-4.42 (m, 2H, H-6', 6''), 4.70 (br s, 2H, NH_2, exchangeable with D_2O), 5.22 (t, 1H, *J* = 10.5 Hz, H-4'), 5.24 (t, 1H, *J* =10.5 Hz, H-2'), 5.40 (t, 1H, *J* = 10.5 Hz, H-3'), 5.70 (d, 1H, *J* =10.5 Hz, H-1'); Anal. Calcd for $C_{23}H_{27}N_3O_{10}S_2$ (569.60): C, 48.50;H, 4.78;N, 7.38; S, 11.26 %; Found: C, 48.35; H, 4.79;N, 7.37; S, 11.25 %.

Synthesis of 9a-e

A saturated solution of ammonia in methanol (30 mL) was added to a solution of the appropriate **7a-e** (10 mmol) in dry methanol (10 mL). The solution was left to stirring at room temperature for (24 h). The solvent was evaporated under reduced pressure, dried under vacuum and crystallized from ethanol to afford the deacetylated product **9a-e**.

3-Methyl-2-(2',3',4',6'-tetrahydroxy-β-D-glucopyranosyl)-3,5,6,7-tetrahydro-4H-cyclopenta[4,5]thieno[2,3-d] pyrimidin-4-one (9a)

Pale yellow oil in yield 56 %. IR (KBr, υ, cm^{-1}): 3421 (4 OH), 1687 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.11-2.90 (m, 2H, CH_2), 2.92 (s, 3H, CH_3), 2.95 (t, 2H, *J* = 5.0 Hz, CH_2), 3.00 (t, 2H, *J* = 5.0 Hz, CH_2), 3.25-3.28 (m, 2H, H-6', 6''), 3.54-3.57 (m, 1H, H-5'), 3.87-3.98 (m, 2H, H-3' + H-4'), 4.45-4.65 (m, 1H, H-2'), 4.70-4.72 (m, 1H, OH), 5.07-5.10 (m, 1H, OH), 5.12-5.14 (m, 1H, OH), 5.30-5.42 (m, 1H, OH), 5.77 (d, 1H, *J* = 10.0 Hz, H-1'); Anal. Calcd for $C_{16}H_{20}N_2O_6S_2$ (400.46): C,47.99; H, 5.03; N, 7.00;S,16.00 %; Found: C, 47.79; H, 5.00; N, 6.99; S, 16.13 %.

3-Ethyl-2-(2',3',4',6'-tetrahydroxy-β-D-glucopyranosyl)-3,5,6,7-tetrahydro-4H-cyclopenta[4,5]thieno[2,3-d]-pyrimidin-4-one (9b)

Pale brown oil in yield 66 %. IR (KBr, υ, cm^{-1}): 3433 (4 OH), 1685 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): δ 1.27 (t, 3H, *J* =5.0 Hz, CH_3), 2.38-2.45 (m, 2H, CH_2), 2.51 (t, 2H, *J* = 5.0 Hz, CH_2), 2.81 (t, 2H, *J* =5.0 Hz, CH_2), 3.22-3.25 (m, 2H, H-6', 6''), 3.51-3.55 (m, 1H, H-5'), 3.84-3.95 (m, 2H, H-3' + H-4'), 4.42 (q, 2H, *J* = 5.0 Hz, CH_2), 4.44-4.61 (m, 1H, H-2'), 4.69-4.71 (m, 1H, OH), 5.00-5.03 (m, 1H, OH), 5.05-5.09 (m, 1H, OH), 5.22-5.40 (m, 1H, OH), 5.72 (d, 1H, *J* =10.0 Hz, H-1'); Anal. Calcd. for $C_{17}H_{22}N_2O_6S_2$ (414.49): C, 49.26; H, 5.35; N, 6.76; S, 15.47 %; Found: C, 49.11; H, 5.32; N, 6.79; S,15.49 %.

3-Cyclohexyl-2-(2',3',4',6'-tetrahydroxy-β-D-glucopyranosyl)-3,5,6,7-tetrahydro-4H-cyclopenta[4,5]thieno-[2,3-d]pyrimidin-4-one (9c)

Pale brown oil in yield 70 %. IR (KBr, υ, cm^{-1}): 3429 (OH), 1698 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.63-1.65 (m, 4H, $2CH_2$), 1.87-2.00 (m, 6H, $3CH_2$), 2.36 (m, 2H, CH_2), 2.49 (t, 2H, *J* = 5.0 Hz, CH_2), 2.97 (t, 2H, *J* = 5.0 Hz, CH_2), 3.28 (m, 1H, CH), 3.30-3.32 (m, 2H, H-6', 6''), 3.50-3.56 (m, 1H, H-5'), 3.82-3.91 (m, 2H, H-3' + H-4'), 4.43-4.59 (m, 1H, H-2'), 4.65-4.70 (m, 1H, OH), 5.04-5.08 (m, 1H, OH), 5.11-5.15(m, 1H, OH), 5.25-5.48 (m, 1H, OH), 5.78 (d, 1H, *J* =10.0 Hz, H-1'); Anal. Calcd: for $C_{21}H_{28}N_2O_6S_2$ (468.58): C, 53.83; H, 6.02; N, 5.98; S, 13.68 %; Found: C, 53.71;H, 6.00; N, 5.99; S, 13.69.

3-Benzyl-2-(2',3',4',6'-tetrahydroxy-β-D-glucopyranosylthio)-3,5,6,7-tetrahydro-4H-cyclopenta[4,5]thieno-[2,3-d]pyrimidin-4-one (9d)

Pale brown oil in yield 75 %. IR (KBr, υ, cm^{-1}): 3433 (OH), 1680 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.36 (m, 2H, CH_2), 2.50 (t, 2H, *J* = 5.0 Hz, CH_2), 3.16 (t, 2H, *J* = 5.0 Hz, CH_2), 3.33-3.35 (m, 2H, H-6', 6''), 3.55-3.60 (m, 1H, H-5'), 3.83-3.94 (m, 2H, H-3' + H-4'), 4.47-4.62 (m, 1H, H-2'), 4.68-4.74 (m, 1H, OH), 5.19-5.28 (m, 1H, OH), 5.31-5.35 (m, 1H, OH), 5.39-5.49 (m, 1H, OH), 5.70 (s, 2H, N-CH_2), 5.74 (d, 1H, *J* =10.0 Hz, H-1'), 7.27-7.95 (m, 5H, Ar-H); Anal. Calcd for $C_{22}H_{24}N_2O_6S_2$ (476.56): C, 55.45; H, 5.08; N, 5.88; S, 13.45 %; Found: C, 55.31; H, 5.05; N, 5.90;S,13.48.

3-Amino-2-(2',3',4',6'-tetrahydroxy-β-D-glucopyranosylthio)-3,5,6,7-tetrahydro-4H-cyclopenta[4,5]thieno-[2,3-d]pyrimidin-4-one (9e)

Yellow oil in yield 56 %. IR (KBr, υ, cm^{-1}): 3439 (OH), 1689 (C=O).^{1}H NMR (DMSO-d_6, δ ppm): 2.31-2.93 (m, 2H, CH_2), 2.96 (t, 2H, *J* = 5.0 Hz, CH_2), 3.00 (t, 2H, *J* = 5.0 Hz, CH_2), 3.22-3.25 (m, 2H, H-6', 6''), 3.50-3.55 (m, 1H, H-5'), 3.83-3.91 (m, 2H, H-3' + H-4'), 4.40-4.61 (m, 1H, H-2'), 4.71-4.85 (m, 1H, OH), 5.04-5.9(m, 1H, OH), 5.14-5.23 (m, 1H, OH), 5.38-5.48 (m, 1H, OH), 5.70 (br s, 2H, NH_2, exchangeable with D_2O), 5.76 (d, 1H, *J*=10.00Hz, H-1'); Anal. Calcd for $C_{15}H_{19}N_3O_6S_2$ (401.45): C,44.88; H, 4.77; N, 10.47; S, 15.97 %; Found: C, 44.79; H, 4.78; N,10.49; S, 15.90 %.

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BIOLOGICAL EVALUATION

In vitro antioxidant activity

1,1-Diphenyl-2-picryl hydrazyl (DPPH) was purchased from Sigma Chem. Co. (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) and methanol were of HPLC grade and all other reagents and chemicals were of analytical reagent grade. Antioxidant activity of each compound and standards (ascorbic acid and rutin) was assessed based on the radical scavenging effect of stable DPPH free radical [**36**]. 10 µl of each tested compound or standard (from 0.0 to 100 µM) was added to 90 µl of a 100 µM methanolic solution of DPPH in a 96-well microliter plate (Sigma-Aldrich Co., St. Louis, MO, US). After incubation in dark at 37°C for 30 min, the decrease in absorbance of each solution was measured at 520 nm using an ELISA micro plate reader (Model 550, Bio-Rad Laboratories Inc., California, USA). Absorbance of blank sample containing the same amount of DMSO and DPPH solution was also prepared and measured. All experiments were carried out in triplicate. The scavenging potential was compared with a solvent control (0% radical scavenging) and the standard compounds. Radical scavenging activity was calculated by the following formula:% Reduction of absorbance = [(AB - AA) / AB] x 100, where: AB – absorbance of blank sample and AA – absorbance of tested compound (t = 30 min). The concentration of each compound required to scavenge 50% of DPPH (IC_{50}) was determined as well [**37,38**].

In vitro anticancer activity:

Cell culture of MCF-7 (breast carcinoma cell line) and Caco-2 (Colorectal adenocarcinoma) were purchased from the American Type Culture Collection (Rockville, MD) and maintained in RPMI-1640 and in DMEM medium respectively. Both media were supplemented with 10% heat-inactivated FBS, 100U/ml penicillin and 100U/ml streptomycin. The cells were grown at 37°C in a humidified atmosphere of 5% CO2.

MTT cytotoxicity assay:

The cytotoxicity activity against MCF-7, Caco-2 and Wish (normal cell line) human cell lines was estimated using the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, which is based on the cleavage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells [**39,40**]. Cells were dispensed in a 96 well sterile microplate (5 x 10^4 cells/well), and incubated at 37°C with 100 µM/ml of each tested compound or Doxorubicin (positive control) for 48 h in a serum free medium prior to the MTT assay. After incubation, media were carefully removed, 40 µL of MTT (5 mg/mL) were added to each well and then incubated for an additional 4 h. The purple form azan dye crystals were solubilized by the addition of 200 µL of acidified isopropanol. The absorbance was measured at 570 nm using a microplate ELISA reader (Biorad, USA). The relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells.

Statistical analysis:

All experiments were conducted in triplicate (n = 3). All the values were represented as mean ± SD. Significant differences between the means of parameters as well as IC_{50}s were determined by probit analysis using SPSS software program (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Chemistry

Derivative of dithiocarbamate **2** was generated by reacting the β-enamino ester **1** [35] with carbon disulfide, sodium hydroxide and then dimethyl sulfate in DMSO (Scheme 1). The disappearance of two peaks between 3413 and 3293 cm^{-1} and the appearance of a single peak at 3419 cm^{-1} indicated the conversion of primary amine to secondary amide. The spectrum of ^1H NMR showed SCH_3 protons singlet at δ 2.68. Product **2** was easily converted to thioureido derivatives **3a-e** in good yields upon treatment with different amines in CH_2Cl_2 at room temperature (Scheme 1). The structures of thioureido derivatives **3a-e** were elucidated on the basis of their spectral data (see experimental). Aqueous alkaline hydrolysis of the thioureido derivatives **3a-e**

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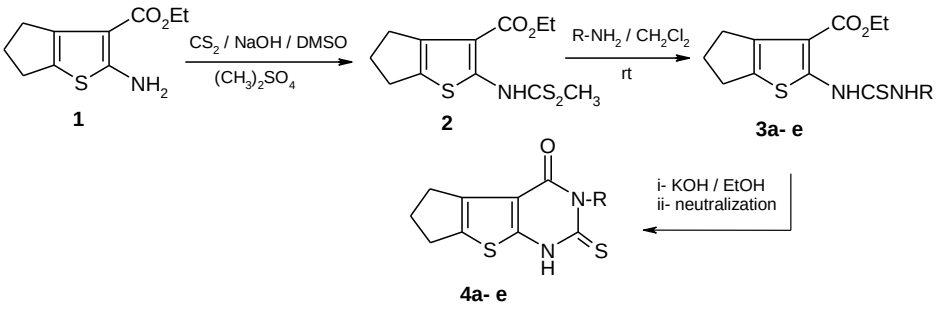
led to the formation of the cyclized product **4a-e** in 65 - 71 % yields (Scheme 1). Spectral and analytical data of compound **4a-e** are in accordance with the proposed structure.



R = (**a**) CH_3, (**b**) CH_2CH_3, (**c**) C_6H_{11} (**d**) $CH_2C_6H_5$, (**e**) NH_2

Scheme 1

Acetobromo-α-D-galucose [**41**] reacted with the potassium salt of **4a-e** to afford the corresponding *S*-glycosides **7a**-e (Scheme 2). Methyl iodide was employed as model alkylating agent of **4a**-**e**. So, treatment of potassium salt **4e** with methyl iodide yielded **6e** in 70 % Yield (Scheme 2). The compound **6e** was elucidated by (IR, MS, ^{1}H NMR). In ^{1}H NMR showed signal due to the SCH_3 proton at 2.49 ppm (see experimental). The data of analyses in addition to the mode of the reaction showed the place of the alkylation was the sulfur atom not the nitrogen atom. Similarly, glycosylation of compounds **4a-e** with acetobromo-α-D-galucose afforded the protected *S*-glycosylated nucleosides **7a-e** (Scheme 2).The structure of the isolated products **7a-e** were established by elemental analyses and (IR, ^{1}H NMR, ^{13}C NMR). The ^{1}H NMR spectrum of **7d** display the anomeric proton of the moiety of glucose as a doublet at δ 5.74 with J = 10.50 Hz denoting *β*-configuration of the anomeric center. The other protons of the ring of the glucopyranosyl sugars resonated at δ 4.1 - 5.4.The four acetoxy groups showed four singlets at δ 1.82 - 2.03. The ^{13}C NMR showed the correct number of the expected sp^2 and sp^3 carbons. The IR data of the compound **7d** didn't appear the signal of a thioxo group but displayed signals at 1680 cm^{-1} for the C=O group. Frequencies of the stretching vibration of the acetate carbonyl groups displayed at 1745 cm^{-1}. The spectrum of UV of compound **7d** excluded the production of *N*-glycoside and proved the formation of *S*-glycoside derivative. Compounds **7d** and the *S*-methyl of compound **6e** displayed UV absorption at 325 nm (see experimental).

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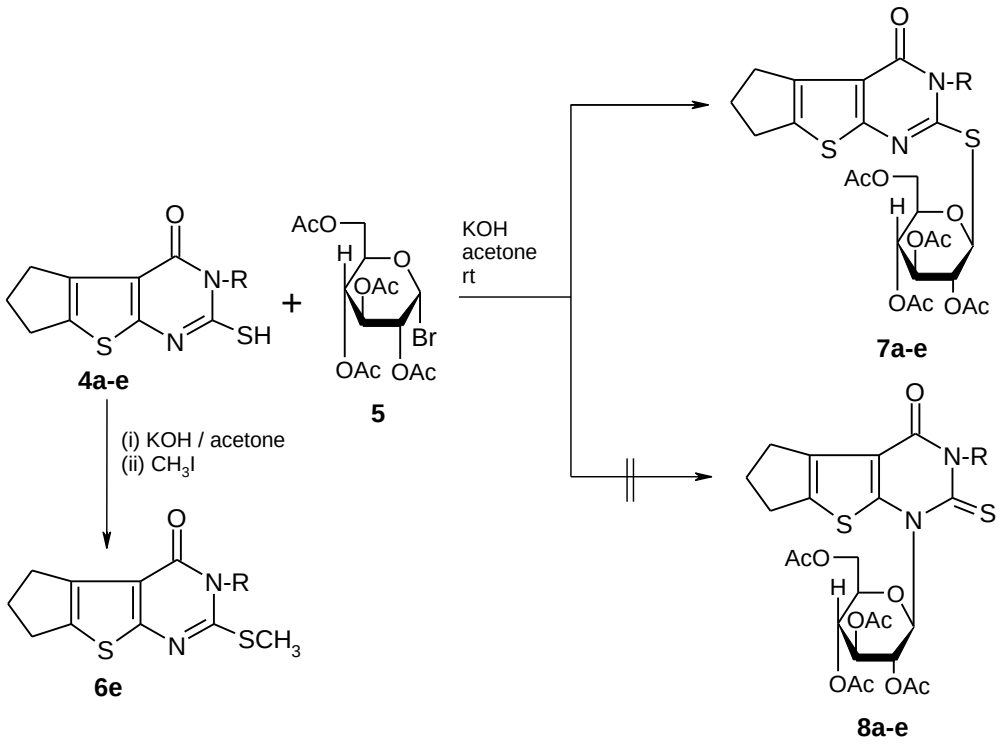
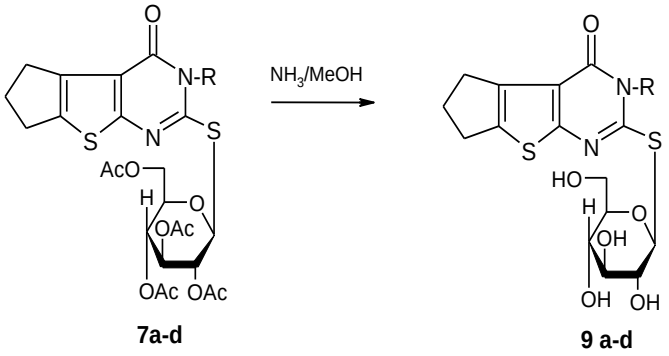


R = (**a**) CH_3 (**b**) CH_2CH_3 (**c**) C_6H_{11} (**d**) $CH_2C_6H_5$ (**e**) NH_2

Scheme 2

Deprotection of **7a-e** with methanolic ammonia at room temperature furnished the corresponding deprotected thioglycosides **9a-e** (Scheme 3). The spectra of IR of **9a-e** demonstrated absorption bands between 3421-3433 cm^{-1} assignable to the (OH) groups, ^1H NMR of **9a-e** displayed no acetyl proton signals but showed the predictable base moiety protons in addition to the sugar moiety protons.



R = (**a**) CH_3 (**b**) CH_2CH_3 (**c**) C_6H_{11} (**d**) $CH_2C_6H_5$ (**e**) NH_2

Scheme 3

The discrimination between the *S*- and *N*- glycoside was confirmed based on comparison of their ^1H and ^{13}C NMR spectra to the published data of similar compounds. [**29,33,42-47**]. C=S Chemical shifts of δ 172 ppm were reported for cycloalkyl[4,5]thieno[2,3-*d*]pyrimidin-4-one-2-thione, while 2-alkylthio-cycloalkyl[4,5]-thieno[2,3-*d*]pyrimidin-4-one show chemical shifts of C-2 around δ 159 ppm.

Antioxidant activities

The newly product were tested in *vitro* for antioxidant activities against DPPH radicals (Figure 1) .The results indicated that all the tested compounds displayed dose dependent DPPH inhibition activities. The results were expressed as IC_{50} value and summarized in table (1). The activities of the organic compounds

displayed in that order: **4C**> rutin Vit C> **7b**>**7c**>**3b**>**4a**>**4e**>**7c**>**4d**>**7c**>**3a**>**2**>**3c**>**6c**. Comparison the activity of the synthesized compounds and the activity of well-known potent DPPH inhibitors (rutin and vit C). It is obvious that the good scavenging properties was obtained by compound **4c** (IC_{50} value of 8.625 µM) which is much higher than that of rutin and vitamin C (IC_{50} values of 27.3 and 48.7, respectively). The activity result of compound **4c** reflected the importance of attachment of a cyclohexyl moiety to the ring system of thienopyrimidine. However, the rest of the compounds displayed lower activity comparison to the two standard compounds.

Table1: The Antioxidant activities of the newly synthesized compounds.

COMPOUND	IC_{50} ± SD	COMPOUND	IC_{50} ± SD
2	127.3 ± 5.9	**6e**	178.7±11.2
3a	117.4 ± 6.1	**7a**	77.6 ± 5.9
3b	91.5 ± 5.5	**7b**	63.4 ± 4.7
3c	142.4± 10.1	**7c**	116.6 ± 9.6
4a	94.6 ± 9.2	**7d**	107.1 ± 4.9
4c	8.6 ± 2.3	**Rutin**	27.3 ± 3.9
4d	111.6± 10.1	**Vit C**	48.7 ± 6.1
4e	105.2 ± 7.8		

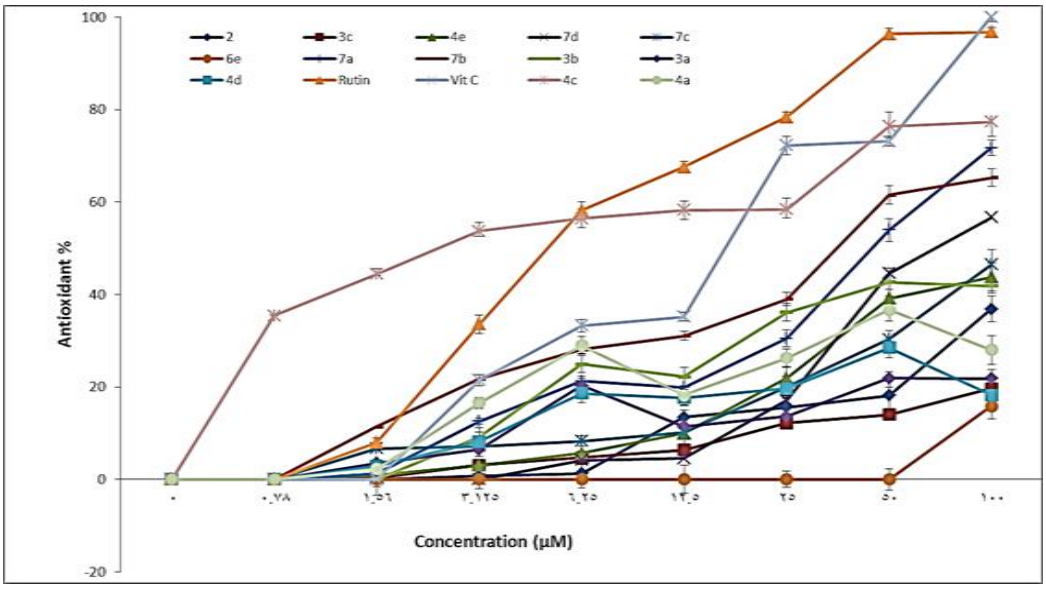


Fig 1: antioxidant activity using DPPH

Anti-Tumor Activity

Using MTT assay the synthesized products were tested in *vitro* for their anti-tumor activities against MCF-7 (human breast carcinoma cell line), Caco-2 (human colorectal adenocarcinoma) and wish (human normal cell lines). The results of the intact cells were compared to the control (Figure 2).

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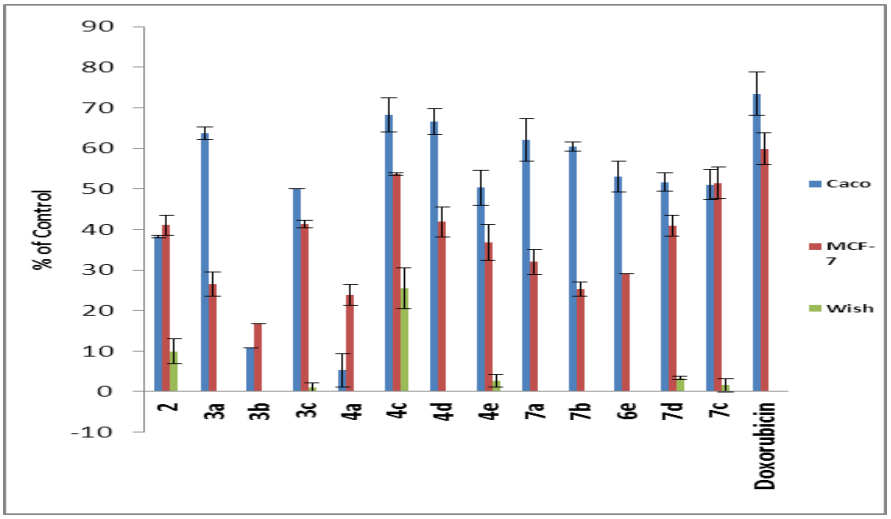

Fig 2: The anticancer of compounds using a MTT assay against MCF-7 and Caco-2 cell line

The activities of the derivatives against carcinoma cells were compared with Doxorubicin® cytotoxicity. The gained results displayed that these compounds exhibited dose-dependent anticancer activities against the two cancer cells. All the tested compounds did not displayed any remarkable cytotoxicity against the human cell line (Table 2).

Table 2: Anticancer activity of compounds against human breast carcinoma (MCF-7) and human colorectal adenocarcinoma (Caco-2) cell lines using (MTT)assay.

	Caco-2	MCF-7		Caco-2	MCF-7
2	38.23529	41.02523	**4e**	50.3268	36.77025
3a	63.72549	26.51793	**6e**	62.0915	32.01062
3b	10.8329	16.74369	**7a**	60.45752	25.24303
3c	50	41.38645	**7b**	52.94118	29.18459
4a	5.317297	23.88845	**7c**	50.98039	51.46879
4c	68.30065	53.68924	**7d**	51.63399	40.88712
4d	66.66667	41.85392	**Doxorubicin**	73.43609	59.88048

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