Received: 03 June 2021 | Revised: 11 July 2021 | Accepted: 18 July 2021
DOI: 10.22034/ecc.2021.293403.1197
Eurasian Chemical Communications
SAMI

FULL PAPER

Synthesis of C- glycoside analogs containing thiouracil derivatives and evaluated as antibacterial, antifungal and antioxidant activities

Taghreed S. Hussein* | **Mohammed R. Ahamad**

Department of Chemistry, College of Science, Baghdad University, Baghdad, Iraq

***Corresponding Author:**
Taghreed S. Hussein
Email: Ted38456@gmail.com
Tel.: + 009647722806989

This work focused on the synthesis of C-glycoside that contained thiouracil unite, which were synthesized from glucon-2-carbothioate thiouracil derivatives **[C_1-C_4]** via reaction of gluconic acid, thionyl chloride, and thiouracil derivatives in a one-pot reaction in the presence of triethylamine as a catalyst. Furthermore, we performed synthesis of 2-(glucofuranose) thiouracil derivatives **[C_5, C_6]** via condensation reaction of D-glucurone, ethyl cyanoacetate, and thiourea or phenyl thiourea in presence of potassium carbonate, and studying the possibility for their application as antifungal, antibacterial, and antioxidant.

KEYWORDS

C-glycosides; thiouracil; thiourea; phenyl thiourea; biological activity.

Introduction

The metabolic stability of these compounds finds application in the inhibition of carbohydrate processing enzymes [1], C-glycosides being able to fit into the active site of glycosidases and glycosyltransferases without being modified by the enzyme. This behavior also allows mapping the active site to study the recognition and binding processes [2], and to investigate the mechanism of action of these enzymes. The metabolic stability of C-glycosides is also exploited in the generation [3] of glycomimetic drugs resistant to in vivo hydrolysis [4], such as integrin ligands or glycemic immunostimulators [5]. Those compounds showed a variety of interesting biological properties such as antitumor, antiviral, or antibacterial activities. The combination of metabolic stability and relevant biological activities generated a great interest in the synthesis of a variety of C-glycosides designed to inhibit specific enzymes, act as agonists or anti-agonists in precentral phenomena, and induce an immunogenic response. Aryl C-glycosides, in particular anthraquinone-carbohydrate hybrids such as undamynone B8, have shown DNA-binding properties and cytotoxicity [6]; therefore, they can be promising antitumor agents. C-Glycosides presenting reactive α-Bromo ketone functionality have been described as irreversible inhibitors of glycosidases. More sophisticated C-glycosidic structures have been synthesized to mimic NDP-sugars, hence act as specific inhibitors of glycosyltransferases. C-Glycosides related to tumor-associated antigens are another relevant synthetic target [7]. Mimics of GalNAcα1-O-Ser (Tn antigen) linked to immunogenic peptides to elicit both B- and T-immune responses have been synthesized [8].

To achieve increased stability, ligation has been applied to the site-specific attachment of the sugar moiety. Chemo selectivity was obtained by coupling a thiouracil group. It is well known that thiouracil derivatives are of great [9,10] biological attention, especially as Anti-HIV, antimicrobial [11], anti-inflammatory [12], and antitumor agents [13,14].

Experimental

Instruments

Melting points have been registered using the melting point equipment of the Gallen Kamp hot stage and adjusted. Fourier Transform Infrarouge Species SHIMADZU (8300) infrarouge, Japan, in range (4000-600) cm^{-1} were used to record spectra of infrarot transform in the Fourier Transform spectrometer SHIMADZU (8300), Japan. Tetramethyl saline as internal standard and DMSO-d6 as a solvent were recorded using Bruker, Ultrasheild (500) MHz nuclear magnetic resonance (Germany).

Methods

Synthesis of S-(thiouracil derivatives) gluconthioate [C_1-C_4].[15]

Gluconic acid (0.001 mol) was added to 0.001 mol of thiouracil derivatives and 3 mL of triethylamine in dimethylformamide, then (0.001 mol) of $SOCl_2$ was added at 0 °C in one pot. The mixture was stirred for 5–20 minutes at 25 °C, then the mixture was refluxed for 2hrs. The resulting product was washed with 1 N HCl and then with 1 N NaOH. The organic phase was dried by (Na_2SO_4) and evaporated to dryness to produce compounds **[C_1-C_4].**

Compound [C1]: Yield was (73%), with mp: (166–169 °C), Rf: (0.27), IR, (cm^{-1}): 3453 (OH), 3126 (NH), 2929 (CH), 1654 (C=O) thiol, 1629(C=O) amide.

Compound [C2]: Yield was (66%), with mp: (234-235 °C), Rf: (0.35), IR, (cm-1): 3448 (OH), 3161 (NH), 2212 (CN), 1676 (C=O) thiol, 1625 (C=O) amide.

Compound [C3]: Yield was (63%), with mp: (254-256 °C), Rf: (0.27), IR, (cm^{-1}): 3431 (OH), 3058 (NH), 2275 (CN), 1642 (C=O) thiol, 1610 (C=O) amide, 1519, 1317 sym., asym. (NO_2). ^{1}H NMR (DMSO-d6): 3.25- 3.78 (m,5H, Sugar protons); 4.21- 4.86 (s,4H, OH); 7.90-8.05 (m,4 H, Ar-H); 12.01 (S,1H, NH). ^{13}C NMR: 67.34 -98.06 (5C, CH of sugar) 94.16(1C, CH of sugar); 112.09 (CN); 127.06 -131.46(4C, CH aromatic); 160.13 (C=N); 168.69 (N-C=O); 197.38 (S-C=O).

Compound [C4]: Yield: (66%), mp: (178-181 °C), IR, (cm-1): 3446 (OH), 3197 (NH), 2200 (CN), 1649 (C=O) thiol, 1598 (C=O) amide.^{1}H NMR (DMSO-d6): 1.90- 3.53 (s,6H, OCH_3); 3.35- 3.99 (m,5H, Sugar protons); 4.45-4.56 (s,4H, OH); 6.27- 6.57 (m,4 H, Ar-H); 8.15 (S,1H, NH). ^{13}C NMR: 72.33 -90.02 (5C, CH of sugar) 94.68 (1C, CH of sugar); 113.04 (CN); 111.069-134.12 (4C, CH aromatic); 163.16 (C=N); 164.89 (N-C=O); 194.92(S-C=O).

Synthesis of 6-(Gluco) thiouracil derivatives [C5, C6]:[16]

A mixture of the D-glucurone (0.01 mol), ethyl cyanoacetate (0.01 mol), thiourea or phenyl thiourea (0.01 mol), and potassium carbonate (0.01 mol), in 30 mL ethanol, was heated for 12 h under reflux. On cooling, the separated precipitate was filtered, washed with ether, and dried. The obtained solid was added to water (20 mL), and the mixture was heated at 80–90 °C until a clear solution was obtained. After cooling, the solution was acidified with acetic acid, and stirring was continued for 30 min. The deposited precipitate was filtered, washed with cold water, dried, and crystallized from ethanol to produce compounds **[C5, C6].**

Compound [C5]: Yield was (77%), mp: (66-68 °C), IR, (cm^{-1}): 3433 (OH), 3255 (NH), 2244 (CN), 1604 (C=O) amide. 1 H NMR (DMSO-d6): 1.98, 2.08 (d,2H, CH_2) 3.20- 3.41 (m,5H, Sugar protons); 4.37- 4.81 (s,4H, OH);

8.53, 12.76 (S,2H, NH). 13C NMR: 67.34 -98.06 (5C, CH of sugar) 94.16(1C, CH of sugar); 112.09 (CN); 127.06 -131.46(4C, CH aromatic); 160.13 (C=N); 168.69 (N-C=O); 197.38 (S-C=O).

Compound [C6]: Yield was (75%), mp: (98-101 °C), Rf: (0.24), IR, (cm^{-1}): 3423 (OH), 3182 (NH), 3082 (Ar-CH), 2124 (CN), 1612 (C=O).

Assessment of biological activity

Antibacterial and antifungal activities

Biological activity of some new free C-glycoside analogs were tested in *vitro* for two Gram-positive microbes *(Staphylococcus aureus, Bacillus subtilis)*, three Gram-negative microbes *(Pseudomonas Aeuroginosa, Escherichia Coli, Klebsiella pneumoniae),* and fungi *(Macrosporium)* with concentration (0.025 g/mL) using conventional cup-plate agar diffusion method [17]. The inhibitory zone for the spread of microorganisms on the platform incorporates this way of communication. The results were shown as the average diameter of bacterial or fungal growth in inhibition zones about mm for every investigated drug [18].

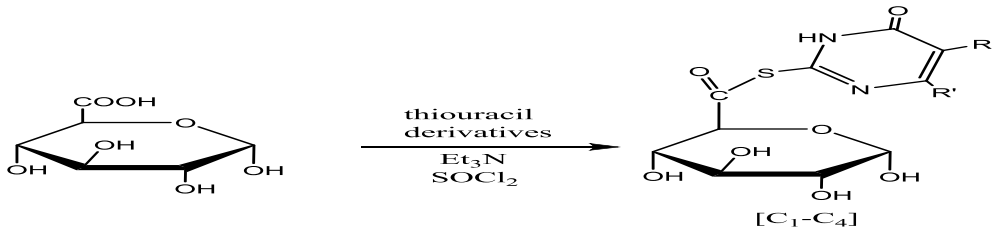
Antioxidant activity

The scavenging measurements of some synthesis compounds were estimated using a constant DPPH assay for determining the IC50 values. Using three unlike concentrations (10, 5, and 2.5 µg/mL) of each compound. The absorbance was measured at 517 nm for each compound [19,20]. The antioxidant activity of the compounds was compared with ascorbic acid as standard.

Results and discussion

Method

C-glycosides were accessed by two methods. First method was via reaction of gluconic acid, thionyl chloride, and thiouracil derivatives in one-pot reaction in the present of triethylamine as catalysis as shown in ***Equation (3.1).***



R= H, CN
R'= C_3H_7, C_6H_5, P_NO_2 C_6H_4, 2,4 OCH_3 C_6H_3

EQUATION (3.1)

The second method was *via* condensation reaction of D-glucurone, ethyl cyanoacetate, thiourea or phenyl thiourea, and potassium carbonate is shown in ***Equation (3.2)***.

R= H or Ph

[C_5,C_6]

EQUATION (3.2)

Some new compounds were fully characterized by spectroscopic analysis (IR, ^{1}H NMR, and ^{13}CNMR). The results are consistent with the proposed structures.

Biological assessment

Antibacterial and antifungal activities

Compounds [C1, C4, C6] have been tested as an antimicrobial agent for two types of Gram-positive bacteria, three types of Gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia*) and yeast-like pathogenic fungi, against a panel of standard pathogenic strains Compounds [C1, C4, C6] (*Macrosporium*). The screening was performed with the agar disc diagram. The chemicals examined showed considerable action with the antibiotic and antifungal medicines of broad scope. Compound **[C1]** shows high effectiveness compared with control for all types of bacteria and fungi. Compound **[C4]** shows high effectiveness compared with control for all types of bacteria and fungi except *Pseudomonas aeruginosa* does not give inhibition. All results are shown in Table 1.

TABLE 1 Activity of antibiotics test of some prepared compounds

Inhibition zone diameter (mm)/Concentration 0.025 g/mL						
	fungi	Gram-negative bacteria			Gram-positive bacteria	
No.	Macros-porium	klebsiella pneumonia	Escherichia coli	Pseudomonas aeuroginosa	Bacillus subtilis	Staphyloco ccus aureus
C1	13	15	17	10	15	13
C4	15	13	14	-Ve	15	15
C6	10	12	12	9	14	14
L	9	12	12	9	11	12
P	-	9	9	9	9	12
DMSO	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve

Solvent: DMSO;
(L= luporal capsule, P = propyl thiouracil) as control Inhibition zone: (-) no zone of inhibition; (3–6) slight; (11-20); (7-10) restrained; (11-20) strong and (20-30) very strong.

Antioxidant active

The newly synthesized compounds showed antioxidant activity against DPPH free radicals and gave a good scavenging percentage. So, the compounds that were tested illustrated antioxidant properties were selected for further testing. Accordingly, inhibitory concentrations (IC_{50}) values were recorded and tabulated in Table 2; accordingly, we applied the anti-oxidant activity classification which depends on IC_{50} range values published by Phongpaichi [21]. See Table 3 for more details about these ranges. So, all the prepared compounds are considered to be of good activity because the IC50 value is relatively low.

TABLE 2 DPPH Radical Scavenging Assay

No.	Ascorbic acid 100 µg/mL	IC50	% Scavenging Activity at Different Concentrations		
			100 µg/mL	50 µg/mL	25 µg/mL
C1	93.44	58.01	83.60	47.54	22.95
C3	99.9	49.198	95.24	59.34	33.93
C5	98.36	48.971	93.77	62.45	36.06

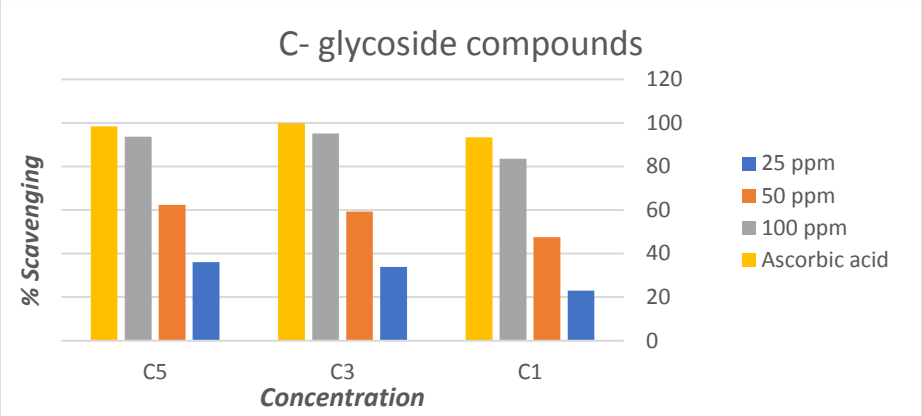


FIGURE 1 Effect of the synthesized compounds toward DPPH assay

TABLE 3 Antioxidant Activity according to Phongpaichit [21]

IC50 (µg/mL	Mark
10-50 µg/mL	Strong Antioxidant Activity
50-100 µg/mL	Intermediate Antioxidant Activity
>100 µg/mL	Weak Antioxidant Activity

Conclusion

In conclusion, new selective C-glycosides continuing thiouracil derivatives were synthesized in good yields. The new C-glycosides formed were structurally characterized and tested for biological activity against fungi and bacteria species and antioxidant activities. Some of the tested products showed good and moderate activity and the results were reported.

Acknowledgments

We thank Prof. Dr. Mohammed Rifat. Ahamad for his valuable scientific information. I also thank the Ministry of Higher Education and Scientific Research, the University of Baghdad, and the College of Science.

Orcid:

Taghreed S. Hussein:
https://www.orcid.org/000-567-5678-5677

References

[1] G.J. Davies, M. Dauter, A.M. Brzozowski, M.E. Bjørnvad, K.V. Andersen, M. Schülein, *Biochemistry,* **1998**, *37*, 1926-1932. [crossref], [Google Scholar], [Publisher]

[2] W. Pan, C. Ansiaux, S.P. Vincent, *Tetrahedron Lett.,* **2007**, *48*, 4353-4356. [crossref], [Google Scholar], [Publisher]

[3] S. El Bkassiny, I.S. N'Go, C.M. Sevrain, A. Tikad, S.P. Vincent, *Org. Lett.,* **2014**, *16*, 2462-2465. [crossref], [Google Scholar], [Publisher]

[4] A. Pałasz, D. Cież, B. Trzewik, K. Miszczak, G. Tynor, B. Bazan, *Top. Curr. Chem.,* **2019**, *377*, 1-84. [crossref], [Google Scholar], [Publisher]

[5] A. Caravano, D. Baillieul, C. Ansiaux, W. Pan, J. Kovensky, P. Sinaÿ, S.P. Vincent, *Tetrahedron,* **2007**, *63*, 2070-2077. [crossref], [Google Scholar], [Publisher]

[6] T. Bililign, B.R. Griffith, J.S. Thorson, *Nat. Prod. Rep.,* **2005**, *22*, 742-760. [crossref], [Google Scholar], [Publisher]

[7] P.M. Foley, A. Phimphachanh, E.S. Beach, J.B. Zimmerman, P.T. Anastas, *Green Chem.,* **2011**, *13*, 321-325. [crossref], [Google Scholar], [Publisher]

[8] S. Yuan, S. Yin, M. Liu, J.-Q. Kong, *Scientific reports,* **2018**, *8*, 1-13. [crossref], [Google Scholar], [Publisher]

[9] E. Abdelghani, S.A. Said, M. Assy, A.M.A. Hamid, *J. Iran. Chem. Soc.,* **2015**, *12*, 1809-1817. [crossref], [Google Scholar], [Publisher]

[10] (a) G. Waghmare, S. Junne, S. Shinde, A. Waghamare, S. Kuberkar, *Chem Sci Trans.,* **2013**, *2*, 1-4. [crossref], [Google Scholar], [Publisher] (b) V.A. Adole, *J. Appl. Organomet. Chem*., **2021**, *1*, 29-40. [crossref], [Google Scholar], [Publisher] (c) S.B Biradar, D.V. Narte, R.P. Kale, K.I. Momin, M.S. Sudewad, K.C. Tayade, D.G. Palke, *J. Appl. Organomet. Chem*., **2021**, *1*, 41-47. [crossref], [Google Scholar], [Publisher]
[11] C. Mugnaini, F. Manetti, J.A. Esté, I. Clotet-Codina, G. Maga, R. Cancio, M. Botta, F. Corelli, *Bioorg. Med. Chem. Lett.,* **2006**, *16*, 3541-3544. [crossref], [Google Scholar], [Publisher]
[12] D.J.L. BAI, *Int. J. Inv. Pharm. Sci*., **2014**, *2*, 759-766. [Pdf], [Google Scholar], [Publisher]
[13] X. Zhang, X. Zhou, R.L. Kisliuk, J. Piraino, V. Cody, A. Gangjee, *Bioorg. Med. Chem.,* **2011**, *19*, 3585-3594. [crossref], [Google Scholar], [Publisher]
[14] N. Sekhar, P.V. Acharyulu, Y. Anjaneyulu, *Tetrahedron Lett.,* **2011**, *52*, 4140-4144. [crossref], [Google Scholar], [Publisher]
[15] (a) A. Leggio, E. Belsito, G. De Luca, M. Di Gioia, V. Leotta, E. Romio, C. Siciliano, A. Liguori, *Rsc Advances,* **2016**, *6*, 34468-34475. [crossref], [Google Scholar], [Publisher]
(b) M.V. Gaikwad, *J. Appl. Organomet. Chem.,* **2021**, *1*, 59-65. [crossref], [Google Scholar], [Publisher]
[16] M.A. Al-Omar, A.R.M. Al-Obaid, N.R. El-Brollosy, A.A. El-Emam, *Synth. Commun.,* **2010**, *40*, 1530-1538. [crossref], [Google Scholar], [Publisher]
[17] R.W. Franck, *Comptes Rendus Chimie,* **2012**, *15*, 46-56. [crossref], [Google Scholar], [Publisher]
[18] R. Kalkotwar, R. Saudagar, *Asian J. Pharm. Res.,* **2013**, *3*, 159-165. [Google Scholar], [Publisher]
[19] M. Jabir, U.I. Sahib, Z. Taqi, A. Taha, G. Sulaiman, S. Albukhaty, A. Al-Shammari, M. Alwahibi, D. Soliman, Y.H. Dewir, H. Rizwana, *Int. J. Nanomedicine,* **2020**, *15*, 9025. [crossref], [Google Scholar], [Publisher]
[20] S. Al-Musawi, S. Albukhaty, H. Al-Karagoly, G.M. Sulaiman, M.S. Jabir, H. Naderi-Manesh, *Adv. Nat. Sci.: Nanosci. Nanotechnol.,* **2020**, *11*, 045009. [crossref], [Google Scholar], [Publisher]
[21] N. Jadid, D. Hidayati, S.R. Hartanti, B.A. Arraniry, R.Y. Rachman, W. Wikanta, *AIP Conference Proceedings 1854*, **2017**, 020019. [crossref], [Google Scholar], [Publisher]

How to cite this article: Taghreed S. Hussein*, Mohammed R. Ahamad. Synthesis of C- glycoside analogs containing thiouracil derivatives and evaluated as antibacterial, antifungal and antioxidant activities. *Eurasian Chemical Communications*, 2021, 3(8), 584-589. **Link:** http://www.echemcom.com/article_134303.html