Design, Synthesis and Cytotoxic Evaluation of Novel Heterocyclic Thioglycosides

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Abstract

A novel series of 2-thioximidazolidin-4-one and Benzothiazole thioglycosides were synthesized via one-pot reaction of the 2-thioximidazolidin-4-one and Benzothiazole thiolate salts, respectively with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromides. The cytotoxic activity of compound 7, 8a, 8b, 10a, 10b, 15a and 15b were evaluated against MCF-7 cell lines (Breast carcinoma cell lines) showing high to moderate anti-tumor activities. Moreover molecular modeling of these compounds revealed that they have high binding affinity through hydrophobic-hydrophobic interaction and moderate selectivity through the hydrogen bond interaction with the atypical nucleotide binding pocket in the amino terminus of HSP90.

Keywords: 2-thioximidazolidin-4-one; Benzothiazole; Thioglycosides; Cytotoxic activity; HSP90

Introduction

According to the world health organization (WHO), cancer is an important health problem that claims the level of more than 7 million people worldwide on an annual basis [1,2]. Because of limitation of surgery and radiotherapy in effecting a cure of cancer, chemotherapy has been of increasingly important [1,2]. Therefore, identification of novel potent, selective, and less toxic anticancer agents remains one of the most pressing health problems.

In the vast cancer chemotherapeutic space, glycosides have played a very important role as established cancer chemotherapeutic agents, either in their naturally, semi-synthetically, or synthetically forms [3-32]. As cited above, among the natural glycosides based anticancer the antibiotic doxorubicin, anthracycline O-glycoside, ranks among the most effective anticancer drug for acute myelocytic leukemia [5-7]. Furthermore, many sugar modified nucleoside analogs are clinically useful chemotherapeutics [3]. For example, capcetabine [14], N-nucleoside and C-nucleoside, are applied in the treatment of metastatic breast cancer and hairy cell leukemia, respectively. Recently, a number of S-glycosides, a new non classical class of nucleosides, have been proved to be potential anticancer agents against many cancer cell lines [17-22]. Elgemeie et al. described the synthesis of series of heterocyclic thioglycosides, pyridine [33], benzisoquinoline [16] and pyrimidinethione [34] thioglycosides and revealed their potential antitumor activities. A novel dihydropyridine S-glycoside B was identified as strong P-glycoprotein antagonist with significant cytotoxic activity against human colon carcinoma cells [20]. Moreover, the triazin S-glycoside C was found to have significant cytotoxic activity against various cancer cell line especially liver carcinoma HEPG-2 and breast carcinoma MCF-7 cell lines [34].

On the other hand, results of in vivo screening of oleyl glycoside derivatives showed that the oleyl-α-thioglucose D reduces tumor volume in nude mice bearing an implanted C6 glioma, while the O-glycosyl derivative is inactive that highlighting the importance of using enzyme resistant glycosides [35].

Heat shock protein 90 (HSP90) represents an exciting target for the treatment of cancer, as inhibition of this chaperone can affect multiple proteins that are directly associated with all six hallmarks of cancer [36-40]. Pharmacological inhibition of HSP90 effectively inhibits protein substrates dependent upon HSP90 for conformational maturation, resulting in destabilization of the HSP90-client protein heteroprotein complex, which leads to degradation of substrates through the ubiquitin-proteasome pathway [41,42]. HSP90 has emerged as a promising anti-cancer target, with more than 20 clinical trials currently in progress with small molecules that bind the N-terminal ATP binding site [43]. It was reported that Novobiocin E, O-glycoside antibiotic shown to bind adjacent to the ATP-binding site of bacterial gyrase B and to interfere with nucleotide binding, was also able to interact with HSP90 catalytic site C-Terminal, albeit with lower affinity than with gyrase B, and to disrupt the chaperone activity of HSP90 in a manner similar to radicicol-HSP90 inhibitor antitumor drug [44,45]. Modifications to both the coumarin core and benzamide side chain have been pursued, resulting in the production of preliminary structure-activity relationships (SAR) showing that both the six membered sugar moiety at position 7 and the amide linker are essential for the highest activity in comparison with other analogues [46,47]. A series of triazole-containing novobiocin analogues has been designed, the anti-proliferative effects of these compounds were evaluated against breast cancer cell lines, and the manifested activities were similar to their amide-containing counterparts [48].

In view of these observations and with the aim of identifying new anticancer agents with improved pharmacokinetic and safety profile, it was considered valuable to synthesize some new non classical nucleoside derivatives incorporating heterocyclic other than coumarin and/or functionalized aryl derivatives carrying carbohydrate residues through S-glycosidic bond as anti-cancer agents especially against breast carcinoma which could be capable of inhibiting HSP90 function by potential interaction towards the binding pocket in the amino terminus of HSP90.

A library of 2-thioximidazolidin-4-one and Benzothiazole

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thioglycosides and Novobiocin was energy minimized using semi-empirical (PM3) calculations. The catalytic domain of HSP90 was obtained from protein data bank (PDB) and was prepared for docking using Open Eye’s Fred Receptor program Open Eye Omega application was used to generate different conformations of each ligand, in order to achieve flexible ligand docking, to be used in the docking simulations, this software package is able to perform consensus scoring which is essential filtering technique used to obtain more accurate predictions i.e. the lower consensus score, the better binding affinity of the ligands towards the receptor. It was done in Faculty of Science, South Dakota University, USA. This study revealed that some 2-thioxoimidazolidin-4-one thioglycosides such as 7, 8a, 8b, 10a and 10b have hydrophobic – hydrophobic interaction towards ATP-binding site of HSP90 (2BZ5) and also for example 8a and 8b has hydrogen bonds coming from oxygen of acetylated hydroxyl moiety at C-2` with Phe 138:A, while 7 has no hydrogen bonds as shown at Figures 1 and 2 and other derivatives 10a and 10b showed different binding mood between the 2-thioxoimidazolidin-4-one ring itself and the receptor 2BZ5 as for 10a has 2 hydrogen bonds at the amino group flanked between carbonyl and sulfur linked to GLY 97: A, the other one between Carbonyl and Thr 184:A but 10b has only one hydrogen bond with the amino group between carbonyl and Thr 184:A (Figures 3 and 4).

And for benzothiazole thioglycosides 15a and 15b showed different binding to 2BZ5 as 15b has 4 hydrogen bonding at Nitrogen of thiazole ring along with GLY 97:A, CN group along with ASN 106:A, Oxygen of acetylated hydroxyl group located at C- 2’ the alpha carbon next to methyl group along with THR 184:A and Carbonyl group of acetylated hydroxyl group at C- 3’ along with ASN 51:A while 15a has only one hydrogen bond at Carbonyl group of acetylated hydroxyl group at C- 3’ along with ASN 51:A (Figure 5).

Molecular modeling comparative consensus score of Benzothiazole and 2-thioxoimidazolidin-4-one thioglycosides are listed in Table 1.

From these finding we found that some derivatives Benzothiazole and, 2-thioxoimidazolidin-4-one thioglycosides has high binding affinity through hydrophobic-hydrophobic interaction and moderate selectivity through the hydrogen bond interaction with the atypical nucleotide binding pocket in the amino terminus of Hsp90.

**Chemistry**

Here we report the reaction of sodium ethylene-1-thiolate salts with halosugars. (Scheme 1) shows the synthesis of 2-thioxoimidazolidin-4-one thioglycosides 7 and 8a,b starting with 2-thioxoimidazolidin-4-one 1.

Reaction of compound 1 with carbon disulfide in presence of sodium ethoxide afforded the sodium dithiolate salts 2. The Sodium 2-thioxoimidazolidin-4-one -5-methylene-dithioketals 2 was readily mono alkylated to give the stable sodium salts of mono alkylthio derivatives. Thus, one equivalent of methyl iodide or 4-chlorophenacyl bromide gave the viable sodium -2-thioxoimidazolidin-4-one-5-
(methylthio) [2-oxo-2- (4-chlorophenylethyl)thio]-methylene thiolate 8 and 9 which without isolation, were treated with tetra-O-acetylated gluco/galacto-pyranosyl bromides 6 in ethanol at room temperature to give the corresponding S-glucosides 7, 8a and S-galactoside 8b.

The structures of the newly synthesized thioglycosides were deduced by their elemental analyses and spectral data (1H NMR, 13C NMR). For example, the 1H NMR spectrum of compound 7 indicated the anomeric proton as a doublet at δ5.45-5.48 ppm with coupling constant $J_{1',2'} = 9.8$ Hz indicating H-1’ to be trans-diaxial to H-2’ confirmed the β configuration of the glycosidic bond. The other six glucose protons and the four acetyl groups protons resonated at δ 3.82-5.39 and 2.01-2.12 ppm, respectively. Furthermore, the 13C NMR spectrum of the same compound represented a signal at δ 85.5 ppm corresponding to the anomeric C-1’ atom which also confirmed the β configuration and five signals appearing at δ 61.8, 68.4, 74.4 and 77.7ppm that were assigned to C-6’, C-4’, C-2’, C-3’ and C-5’, respectively, in addition to other signals appearing at δ 20.58 – 20.73, 29.7, 169.2-176.5, 210.35ppm corresponding to CH$_3$ sugar acetyl group, SCH$_3$, CO, and C=S, respectively.

The titled compounds 10a, b were prepared by heating the starting hydantoin 1 with phenylisothiocyanate in ethanol in the presence of potassium hydroxide to give the viable intermediate potassium -2-thioxoimidazolidin-4-one-5-(phenylamino)-methylene thiolate salt 9 which without isolation, was treated with the blocked gluco-and galactopyranosyl bromides 6 in ethanol at room temperature to give the corresponding S-glucoside 10a and S-galactoside 10b, in 90% and 83% yield.

The structures of the reaction products 10a, b were substantiated by their elemental analyses and spectral data (IR, 1H NMR). Their IR spectra indicated the presence of a stretching band at 1748 and 1754 cm$^{-1}$ for acetyl C=O group of sugar. The 1H NMR spectrum of 13b showed the anomeric proton as a doublet at δ 6.07 ppm with coupling
analytical data for 15a revealed a molecular formula C_{30}H_{29}N_{3}O_{9}S_{2}. The \(^1\)H NMR spectrum showed the anomeric proton as a doublet at δ 5.2 ppm. The spectrum showed also the 5 aromatic protons as a multiplet at δ 7.00-7.57 ppm of the aglycon part.

In this investigation, we introduce a novel synthesis of benzothiazole thioglycosides derivatives as a new class of glycosides. Potassium 2-[(2E)-2-(phenylamino)-1-(cyano)vinyl]-1,3-benzothiazole-2-thiolates salt 14 was chosen as the key intermediate. The sequence of the reactions, followed in the preparation of the designed compounds was summarized in (Schemes 2 and 3). The starting material, Benzothiazole acetonitrile 13, was obtained from the reaction of 2-amino thiophenol 11 and Acetic acid and malononitrile 12 in absolute ethanol [48]. Thus, it has been found that benzothiazole acetonitrile 13 reacted with phenylisothiocyanate in KOH-EtOH to give the corresponding stable Potassium 2-[(2E)-2-(phenylamino)-1-(cyano)vinyl]-1,3-benzothiazole-2-thiolates salt 14. The latters react with 2,3,4,6-tetra-O-acetyl-\(\alpha\)-D-glucopyranosyl- and galactopyranosyl bromides 6 in ethanol at room temperature to give in a high yield the corresponding S-glucosides 15a or S-galactosides 15b, respectively.

The structures of the reaction products 15a,b were established by their elemental analyses and spectral data \(^1\)H NMR. As an example, the analytical data for 15a revealed a molecular formula C_{30}H_{29}N_{3}O_{9}S_{2}. The \(^1\)H NMR spectrum showed the anomeric proton as a doublet at δ 5.2 ppm. The coupling constant \(J_{H-1',2'}=9.8\) Hz indicated H-1' to be trans-diaxial to H-2'. The other six glucose protons resonated at 3.34-5.18 ppm and the four acetyl groups appeared as four singlets at δ 1.88-1.99 ppm.

In summary, we have achieved the synthesis of heterocyclic thioglycosides by the reaction of the thiolate salts with \(\alpha\)-glycosyl halides. These heterocyclic glycosides can be utilized as starting materials for the synthesis of other carbohydrate derivatives.

\[ \text{Scheme 2: Synthesis of 2-thioxoimidazolidin-4-one thio glycosides derivatives.} \]

\[ \text{Scheme 3: Synthetic pathway for synthesis of benzothiazole thioglycosides.} \]

**Pharmacology**

**Materials and methods**

Potential cytotoxicity effect of the newly synthesized compounds in four concentrations, were evaluated in the National Institute of Cancer, Cairo Egypt by SRB assay [49]. Cells were plated in 96-multiwell plate (104 cells/ well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of plate. Different concentrations of each compound under test (0, 5, 12.5, 25 and 50 \(\mu\)g/ml) were added to the cell monolayer triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37°C and in atmosphere of 5% CO\(_2\). After 48 h, cells were fixed, washed and stained with sulfo-rhodamine B strain. Excess stain was washed with acetic acid and attached stain was recovered with tris EDTA buffer. Color intensity was measured in an ELISA reader. Finally, the relation between surviving fraction and drug conc. is plotted to get the survival curve of each tumor cell line after the specified compound.

**Anticancer screening studies**

Five of the newly synthesized compounds were screened for their anticancer activities against MCF-7 (Breast), IC50 was calculated with regard to saline control group and potency was calculated with regard to percentage of change of Novobiocin and tested compounds, as depicted, in Table 2.

Our SAR study shows that all the tested compounds have high or moderate anti-tumor activity towards Breast cell lines (MCF-7) with IC50 values ranging from 3.99-41.00 \(\mu\)M. For 2-thioxoimidazolidin-4-one-S-glucosides 8a (IC50=2.7 \(\mu\)M and consensus score=251) with [2-oxo-2-(4-chlorophenyl ethyl) thio] moiety at position 4 is more active than 10a (IC50=41 \(\mu\)M and consensus score =431) with [methyl thio] moiety at the same position which are more active than 7 (IC50=41 \(\mu\)M and consensus score =431) with [methyl thio] moiety as it seems that [2-oxo-2-{(4-chlorophenyl ethyl) thio}] improve the hydrophobic-hydrophobic interaction towards the ATP binding site of HSP 90 and thus increase the ability of the ligands to fill the hydrophobic pocket of the target as shown in Figures 1-4, and for 2-thioxoimidazolidin-4-one-S-galactosides 8b and 10b (IC50=4.29 \(\mu\)M, consensus score=252 and IC50=14.67 \(\mu\)M and consensus score=334, respectively) is lower in activity than S-glucosides derivatives in contrarie with Benzothiazole-
S-glucopyranosyl bromide 6a (4.10 g, 0.01 mol) in absolute ethanol (20 ml) was stirred at room temperature for 2 h and then a solution of 2,3,4,6-O-acetyl-α-D-glucopyranosyl bromide 6a (4.10 g, 0.01 mol) in absolute ethanol (20 ml) was added. The reaction mixture was stirred at room temperature for 18 h, evaporated under reduced pressure and the residue was washed with distilled water, then the title compound was separated in pure form by silica column using mobile phase CHCl₃:methanol (9:5.5:0.5).

General procedure:
A mixture of 2 (0.98 g, 0.01 mol) and methyl iodide (0.61 ml, 0.01 mol) in absolute ethanol (20 ml) was refluxed for 30 min. After cooling: carbon disulphide (0.8 ml, 0.01 mol) was added. The reaction mixture was stirred at room temperature for 1 h. The solution was evaporated and the formed solid product was collected and recrystallized from ethanol to give the title compound 2a,b in 70-75% yield.

(Z)-5-[(2`,3`,4`,6`-tetra-O-acetyl-β-D-glucopyranosylthio) (methylthio)methylene]-2-thioxoimidazolidin-4-one. (7)

General procedure:
A mixture of 2 (0.98 g, 0.01 mol) and methyl iodide (0.61 ml, 0.01 mol) in absolute ethanol (20 ml) was stirred at room temperature for 2 h and then a solution of 2,3,4,6-O-acetyl-α-D-glucopyranosyl bromide 6a (4.10 g, 0.01 mol) in absolute ethanol (20 ml) was added. The reaction mixture was stirred at room temperature for 18 h, evaporated under reduced pressure and the residue was washed with distilled water, then the title compound was separated in pure form by silica column using mobile phase CHCl₃:methanol (9:5.5).
Potassium 2-[2E]-2-(phenylamino)-1-(cyano) vinyl 1,3-benzothiazole-2-thiolates. (14).

General procedure:
A mixture of 13 (0.01 mol) and Phenyl isothiocyanate (0.01 mol) was stirred for 60 minutes in ethanol (25 ml) containing potassium hydroxide (0.01 mol) and then evaporated. The separated residue was filtered and then recrystallized from ethanol to give the titled compound 14 in 60%-70% yield.

2-[2E]-2-(phenylamino)-1-cyano-2-(‘2’,‘3’,‘4’,‘6’-tetra-O-acetyl-β-D-gluco-and/or galactopyranosilythio) vinyl][1,3-benzothiazole. (15a&b).

General procedure: A solution of compound 14 (0.01 mol) in absolute ethanol (30 ml) and a solution of 2,3,4,6-tetra-O-acetyl –α-D-gluco- and/or galactopyranosyl bromides (0.01mol) in 10 ml aceton was stirred at room temperature for 6 hour. The solution was evaporated and the formed residue was washed with distilled water to remove the formed potassium bromide salt. The resulting product was recrystallized from ethanol.

2-[2E]-2-(phenylamino)-1-cyano-2-(‘2’,‘3’,‘4’,‘6’-tetra-O-acetyl-β-D-gluco-pyranosilythio) vinyl][1,3-benzothiazole. (15a): Yellow solid; yield 83%; m.p. 196-8 ºC; 1H NMR( 500MHz d ppm 56.10; H, 4.35; N,6.43. Found: C, 56.26; H, 4.56; N, 6.46.

2-[2E]-2-(phenylamino)-1-cyano-2-(‘2’,‘3’,‘4’,‘6’-tetra-O-acetyl-β-D-galactopyranosilythio) vinyl][1,3-benzothiazole. (15b): Yellow solid; yield 88%; m.p. 204-6 ºC; 1H NMR( 500MHz d ppm CDCl3) 1.88-1.99 (4s, 12H, 4 x CH3CO), 3.34 ( s, 2H, 6`-H2), 3.38-3.66 (m, 2H, J=9.64 4`-H, 5`-H), 3.98 (d, 1H, 3`-H), 4.79-5.18 (m, 1H, 2`-H), 5.20-5.23 (d, 1H, J=9.5 1`-H), 7.33-8.08 (m, 9H,C6H5, C6`H4`), Anal.Calc For C30H29N3O9S2 (640.47): C, 56.26; H, 4.56; N, 6.56. Found: C, 56.33; H, 4.50; N, 6.77.

Conclusion
We have achieved the synthesis of 2-thioximidazolidin-4-one and Benzothiazole derivatives having cyclic carbohydrate residues through S-glycosidic bond formation in an efficient manner. Pharmacological evaluation of compounds 7, 8a, 8b, 10a, 10b, 15a and 15b against cell lines MCF-7 revealed them to possess high or moderate anti-tumor activities. Hence, they could be potential drug candidates for cancer treatment.

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References


