Synthesis and in vitro anti-tumor activity of new imidazole and thienoimidazole thioglycosides

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Abstract: A facile, convenient and high yielding synthesis of novel imidazole and thienoimidazole thioglycosides via one-pot reaction of the potassium thiolate salts of aglycon part - prepared from readily available starting materials - with 2,3,4,6-tetra-O-acetyl-*α*-D-gluco- and galactopyranosyl bromides . Pharmacological evaluation of compounds **7a**, **7b**, **6a**, **6b**, **6c**, **10a**, **10c**, **12a** and **12c** in vitro against (HEPG2)cell line (liver carcinoma cell line) showing moderate-low anti-tumor activities with IC50 values ranging from 67.3- >100 (μMol) .
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1. Introduction

The incidence and mortality of cancer patients have become one of the important issues discussed worldwide. Unfortunately, development of resistance to chemotherapeutic agents is a common obstacle in the treatment of different types of cancers **[1,2]** Several important drugs including tamoxifen (TAM), 5- flurouracil (5FU), adriamycin (ADR) and vincristin (VCR) with different structures and mechanisms of anti-tumor activities fail to end these problems completely. Due to the several side effects, drug resistance and failure of anti-tumor drugs to exert their effects in certain cases of cancers **[3 -5]** , looking for new chemotherapeutic agents with synthetic or natural origins is one of the hot topics in cancer research laboratories.

Recently, a number of heterocyclic thioglycosides have emerged that possess interesting cytotoxic activity as pyridine thioglcosides, benzisoquinoline thioglycosides, pyrimidine thioglycosides, thiophene thioglycosides, imidazole thioglycosides and thienopyrazole thioglycosides **[6-10].** It was reported that the dihydropyridine derivative exhibits strong P-glycoprotein (Pgp) antagonist effect and possesses activity against human colon carcinoma cells **[14]**

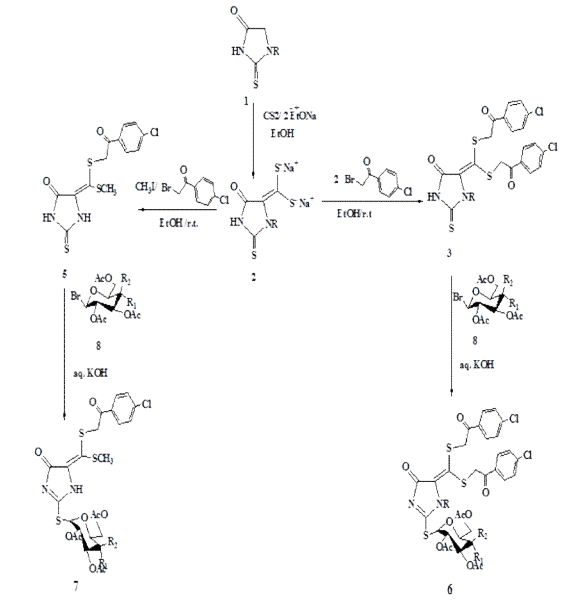
In view of the above mentioned findings and our previous reports **[11-13]** the purpose of the present work was to design, synthesize and investigate the anti-tumor activity of some novel imidazole and thieno imidazole derivatives carrying carbohydrate residues through S-glycosidic bond formation.

2-Chemistry

Here we report novel synthesis of imidazole thioglycoside derivatives. 2-thiohydantoin **1** was refluxed with carbon disulfide and sodium ethoxide in ethanol for 30 minutes to give the corresponding stable sodium 2-thiohydantoin-5-methylenedithiolate **2**. The latter react either with 2 mol P-chloro phenacyl bromide or with1 mol P-chloro phenacyl bromide and 1 mol methyl chloride and then react with 2,3,4,6-tetra-*O*-acetyl-*α*-D-gluco- and galactopyranosyl bromides **8** in ethanol at room temperature to give the corresponding S-glucosides **6a, 7a** and **6c** or S-galactosides **6b**, and **7b** in high yield as shown in (**Scheme 1).**

The structures of the reaction products **6a,b** were established by their elemental analyses and spectral data (IR,^{1}H NMR). As an example, the analytical data for **6b** revealed a molecular formula $C_{27}H_{29}N_2O_{11}S_3Cl$. The ^{1}H NMR spectrum showed the anomeric proton as a doublet at δ5.7 ppm. The coupling constant $J_{1',2'}$ = 9.9 Hz indicated H-1′to be trans-diaxial to H-2′. The other six glucose protons resonated at 3.48-5.05 ppm and the four acetyl groups appeared as four singlets at δ 1.96-2.05 ppm.

When compound **2a,b** were di-alkylated with two p-chloro phenacyl bromide it gave (*5Z*)-5-bis-[(2-oxo-2-(4-chlorophenyl ethyl) thio) methylene]-2-thiohydanyoin and/or *N*-phenyl -2-thiohydantoin **3 a, b** which was then reacted with 2,3,4,6-tetra-*O*-acetyl-*α*-D-gluco- and galactopyranosyl bromides **8** in acetone and aqueous KOH at room temperature to give the corresponding S-glucosides **6a** or S-galactosides **6b,c.**



6	R_1	R_2	R
a	H	OAc	H
b	OAc	H	H
c	H	OAc	Ph

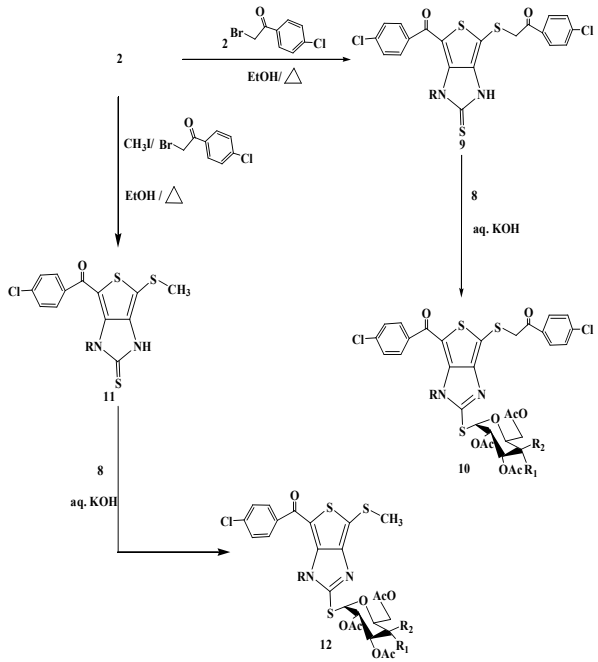
7,8	R_1	R_2
a	H	OAc
B	OAc	H

Scheme1. Synthetic pathway for Imidazole thioglycosides

The structures of the reaction products **6 a, b and c** were established by their elemental analyses and spectral data (IR, ^{1}H NMR). As an example, the analytical data for **6c** revealed a molecular formula $C_{40}H_{36}N_2O_{12}S_3$ Cl_2. The ^{1}H NMR spectrum showed the anomeric proton as a doublet at δ5.24 ppm. The coupling constant $J_{1',2'}$ = 9.6 Hz indicated H-1´to be trans-diaxial to H-2´. The other six glucose protons resonated at 3.81-5.04 ppm and the four acetyl groups appeared as four singlets at δ 1.88-2.06 ppm.

As a further modification of the aglycon moiety, the thieno-imidazole thioglycosides **10a-d** and **12a-d** were synthesized as shown in (Scheme2). Compound **2a,b** was refluxed with one methyl iodide and one p-chloro phencyl bromide to give 4-[methylthio]-6-[(4-chlorophenyl)-oxo-methyl]-2-thio-2,3 dihydro-1-H and/or phenyl -thieno [3,4,d] imidazole **11 a,b .**The latter react with 2,3,4,6-tetra-O-acetyl-α-D-gluco- and galactopyranosyl bromides **8** in acetone and aqueous KOH at room temperature to give the corresponding S-glucosides **12a,c** or S-galactosides **12b,d** , in high yield as shown in **(Scheme 2).**

The structures of the reaction products **12a-d** were established by their elemental analyses and spectral data (IR, ^{1}H NMR). As an example, the analytical data for **12c** revealed a molecular formula $C_{33}H_{31}N_2O_{10}S_3Cl$



10 , 12,	R1	R2	R
a,	H	OAc	H
b ,	OAc	H	H
c,	H	OAc	Ph
d ,	OAc	H	Ph

Scheme 2. Synthethitic pathway for Thienoimidazole thioglycosides

The ^{1}H NMR spectrum showed the anomeric proton as a doublet at δ5.26 ppm. The coupling constant $J_{1',2'}$ = 9.9 Hz indicated H-1´to be trans-diaxial to H-2´. The other six glucose protons resonated at 3.63-5.02 ppm and the four acetyl groups resonate at 1.90-2.05. The ^{13}C NMR spectrum of **12c** contained a signal at δ 85.87 corresponding to the C-1' atom and five signals appearing at δ 61.19, 67.52, 68.87, 72.92 and 74.87 that were assigned to C-6', C-4', C-2', C-3' and C-5', respectively.

Compound **2a,b** was refluxed with two p-chloro phenacyl bromide to give 4-[2-(4-chlorophenyl)-2-oxo-ethylthio]-6-[(4-chlorophenyl)-oxo-methyl]-2-thio-2,3 dihydro-1-H and/or phenyl -thieno [3,4,d] imidazole **9 a,b** The latter react with 2,3,4,6-tetra-O-acetyl-α-D-gluco- and galacto-pyranosyl bromides **8** in acetone and aqueous KOH at room temperature to give the corresponding S-glucosides **10a,c** or S-galactosides **10b,d** , in high yield as shown in **(Scheme 2).**

The structures of the reaction products **10a-d** were established by their elemental analyses and spectral data (IR, ^{1}H NMR). As an example, the analytical data for **10a** revealed a molecular formula $C_{34}H_{31}N_2O_{11}S_3Cl_2$. The ^{1}H NMR spectrum showed the anomeric proton as a doublet at δ5.30 ppm. The coupling constant $J_{1',2'}$ = 9.5 Hz indicated H-1´to be trans-diaxial to H-2´. The other six glucose protons

resonated at 3.76-4.67 ppm and the four acetyl groups resonate at 1.96-2.16. The ^{13}C NMR spectrum of **10a** contained a signal at δ 85.83 corresponding to the C-1' atom and five signals appearing at δ 61.35, 68.83, 69.65, 72.69 and 74.75 that were assigned to C-6', C-4', C-2', C-3' and C-5', respectively.

3- Pharmacology

3.1. Materials and methods

Potential cyto-toxicity effect of the newly synthesized compounds in four concentrations, were evaluated in Faculty of Pharmacy – Alberta University –Canada by MTT assay **[15]**. 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 (0.25, 50 and 100 μM) were added to the cell monolayer octet wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 24 h at 37 ºC and in atmosphere of 5% CO2. After 24 h, the medium was removed and replaced with cell culture medium containing 1.2 mM MTT dissolved in phosphate buffered saline, after 2 h of incubation the crystals formed were dissolved in isopropanol. The intensity of the color formed was measured at wave length 550 nm using Bio-Tek El 312e micro plate 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.

3.2. Anticancer screening studies

Nine of the newly synthesized compounds were screened for their anticancer activities against HEPG2 (Liver), IC50 was calculated with regard to DMSO control group, in **Table 1**.

Table 1: Cytotoxcity of the synthesized candidates on Liver cancer cell line HEPG2

Compound No.	IC50 ± SD (μMol) Liver cancer cell line HEPG2
6a	90.2 ± 49.5
6b	74.7 ± 59.2
6c	79.8 ± 50.1
7a	71.2 ± 47.2
7b	67.3 ± 107.5
10a	76.9 ± 60.8
10c	88.9 ± 85.0
12a	76.3 ± 38.4
12c	114.7 ± 82.5

Our SAR study shows that the imidazole thioglycosides have moderate anti-tumor activity towards Liver cell lines (HEPG2) with IC50 values ranging from 67.3-90.2 (μM) while the tested thieno imdazole thioglycosides have low anti-tumor activity towards Liver cell lines (HEPG2) with IC50 values ranging from 76.3- >100 (μM)

4. Conclusion

We have achieved the synthesis of imidazole and thienoimidazole derivatives having cyclic carbohydrate residues through S-glycosidic bond formation in an efficient manner. Pharmacological evaluation of compounds **6a, 6b, 6c, 7a, 7b, 10a, 10c, 12a** and **12c** against cell lines HEPG2 (Liver) revealed them to possess moderate anti-tumor activities. Hence, they could be a potential drug candidate for cancer treatment.

5- Experimental

All melting points were uncorrected on a Gallenkamp melting point apparatus. The IR spectra were recorded (KBr disk) on a Perkin Elmer 1650 FT-IR instrument. The ^1H NMR spectra were recorded on a Varian 300 MHz spectrometer in $(CD_3)_2SO$ and $CDCl_3$ using $Si(CH_3)_4$ as an internal standard at Alberta University, Faculty of Pharmacy, Canada. Elemental analyses were obtained from the Micro analytical Data Center at Cairo University, Egypt. Progress of the reactions was monitored by TLC using aluminum sheets coated with silica gel F254 (Merck). Viewing under a short-wavelength UV lamp effected detection. All evaporations were carried out under reduced pressure at 35 ^0C.

Compound **1** was prepared following reported procedures. **[16]**

5.1. Sodium 2-thiohydantoin-5-methylenedithiolates (2)

A solution of the 2-thiohydantoin 1 (0.01 mol) and sodium ethoxide (0.46g., 0.02 mol) in absolute ethanol (20 ml) was refluxed for 3o min . The reaction was cooled in ice; and carbon disulphide (0.8 ml, 0.01 mol) was added . The reaction mixture was stirred at room temperature for 1 hr, then the solution was evaporated and the formed product was collected by filtration in 70-75% yield.

5.2. (*5Z*)-5-bis-[(2-oxo-2-(4-chlorophenyl ethyl) thio) methylene]-2-thiohydanyoin and/or *N*-phenyl -2-thiohydantoin (3 a,b)

A solution of the thiohydantoin 2 (0.01 mol) and P- chloro phenacyl bromide (4.68 g, 0.02 mol) in ethanol (20 ml) was stirred at room temperature for 2 hours . The solution was then evaporated and the formed solid product was collected by filtration and re-crystallized from absolute methanol to give the tile compound in 60% yield.

5.3. (*5E*)-[2-oxo-2-((4-chlorophenyl ethyl) thio)-2-(methylthio) methylene]-2-thiohydanyoin (4)

A solution of the thiohydantoin 2 (0.01 mol) and methyl iodide (1.4 ml, 0.01 mol) and P- chloro phenacyl bromide (2.34 g, 0.01 mol) in ethanol (20 ml) was stirred at room temperature for 2 hours . The solution was then evaporated and the formed solid product was collected by filtration and re-crystallized from absolute methanol to give the tile compound in 55% yield.

5.4. General procedure for synthesizing of 6a, 6b and 6c:

The compound (3 , 0.01 mol) was added on a solution of Potassium Hydroxide (0.01 mol) in water (10 ml.), a solution of 2,3,4,6-O-acetyl-α-D-gluco or galactopyranosyl bromide (4.1 g, 0.01 mol) in acetone(20 ml) was added. The reaction mixture was stirred at room temperature for 18 hours, and then evaporated under reduced pressure and the residue was washed with distilled water to remove the Potassium bromide formed , and then the compound was separated by silica column using mobile phase $CHCl_3$: Methanol (9.5 : 0.5) .

5.4.1 (*5Z*)-5-bis-[(2-oxo-2-(4-chlorophenyl ethyl) thio) methylene]-2-(2`,3`,4`,6`-tetra-*O*-acetyl – *β* -D-glucopyranosyl thio)-1H-imidazol-4-(5H)-one (6a).

Brown solid; yield 55%; m.p. 115 ^oC; IR (KBr cm^{-1}) 3213 (NH), 1745.6 (4 x CH_3CO), 1690 (CO) ; ^1H NMR(300MHz δ ppm) 1.89-1.99 (4s, 12H, 4 x CH_3CO) 3.99-4.12 (m, 3H, *J*=9.21, 5`-H, 6`-H_2), 4.59-4.71 (m, 2H, *J*=9.35 4`-H, 3`-H),4.87-4.90 (m, 4H,2 x CH_2), 5.22 (t, 1H, 2`-H) 5.34 (d, *J*=9.55 1H,1`-H), 7.26-8.21 (m, 8H, 2 x C_6H_4), 11.8 (s, 1H, NH) ; Anal.Calcd For. $C_{34}H_{32}N_2O_{12}S_3$ Cl_2 (827.737): C, 49.33; H, 3.89; N, 3.38. Found: C, 49.43; H, 4.00; N, 3.45.

5.4.2 (*5Z*)-5-bis-[(2-oxo-2-(4-chlorophenyl ethyl) thio) methylene]-2-(2`,3`,4`,6`-tetra-*O*-acetyl – *β* -D-galactopyranosyl thio)-1H-imidazol-4-(5H)-one. (6b).

Brown solid; yield 61%; m.p. 135 ^oC; IR (KBr cm^{-1}3248 (NH), 1745.6 (4 x CH_3CO) , 1693 (CO) ; ^1H NMR(300MHz δ ppm) 1.76-1.97 (4s, 12H, 4 x CH_3CO) 3.64-4.04 (m, 3H, *J*=10.3, 5`-H, 6`-H_2), 4.42-4.47 (m, 2H, *J*=9.43, 4`-H, 3`-H), 4.89-4.95 (m, 4H, 2 x CH_2), 5.11- 5.18 (t, 1H, 2`-H) 5.25-5.26 ( d, 1H, *J*=9.55 1`-H), 7.26-7.80 (m, 8H,2 x C_6H_4) ; Anal.Calcd For $C_{34}H_{32}N_2O_{12}S_3$ Cl_2 (827.737): C, 49.33; H, 3.89; N, 3.38. Found: C, 49.47; H, 3.91; N, 3.25.

5.4.3 (*5Z*)-5-bis-[(2-oxo-2-(4-chlorophenyl ethyl) thio) methylene]-2-(2`,3`,4`,6`-tetra-*O*-acetyl – *β* -D-galactopyranosyl thio)-1-(Phenyl)-imidazol-4-(5H)-one. (6c).

Brown solid; yield 56%; m.p. 93 ^oC; IR (KBr cm-3385(NH), 1754. (4 x CH_3CO), 1654 (CO) ; ^1H NMR(300) MHz δ ppm) 1.88-2.06 (4s, 12H, 4 x CH_3CO) 3.81-4.09 (m, 3H, *J*=9.35, 5`-H, 6`-H_2), 4.7-4.75 (t, 1H,4`-H), 4.89-4.96 ( m, 4H, 2 x $CH_2$) 5.01-5.04 (t, 1H, 3`-H), 5.18-5.24 (m, 2H, *J*=9.13,1`-H,2`-H), 7.29-7.91 (m, 13H, 2 x C_6H_4, N-C_6H_5); Anal.Calcd For. $C_{40}H_{36}N_2O_{12}S_3$ Cl_2 (903.83): C, 53.15; H, 4.08; N, 3.09. Found: C, 53.33; H, 4.09; N, 3.00.

5.5. General procedure for synthesizing of 7a and 7b:

The compound (**4** , 0.01 mol) was added on a solution of Potassium Hydroxide (0.01 mol) in water (10 ml.), a solution of 2,3,4,6-O-acetyl-α-D-gluco or galactopyranosyl bromide (4.1 g, 0.01 mol) in acetone(20 ml) was added. The reaction mixture was stirred at room temperature for 18 hours, and then evaporated under reduced pressure and the residue was washed with distilled water to remove the Potassium bromide formed , and then the compound was separated by silica column using mobile phase $CHCl_3$: Methanol (9.5 : 0.5) **.**

5.5.1 (*5E*)-[2-oxo-2-((4-chlorophenyl ethyl) thio})-2-(methylthio) methylene]-2-(2`,3`,4`,6`-tetra-*O*-acetyl – *β* -D-glucopyranosylthio)-*1H*-imidazol-4-(5H)-one. (7a)

Brown solid; yield 64%; m.p. 136 ^oC; IR (KBr cm^{-1} 3312.1 (NH), 1755.2 (4 x CH_3CO), 1690 (CO) ; ^1H NMR(300MHz δ ppm) 1.94-2.04 (4s, 12H, 4 x CH_3CO), 2.48 (s, 3H, SCH_3), 3.84-4.18 (m, 3H, *J*=9.32 5`-H, 6`-H_2), 4.87-4.99 (m, 2H, *J*=9.87,4`-H,), 5.05 (s, 2H, $CH_2$ ), 5.19 ( t, 1H, 3`-H), 5.34 (t, 1H, 2`-H) 5.75 (d, 1H,1`-H), 7.37-7.93 (m, 4H, C_6H_4), 11.8-12.5 (m, 2H, 2 x NH); Anal.Calcd For. $C_{27}H_{29}N_2O_{11}S_3$ Cl (689.184): C, 47.05; H, 4.24; N, 4.06. Found: C, 46.99; H, 4.13; N, 4.22.

5.5.2 (*5E*)-[2-oxo-2-((4-chlorophenyl ethyl) thio)-2-(methylthio) methylene]-2-(2`,3`,4`,6`-tetra-*O*-acetyl – *β* -D- galactopyranosylthio)-*1H*-imidazol-4-(5H)-one. (7b)

Brown solid; yield 60%; m.p. 98 ^oC; IR (KBr cm^{-1} 3246 (NH), 1744.9 (4 x CH_3CO) , 1695 (CO) ; ^1H NMR(300MHz δ ppm) 1.86-2.05 (4s, 12H, 4 x CH_3CO), 2.47 (s, 3H, SCH_3), 3.48-3.53 (m, 3H, *J*=9.35 5`-H, 6`-H_2), 3.90-3.99 (m, 2H, *J*=9.67,4`-H, 3`-H), 4.9 (s, 2H, CH_2), 5.74 (m, 2H, 2`-H, 1`-H), 7.39-7.91 (m, 4H, C_6H_4);Anal.Calcd For. $C_{27}H_{29}N_2O_{11}S_3$ Cl (689.184): C, 47.05; H, 4.24; N, 4.06. Found: C, 47.21; H, 4.33; N, 4.19.

5.6 4-[2-(4-chlorophenyl)-2-oxo-ethylthio]-6-[(4-chlorophenyl)-oxo-methyl]-2-thio-2,3 dihydro-1-H and/or phenyl -thieno [3,4,d] imidazole .(9a,b)

A solution of the thiohydantoin **2** (0.01 mol) and P-chloro phenacyl bromide (4.68 g, 0.02 mol) in ethanol (20 ml) was refluxed for 2 hours . The

solution was then evaporated and the formed solid product was collected by filtration and re-crystallized from absolute methanol to give the tile compound in 70% yield**.**

5.7 4-[methylthio]-6-[(4-chlorophenyl)-oxo-methyl]-2-thio-2,3 dihydro-1-H and/or phenyl - thieno [3,4,d] imidazole .(11 a,b)

A solution of the thiohydantoin **2** (0.01 mol) and methyl iodide (1.4 ml, 0.01 mol) and P- chloro phenacyl bromide (2.34 g, 0.01 mol) in ethanol (20 ml) was refluxed for 2 hours . The solution was then evaporated and the formed solid product was collected by filtration and re-crystallized from absolute methanol to give the tile compound in 63% yield**.**

5.8. General procedure for synthesizing of 12a, 12b, 12c and12d:

The compound **11** (0.01 mol) was added on a solution of Potassium Hydroxide (0.01 mol) in water (10 ml.), a solution of 2,3,4,6-O-acetyl-α-D-gluco or galactopyranosyl bromide (4.1 g, 0.01 mol) in acetone(20 ml) was added. The reaction mixture was stirred at room temperature for 18 hours, and then evaporated under reduced pressure and the residue was washed with distilled water to remove the Potassium bromide formed , and then the compound was separated by silica column using mobile phase $CHCl_3$: Methanol (9.5 : 0.5**) .**

5.8.1 4-(methylthio)-6-[(4-chlorophenyl)-oxo-methyl]-2-(2`,3`,4`,6`-tetra-*O*-acetyl – *β* -D-glucopyranosylthio)-*1H*-thieno [3,4,d] imidazole .(12 a)

Brown solid; yield 65%; m.p. 150 ^oC; IR (KBr cm^{-1} 3248 (NH), 1748.6 (4 x CH_3CO), 1690 (CO) ; ^1H NMR(300MHz δ ppm) 11.91-1.99 (4s, 12H, 4 x CH_3CO), 2.41 (s, 3H, SCH_3), 3.84-4.18 (m, 3H, *J*=9.46,5`-H, 6`-H_2), 4.90 (s, 2H, CH_2) 4.99-5.21 (m, 2H, *J*=10.11, 4`-H, 3`-H), 5.44-5.60 (m, 1H, 2`-H,1`-H), 7.47-8.22 (m, 4H, C_6H_4), 11.87-12.5 (m, 1H, NH); Anal.Calcd For. $C_{27}H_{27}N_2O_{10}S_3Cl$. (671.16): C, 48.31; H, 4.05; N, 4.17. Found: C, 48.35; H, 4.09; N, 4.40.

5.8.2 4-(methylthio)-6-[(4-chlorophenyl)-oxo-methyl]-2-(2`,3`,4`,6`-tetra-*O*-acetyl – *β* -D-galctocopyranosylthio)-*1H*-thieno [3,4,d] imidazole .(12 b)

Brown solid; yield 54%; m.p. 102 ^oC; IR (KBr cm^{-1} 3249 (NH), 1754.1 (4 x CH_3CO) , 1693 (CO) ; ^1H NMR(300MHz δ ppm) 1.70-1.89 (4s, 12H, 4 x CH_3CO), 2.23-2.56 (m, 3H, SCH_3), 3.59-3.96 (m, 3H, *J*=9.35, 5`-H, 6`-H_2), 4.36-4.39 (t, 2H,4`-H), 4.86-4.88 ( t, 1H,3`-H), 5.06 (s, 2H, CH_2) 5.15-5.16 (t, 1H, 2`-H), 5.17-5.18 (d,1H1`-H), 7.13-7.80 (m, 4H, C_6H_4), ; Anal.Calcd For. $C_{27}H_{27}N_2O_{10}S_3Cl$ (671.16): C, 48.31; H, 4.05; N, 4.17. Found: C, 48.22; H, 3.97; N, 4.23.

5.8.3 4-(methylthio)-6-[(4-chlorophenyl)-oxo-methyl]-2-(2`,3`,4`,6`-tetra-*O*-acetyl – *β* -D-glucopyranosylthio)-*1phenyl*-thieno [3,4,d] imidazole .(12 c)

Brown solid; yield 70%; m.p. 117 ^oC; IR (KBr cm^{-1} 3456.2(NH), 1765 (4 x CH_3CO), 1694 (CO); ^1H NMR(300MHz δ ppm) 11.90-2.05 (4s, 12H, 4 x CH_3CO), 2.49 (s, 3H, SCH_3), 3.63-3.96 (m, 3H, *J*=9.46, 5`-H, 6`-H_2), 4.00-4.03 (t, 1H,4`-H), 4.07-4.10 ( t, 1H,3`-H), 4.82 (s, 2H, CH_2) 4.85-5.02 (m, 1H, 2`-H), 5.26 (d,1H, 1`-H),7.31-8.00 (m, 9H, C_6H_4, N-C_6H_5), ^{13}C NMR(300MHz δ ppm) 20.05-20.24 (4 x CH_3), 38.67-40.32 (SCH_2), 40.82-41.01 (SCH_3), 61.19-61.43 (CH_2, C-6`), 67.52 (C-4`), 68.87 (C-2`), 72.92 (C-3`), 74.87 (C-5`), 85.87 (C-1`), 126.41–130.37 (N-C_6H_5, C_6H_4), 160.70 (=C-S), 168.37-168.92 (6 x CO) ; Anal.Calcd For $C_{33}H_{31}N_2O_{10}S_3Cl$ (747.26): C, 53.04; H, 4.18; N, 3.74. Found: C, 53.26; H, 4.27; N, 3.39.

5.8.4 4-(methylthio)-6-[(4-chlorophenyl)-oxo-methyl]-2-(2`,3`,4`,6`-tetra-*O*-acetyl – *β* -D-galactopyranosylthio)-*1phenyl*-thieno [3,4,d] imidazole .(12 d)

Brown solid; yield 64%; m.p. 121 ^oC; IR (KBr cm^{-1} 3385 **(** NH), 1754. (4 x CH_3CO), 1654 (CO); ^1H NMR(300MHz δ ppm) 1.79-2.07 (4s, 12H, 4 x CH_3CO), 2.42 (s, 3H, SCH_3), 3.73-3.78 (m, 3H, *J*=10.34, 5`-H, 6`-H_2), 3.99 (t, 1H,4`-H), 4.11-4.18 ( t, 1H,3`-H), 4.93 (s, 2H, CH_2), 5.12-5.16 (m, 1H, 2`-H), 5.26-5.31 (d,1H1`-H),7.13-7.93 (m, 9H, C_6H_4, N-C_6H_5) Anal.Calcd For. $C_{33}H_{31}N_2O_{10}S_3Cl$. (747.26): C, 53.04; H, 4.18; N, 3.74. Found: C, 53.11; H, 4.33; N, 3.89.

5.9. General procedure for synthesizing 10a, 10b, 10c and 10d:

The compound (**9** , 0.01 mol) was added on a solution of Potassium Hydroxide (0.01 mol) in water (10 ml.), a solution of 2,3,4,6-O-acetyl-α-D-gluco or galactopyranosyl bromide (4.1 g, 0.01 mol) in acetone(20 ml) was added. The reaction mixture was stirred at room temperature for 18 hours, and then evaporated under reduced pressure and the residue was washed with distilled water to remove the Potassium bromide formed , and then the compound was separated by silica column using mobile phase $CHCl_3$: Methanol (9.5 : 0.5) .

5.9.1 4-[2-(4-chlorophenyl)-2-oxo-ethylthio]-6-[(4-chlorophenyl)-oxo-methyl] -2- (2`,3`,4`,6`-tetra-

***O*-acetyl – *β* -D-glucopyranosylthio)-1-*H*-thieno [3,4,d] imidazole .(10 a)**

Brown solid; yield 85%; m.p. 105 ^{o}C; IR (KBr cm^{-1} 3248 (NH), 1754.7 (4 x CH_3CO), 1648 (CO) ; ^{1}H NMR(300MHz δ ppm) 1.96-2.16 (4s, 12H, 4 x CH_3CO) 3.76-3.81 (m, 3H, 5`-H, 6`-H_2), 4.20-4.36 (m, 2H, *J*=9.47,4`-H, 3`-H),4.64-4.67 (m, 2H,CH_2), 5.07-5.30 (m, 2H, *J*=9.55, 2`-H,1`-H), 7.26-8.11 (m, 8H, 2 x C_6H_4) ; ^{13}C NMR(300MHz δ ppm) 19.89-21.14 (4 x CH_3), 38.67-40.72 (SCH_2), 61.35-61.74 (CH_2, C-6`), 68.83-68.99 (C-4`), 69.65 (C-2`), 72.69 (C-3`), 74.75 (C-5`), 85.83 (C-1`), 126.05–131.34 (N-C_6H_5, 2 x C_6H_4), 164.14 (=C-S), 168.75-170.06 (6 x CO) Anal.Calcd For $C_{34}H_{31}N_2O_{11}S_3Cl_2$ (811.82): C, 50.30; H, 3.84; N, 3.45. Found: C, 50.46; H, 3.78; N, 3.53.

5.9.2 4-[2-(4-chlorophenyl)-2-oxo-ethylthio]-6-[(4-chlorophenyl)-oxo-methyl] -2- (2`,3`,4`,6`-tetra-*O*-acetyl – *β* -D-galactopyranosylthio)-*1-H*-thieno [3,4,d] imidazole .(10b)

Brown solid; yield 45%; m.p. 110 ^{o}C; IR (KBr cm^{-1} 3248 (NH), 1754.7 (4 x CH_3CO), 1648 (CO) ; ^{1}H NMR(300MHz δ ppm) 1.60-1.79 (4s, 12H, 4 x CH_3CO) 3.65-3.84 (m, 3H, *J*=9.38, 5`-H, 6`-H_2), 3.99-4.32 (m, 2H, *J*=10.03,4`H, 3`H),4.77-4.81 (m, 2H,CH_2), 4.90-5.04 (m, 2H, *J*=9.79, 2`H, 1`H), 7.10-7.63 (m, 8H, 2 x C_6H_4), 11.90 (s, 1H, NH) ,Anal.Calcd For $C_{34}H_{31}N_2O_{11}S_3Cl_2$ (811.82): C, 50.30; H, 3.84; N, 3.45. Found: C, 50.21; H, 3.98; N, 3.39.

5.9.3 4-[2-(4-chlorophenyl)-2-oxo-ethylthio]-6-[(4-chlorophenyl)-oxo-methyl] -2- (2`,3`,4`,6`-tetra-*O*-acetyl – *β* -D-gluctopyranosylthio)-*1-phenyl*-thieno [3,4,d] imidazole .(10c)

Brown solid; yield 66%; m.p. 85 ^{o}C; IR (KBr cm^{-1} 3325.7(NH), 1748.6 (4 x CH_3CO), 1694 (CO) ; ^{1}H NMR(300MHz δ ppm) 2.00-2.17 (4s, 12H, 4 x CH_3CO) 3.79-3.91 (m, 3H, *J*=10.00, 5`-H, 6`-H_2), 4.15-4.28 (m, 2H, *J*=9.69, 4`-H, 3`-H),4.66-4.70 (m, 2H,CH_2), 5.10-5.13 (t, 1H, 2`-H) 5.22-5.26 (m, 1H,1`-H), 7.10-7.92 (m, 13H, 2 x C_6H_4, N-C_6H_5) , Anal.Calcd For $C_{40}H_{35}N_2O_{11}S_3Cl_2$. (887.918): C, 54.10; H, 3.97; N, 3.15. Found: C, 54.33; H, 3.84; N, 3.13.

5.9.4 4-[2-(4-chlorophenyl)-2-oxo-ethylthio]-6-[(4-chlorophenyl)-oxo-methyl] -2- (2`,3`,4`,6`-tetra-*O*-acetyl – *β* -D-galactopyranosylthio)-*1-phenyl*-thieno [3,4,d] imidazole .(10d)

Brown solid; yield 61%; m.p. 96 ^{o}C; IR (KBr cm^{-1} 3325.7 **(** NH), 1743.2 (4 x CH_3CO), 1664 (CO) ; ^{1}H NMR(300MHz δ ppm) 1.94-2.17 (4s, 12H, 4 x CH_3CO) 3.68-3.76 (m, 3H, *J*=9.44, 5`-H, 6`-H_2), 3.91-4.20 (m, 2H, *J*=9.79, 4`-H, 3`-H),4.56-4.61 (m, 2H,CH_2), 5.07-5.11 (t, 1H, 2`-H) 5.33-5.44 (m, 1H,1`-H), 7.26-7.98 (m, 13H, 2 x C_6H_4, N-C_6H_5) ; Anal.Calcd For $C_{40}H_{35}N_2O_{11}S_3Cl_2$ (887.918): C, 54.10; H, 3.97; N, 3.15 Found: C, 54.14; H, 4.02; N, 3.19.

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