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Synthesis and antimicrobial activity of some *S*- nucleosides and *N*-nucleosides

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ABSTRACT

Many of nucleosides have proved to be active anticancer, antipyretic and inflammatory agents. In the present work the reaction of 5, 6-diphenyl-1, 2, 4-triazine-3-thiol **(1)** with a blocked tri-*O*-acetyl-α-D-arabinosyl bromide **(2)** gave the corresponding nucleoside **(3)**. We synthesized 6-tetra-*O*-acetyl-β-D-glycosidamino-2-methylthiopyrimidin-4-one **(7)**, 5-amino-tetra-*O*-acetyl-β-D-glycosidamino-2-(methylthio)pyrimidin-4-one **(9)**, 3, 4, 5, 6, 7, 8-hexahydro-4-(4-nitrophenyl) quinazoline-2(*1H*) thione and 2-(β-D-glucopyranosylthio)-5, 6, 7, 8-tetrahydro-4-(4-nitrophenyl) quinoazoline **(13)** from pyrimidines derivatives with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl bromide. The structure of the newly synthesized compounds has been established on the basis of their analytical and spectral data. The biological activity of the prepared compounds was also described

KEYWORDS

Nucleosides;
Arabinosyl bromide;
Quinoazoline.

INTRODUCTION

Due to the great importance of triazines as versatile therapeutic agents, the attention of chemists and druggists has been attracted to the investigation of their biological activity. Some 1, 2, 4-triazines have antibiotic[1] and antibacterial activities[2]. The synthesis of these pyrimidine *S*-Nucleosides has been reviewed by many authors[3,4]. Fused ring system incorporated tetrahydrothio pyrimidines have become of considerable interest during the last years. Many of these compounds have proved to be active as anticancer, antipyretic and inflammatory agents[5].

RESULTS AND DISCUSSION

The reaction of 5,6-diphenyl-1,2,4-triazine-3-thiol **(1)** [which was obtained through the condensation of benzil with thiosemicarbazide in the presence of sodium ethoxide] with a blocked tri-*O*-acetyl-α-D-arabinosyl bromide **(2)** in the presence of acetonitrile and mercuric cyanide at 0-5°C gave the corresponding nucleoside 2-(2‘,3‘,5‘-tri-*O*-acetyl-D-arabinosyl)-5,6-diphenyl-3-thio-1,2,4-triazine **(3)** The deacetylation of nucleoside 3 by using alcoholic ammonia gave 2-(D-arabinosyl)-5, 6-diphenyl-3-thio-1, 2, 4-triazine **(4)** (Scheme 1).

A mechanism is given for the reaction that involved quaternization of the nitrogen followed by the elimination of HBr and converted into the thione. This shows that the glycosides are *N*-glycosides and not *S*-glycosides (Scheme 2).

Condensation of ethylcyanoacetate with thiourea in the presence of sodium ethoxide under reflux for 10

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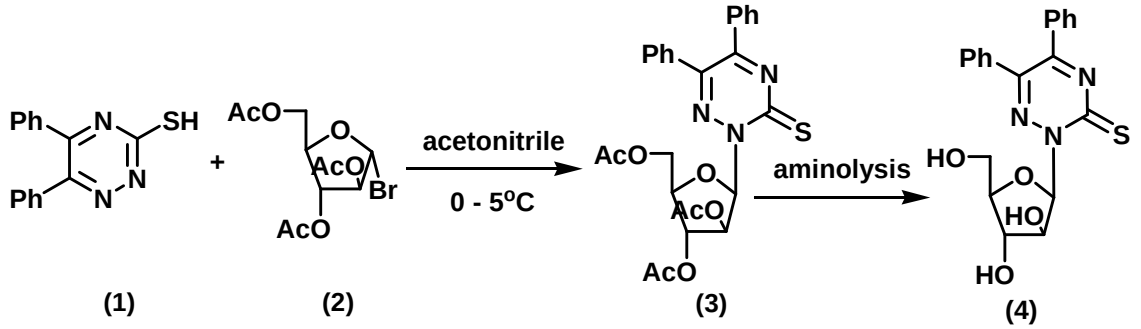
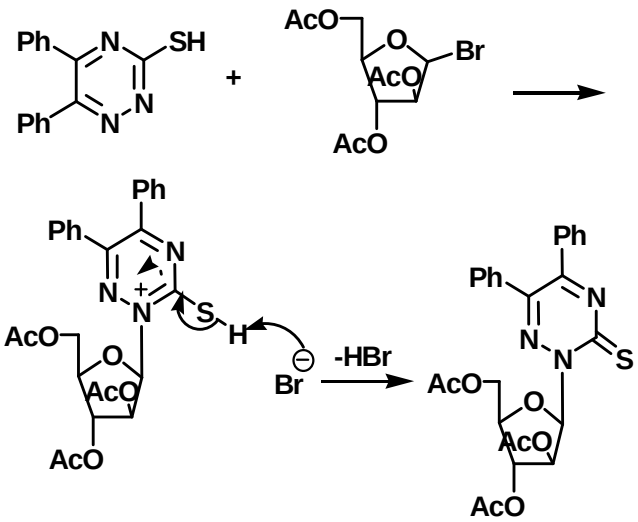


(1) (2) (3) (4)

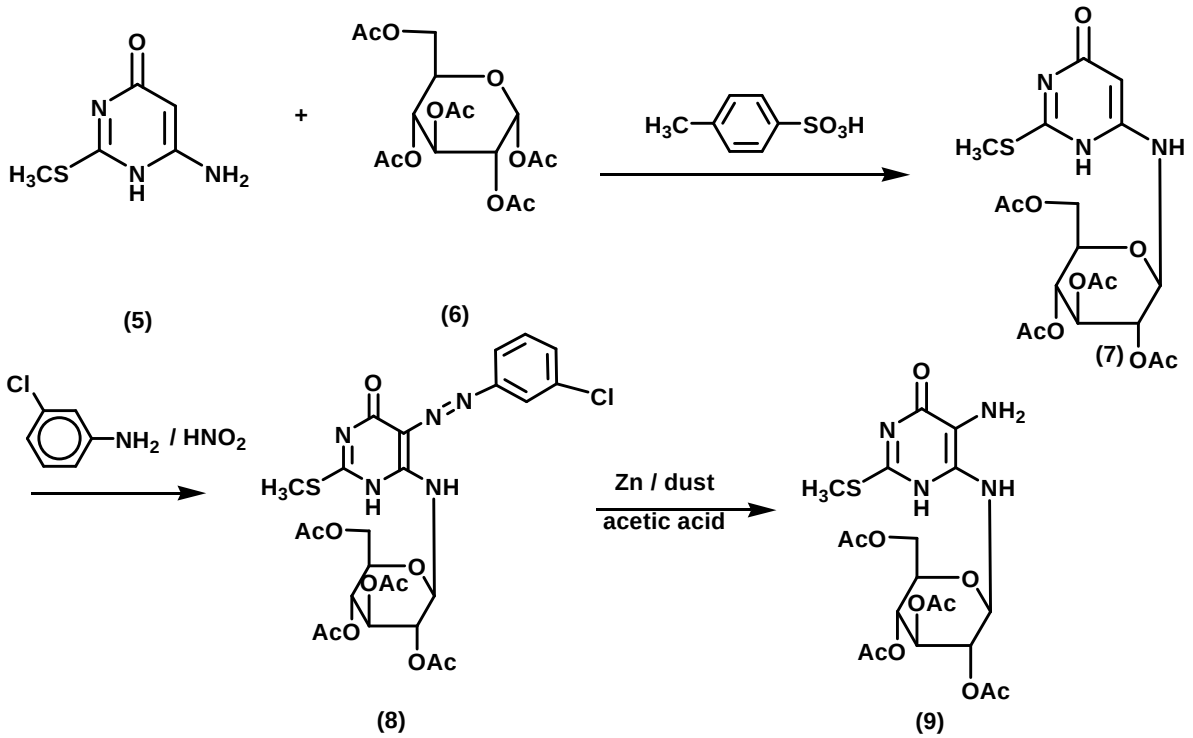
Scheme 1 : Synthetic pathway for the preparation of compounds (3-4)



Scheme 2 : Mechanism for synthesis of compound (3)

hrs, afforded 6-amino-2-thiopyrimidin-4-one which was methylated by methyl iodide in the presence of sodium ethoxide and ethanol to give the known compound 6-amino-2-methylthiopyrimidin-4-one **(5)**[6]. As an extension to our program of nucleosides synthesis, the fusion of 6-amino-2-methylthiopyrimidin-4-one 5 with 1, 2, 3, 4, 6-peta-*O*-acetyl-*β*-D-glycopyranose 6 in the presence of *p*-toluene sulfonic acid as a catalyst for 1 hr., gave 6-tetra-*O*-acetyl- *β* -D-glycosidamino-2-methylthiopyrimidin-4-one 7 according to a reported procedure[7] Scheme 3.

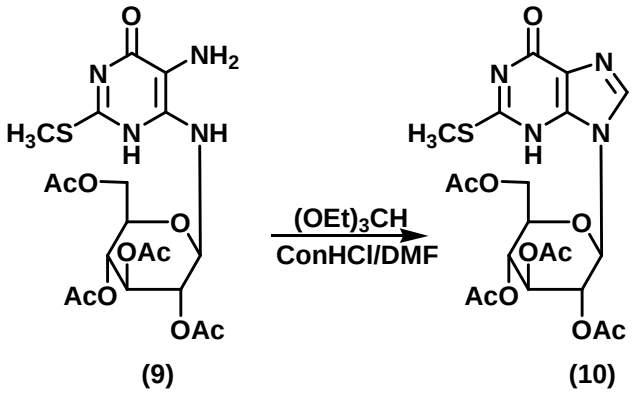
Coupling of compound **(7)** with the corresponding diazonium salt of 3-chloroaniline afforded 5-(3'-chlorobenzeneazo)-6-tetra-*O*-acetyl-*β*-D-glycosidamino-2-methyl- thiopyrimidin-4-one 8 as in-



(5) (6) (7) (8) (9)

Scheme 3 : Synthetic pathway for the preparation of compounds (7-9)

termediate which was catalytically reduced by zinc dust and acetic acid to give 5-amino-tetra-*O*-acetyl-*β*-D-glycosidamino-2-(methylthio)pyrimidin-4-one **(9)**. Treatment of compound **(9)** with triethylorthoformate in the presence of dimethylformamide gave hypoxanthine derivative **(10)**[8] Scheme 4.

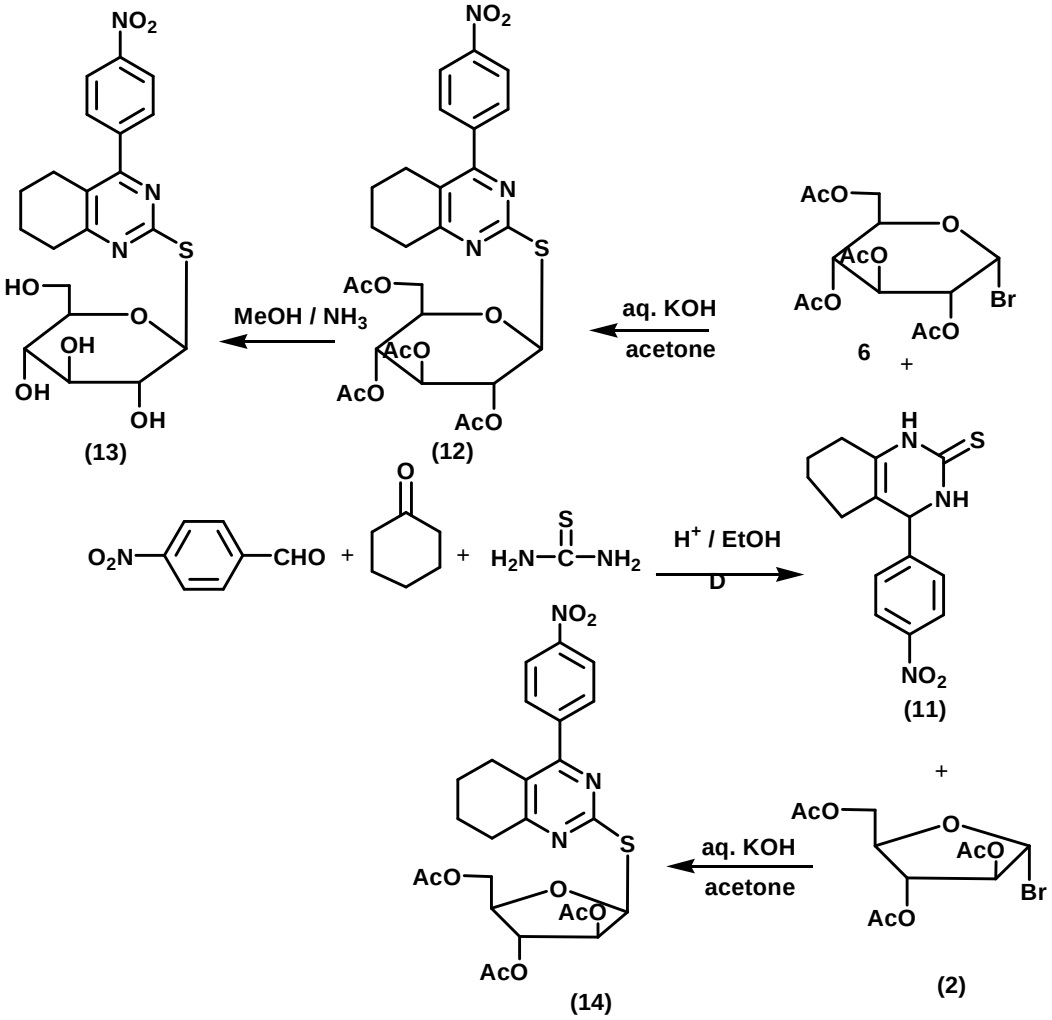


Scheme 4 : Synthetic pathway for the preparation of compound (10)

We report here a novel and convenient method for the synthesis of condensed 2-thiopyrimidinecarbocyclic nucleosides utilizing the 3, 4, 5, 6, 7, 8-hexahydro-4-(4-nitrophenyl) quinazoline-2(*1H*) thione I1. Compound **(11)** was prepared by a one-pot reaction of cyclohexanone, *p*-nitrobenzaldehyde, and thiourea in boiling ethanol containing a catalytic amount of hydrochloric acid.

The first syntheses of pyrimidine nucleosides were achieved by the reaction of the pyrimidine derivative 11 with 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl bromide[10,11] in the presence of aqueous potassium hydroxide and acetone to give 2-(2‘,3‘,4‘,6‘-tetra-*O*-acetyl-*β*-D-glucopyranosylthio)-5,6,7,8-tetrahydro-4-(4-nitrophenyl) quinoazoline 12 through hydrogen bromide elimination and aromatization under the reaction conditions.

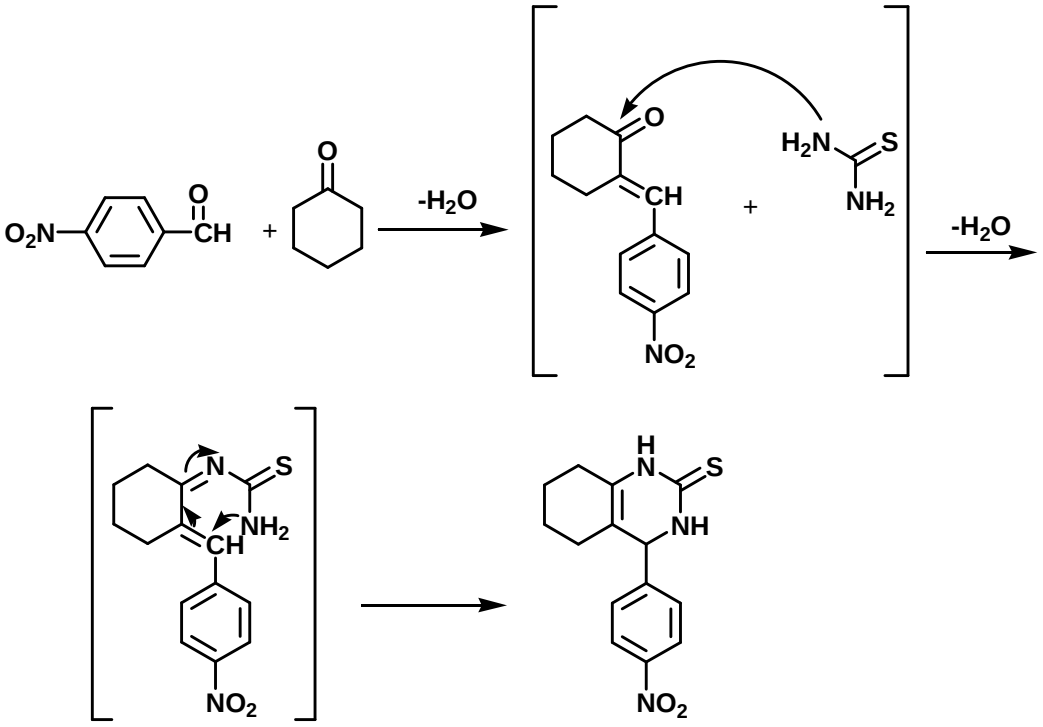
The deacetylation of compound 12 was carried out



Scheme 5 : Synthetic pathway for the preparation of compounds (12-14)

in methanolic ammonia to get the corresponding deblocked nucleoside 2-(β-D-glucopyranosylthio)-5, 6, 7, 8-tetrahydro-4-(4-nitrophenyl) quinoazoline **(13)**. Compound **(11)** was treated also with tri-*O*-acetyl-D-arabinosyl bromide in the presence of potassium hydroxide and acetone to give compound **(14)** (Scheme 5).

A postulated mechanism for this reaction can be given by referring to some recent related reactions[9] as



Scheme 6 : Mechanism for synthesis of compound (11)

follows Scheme 6.

Antimicrobial activity

The synthesized were tested for their antibacterial activity *in vitro* in comparison with gatifloxacin as a reference drug using the standard agar disc diffusion method[12] gainst six bacterial species: *Bacillus cereus* (AUMC B70), *Staphylococcus aureus* (AUMC B71) as representatives of the Gram-positive strains, while the Gram-negative strains were represented by *Escherichia coli* (AUMC B69), *Pseudomonas aeruginosa* (AUMC B72), and *Serratia marcescens* (AUMC B67). Cell suspension of bacterial strains was prepared from 48 h old cultures on nutrient agar (NA) in sterile water. One milliliter of suspension was added to a Petri dish of 9 cm diameter and then 15 mL of NA was poured into the plate. The plate was shaken gently to homogenize the inocula. Sterile 5-mm filter paper disc (Whatman, UK) was saturated with 10 mL of the solution of test compound and gatifloxacin as a reference drug (53 mmol mL–1 in DMSO). In addition, another disc was impregnated with the solvent (DMSO) and served as a negative control. The discs were then dried for 1 h and placed in the center of each plate. The seeded plates were incubated at 35 ± 2 °C for 24–48 h. The radii of inhibition zones (in mm) of triplicate sets were measured and the results are given in TABLE 1.

The examination of data (TABLE 1) reveals that most of compounds showed excellent antibacterial activity when compared with gatifloxacin. From the results, it is obvious that compound 3 showed the highest

TABLE 1 : Results of antibacterial activity of the tested compounds

Compound	Microorganisms				
	Antibacterial activity (in mm/conc. 1mg/ml^{-1})				
	Staphylo coccus aureus	*Serratia marcescens*	*Pseudo monas aeruginosa*	*Escherichia coli*	*Bacillus Cereus*
3	8	7	7	9	8
7	5	2	6	7	6
10	6	4	4	7	4
12	7	8	6	5	7
14	3	4	4	3	6
Gatifloxacin	15	9	10	10	8

degree of inhibition against *Staphylococcus aureus*, *Serratia marcescens, Escherichia coli* EC and *Bacillus subtilis* BS. Moreover, compounds **(10)** and **(14)** have weak inhibition against *Escherichia coli* and *Cereus Bacillus*. While 12 had a considerable degree of inhibition against *Bacillus Cereus and Pseudomonas aeruginosa*

EXPERIMENTAL SECTION

Melting points were determined with an Electro Thermal Mel-Temp II apparatus and are all uncorrected. IR spectra were obtained in the solid state as potassium disc using a Perkin-Elmer model 1430 Spectrometer. ^{1}H NMR were recorded on a Varian/Gemini 200/MHZ spectrometer in DMSO-d_6 as a solvent and TMS as an internal standard (chemical shift in δ, ppm). Mass spectra were measured on an instrument "VG-7035" spectra were recorded at 70 or 15 electron volt. Elemental analysis was performed at the Micro analytical centre, Cairo University, Giza, Egypt.

2- (2', 3', 5'-Tri-*O*-acetyl-D-arabinosyl)-5, 6-diphenyl-3-thio-1, 2, 4-triazine (3)

5, 6-Diphenyl-1, 2, 4-triazine-3-thiol 1 (1.1 g, 4.1 mmol), in acetonitrile (5 ml) was added to a solution of 2, 3, 5-tri-*O*-acetyl-*α*-D-arabinosyl bromide 2 (1.1 g, 4.15 mmol) and (2 mmol) of mercuric cyanide in acetonitrile (10 ml). The reaction mixture was stirred at 0-5°C for 34 h and then the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography with ethyl acetate: ethanol (3:1) to give 3 as yellow crystals (57%), m.p. 232-234°C. IR (KBr): 1746, 1221, 1651 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.37-7.46 (m, 10H, H-ar), 5.95-5.98 (d, 1H, H1'), 4.52-4.41 (m, 3H, H-2', H-3', H-4'), 4.25 (m, 2H, H-5',5''), 1.96-2.61 (3s, 9H, 3OAc) ppm. M.S.: (M$^+$, 523.14) which is equivalent to the molecular formula $C_{26}H_{25}N_3O_7S$. Anal. Calcd. for $C_{26}H_{25}N_3O_7S$: C, 59.65; H, 4.78; N, 8.03. Found. C, 59.83; H, 4.63; N, 8.12.

2- (*α*-*D*-arabinosyl) -5, 6-diphenyl-3-thio-1, 2, 4-triazine (4)

The protected nucleoside 3 (1 mmol) was dissolved in dry methanol and a fairly rapid stream of dry ammonium was passed into the solution for 2 h, with stirring until TLC indicated completion of the reaction. The solution was kept for 12 hrs at room temperature. Removal of the solvent under reduced pressure yielded syrup which was dissolved in dry ethanol and recrystallized by adding a few drops of ethyl acetate. Yield =35%, m.p. 170-175°C. IR (KBr): 3422-3588, 1183, 1652cm^{-1}. M.S.: (M$^+$, 397,) which is equivalent to the molecular formula $C_{20}H_{19}N_3O_4S$ and fragmentation ions at m /e (290, 14.2%), (265, 20.3%) and (178, 100%). Anal. Calcd. for $C_{20}H_{19}N_3O_4S$: C, 60.45; H, 4.78; N, 10.58. Found: C, 60.49; H, 4.84; N, 10.66.

6-Tetra-*O*-acetyl -*β* -D-glycosidamino-2-(methylthio) pyrimidin-4-one (7)

A mixture of 1, 2, 3, 4-penta-*O*-acetyl-â-D-glucopyranose 6 (0.653 g, 1.67 mmol) and 6-amino-2-methylthiopyrimidin-4-one 5 (0.32 g, 2.03 mmol) in the presence of p-toluene sulphonic acid (0.195 g) in tetrahydrofurane was refluxed with stirring for 6 hrs. After the solvent was removed by evaporation in vacuo to dryness, the residue was dissolved in chloroform and washed with aqueous sodium bicarbonate. The organic layer was dried, evaporated to dryness and the residue was recrystallized from ethanol to give yellow crystals yield, 51%, m.p. 210-220°C. IR (KBr): 3150, 1702, 1590, 1078 cm^{-1}; ^{1}H NMR δ 7.72 (s, 1H, NH), 6.52 (d, 1H, H-1'), 5.18(s, 1H, H-5), 4.95 (s, 1H, NH), 4.06-4.89 (m, 6H, H-2',3',4',5',6',6''), 3.51(s, 3H, CH_3), 1.91-2.07 (4s, 12H, 4OAc) M.S.: (M$^+$, 487) which is equivalent to the molecular formula $C_{19}H_{25}N_3O_{10}S$ has fragmentation ions at m/e (441,12.3%), and at m/e (150,100%). Anal. Calc for $C_{19}H_{25}N_3O_{10}S$: C, 46.81; H, 5.13; N, 8.62. Found: C, 46.79; H, 5.21; N, 8.69

5-(3'-Chlorobenzeneazo)-6-tetra-*O*-acetyl-β-D-glycosidamino-2-methyl-thiopyrimidin-4-one (8)

A suspension of compound 7 (0.59 g, 1.2 mmol) in water (30 ml) was treated with a solution of 3-chlorobenzenediazonium chloride, prepared in the usual way from 3-chloroaniline (4 g) in water (120 ml) containing concentrated hydrochloric acid (25 ml) and sodium nitrite (0.7 g). The red azo compound was collected and dried. This crude material was dissolved in pyridine (50 ml), treated with acetic anhydride (37.5 ml) and after standing overnight, excess of acetic anhydride was decomposed with ethanol (60 ml) and sol-

vents removed under reduced pressure. Recrystallization of the residue from benzene gave needles crystals with m.p. 246-247°C. IR (KBr): 1735, 1613, 1321, cm^{-1}. (M^+, 625.5; M^+ +1, 626) which is identical to molecular formula $C_{25}H_{28}N_5O_{10}SCl$ and has fragmentation ion at m/e (443, 45.3%) and at m /e (169,100%) Anal. Calcd. for $C_{25}H_{28}N_5O_{10}SCl$: C, 47.96; H, 4.47; N, 11.19. Found: C, 48.14; H, 4.52; N, 11.27

5- Amino-6-tetra-*O*-acetyl-β-glucosidamino-2-methylthiopyrimidin-4-one (9)

Zinc dust (0.4 g) was added to a mixture of the azo compound 8 (1.5 g, 0.24 mmol), tetrahydrofuran (30 ml), ethanol (30 ml), water (30 ml) and acetic acid (30 ml) at 70°C, and the mixture was stirred at this temperature for 20 min. The reaction mixture was filtered and the filtrate was concentrated to dryness. The residue was purified by silica gel column chromatography with chloroform: methanol (3: 1) to give yield 52%, as foam. IR (KBr): 3124, 1737, 1632 cm^{-1}; the mass spectrum showed the molecular ion peak M^+ at (m/z: 502.14) supporting to the molecular formula $C_{19}H_{26}N_4O_{10}S$ and has fragmentation ion at m/e (443, 45.3%) and at m /e (169,100%) Anal. Calcd for $C_{19}H_{26}N_4O_{10}S$: C, 45.42; H, 5.18; N, 11.15. Found: C, 45.51; H, 5.26; N, 11.20

9-(2‘,3‘,4‘,6‘-Tetra-*O*-acetyl-*β*-D-glucopyranosyl)-2-methylthio-*3H*-hypoxanthin (10)

A mixture of 9 (0.130 g, 0.36 mmol), triethylorthoformate (3 ml), concentrated hydrochloric acid (0.7 ml), and dimethylformamide (1.5 ml) was stirred at 0-5°C for 8 h. and then at room temperature for 8 h. After the solvent was removed under reduced pressure, the residue was purified by silica gel column chromatography with (MeOH: $CHCl_3$) (1: 3) to give a yellowish solid (46%) m.p. 273-275°C. IR (KBr): 1735, 1687, 1613, 1621 cm^{-1} ; ^{1}H-NMR δ 10.25 (s, 1H, NH), 8.35 (s, 1H, H-8), 6.63 (d, 1H, H-1‘), 4.78 (m, 3H, H-2‘,3,4‘), 4.05 (m, 3H, H-5‘,H,6‘,6“), 2.87 (s, 3H,CH_3), 1.96-2.32 (s, 12H, 4OAc). M.S: (M^+, 337) which is equivalent to molecular formula $C_{20}H_{24}N_4O_{10}S$ (512.12). Anal. Calcd for $C_{20}H_{24}N_4O_{10}S$: C, 46.77; H, 4.74; N, 10.81. Found: C, 46.87; H, 4.68; N, 10.93

3, 4, 5, 6, 7, 8-Hexahydro-4-(4-nitrophenyl) quinazoline-2(*1H*)-thione (11)

To a mixture of *p*-nitrobenzaldehyde (1.25 g, 8.28 mmol), cyclohexan-one (1.65 g, 1.68 mmol) and thiourea (1.28 g, 1.68 mmol) in ethanol (50 ml), conc. HCl (2 ml) was added. The mixture was heated under reflux for 4 hrs and then left to stand overnight. The resultant precipitate was filtered off and recrystallized from water-ethanol to afford yellow crystals, yield 76%, m.p. 216-220°C. IR (KBr): 3183, 1697, 1516, 1344, 1198 cm^{-1}; ^{1}H NMR δ 10.09 (s, 1H, NH), 10.22 (s, 1H, NH), 7.57-8.27(d,d, 4H, C_6H_4), 5.18 (s, 1H, CH), 3.31 (t, 4H, $2CH_2$), 2.49 (m, 4H, $2CH_2$); M.S: (M^+ 289) which is corresponding to molecular formula $C_{14}H_{15}N_3O_2S$ and has fragmentation ions at m/e (266, 16.2%), (203, 80.2%) and (166, 100%).. Anal. Calcd for $C_{14}H_{15}N_3O_2S$: C, 58.11; H, 5.23; N, 14.52; Found: C, 58.23; H, 5.41; N, 14.57.

2-(2‘,3‘,4‘,6‘-Tetra-*O*-acetyl-*β*-D-glucopyranosylthio)-5,6,7,8-tetra-hydro-4-(4-nitrophenyl) quinazoline (12)

To a solution of 3,4,5,6,7,8-hexahydro-4-(4-nitrophenyl) quinazoline-2(*1H*)-thion 11 (0.23 g, 0.79 mmol) in aqueous potassium hydroxide (0.73 g in 10 ml of distilled water), was added a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl bromide (0.46 g, 2.0 mmol) in acetone (20 ml). The reaction mixture was stirred at room temperature until the reaction was completed, then evaporated under reduced pressure and the residue was washed with distilled water to remove the formed potassium bromide. The product was dried and recrystallized from ethanol to afford pale yellow crystals, yield 63%, m.p. 206-211°C. IR (KBr): 1736, 1604, 1517,1325, 523 cm^{-1}; ^{1}H-NMR δ 7.61-8.19 (d,d, 4H, C_6H_4), 6.82 (d, 1H, H-1‘), 5.12 (m, 2H, H-6‘,6“), 4.32- 4.47 (m, 4H, H-2‘,3‘,4‘,5‘), 3.17 (t, 4H, $2CH_2$), 2.46 (m, 4H, $2CH_2$), 1.90-2.13 (4s, 12H, 4OAc) ppm; M.S: (M^+ 617.17) which is corresponding to molecular formula $C_{28}H_{31}N_3O_{11}S$. Anal. Calcd for $C_{28}H_{31}N_3O_{11}S$: C, 54.53; H, 5.22; N, 9.65. Found: C, 54.45; H, 5.05; N, 6.80

2- (*β*-D-Glucopyranosylthio)-5, 6, 7, 8-tetrahydro-4-(4-nitrophenyl)-quinoazoline (13)

A solution of nucleoside 12 (1 mmol), in absolute methanol (10 ml) was added at 0°C to a saturated solution of anhydrous NH_3 in absolute methanol (25 ml) and the mixture was stirred at 0°C for 4 hrs., and then at room temperature for an additional 12 hrs, purified

on a silica gel column (MeOH: $CHCl_3$, 1:9, v/v) and then crystallization from methanol to give compound 3 yield 65%, m.p. 175-181°C. ii) IR (KBr): 3400-3521, 1621, 1525, 1352, 551cm^{-1}; M.S: ($M^{+\cdot}$ 449.13) which is corresponding to molecular formula $C_{20}H_{23}N_3O_7S$ has fragmentation ions at m/e at (224, 100%) and (322, 7.0%) Anal. Calcd for $C_{20}H_{23}N_3O_7S$: C, 53.45; H, 5.17; N, 9.35. Found: C, 53.53; H, 5.26; N, 9.39

2-(2‘, 3‘, 5‘-Tri-*O*-acetyl-*β*-D-arabinosylthio)-5, 6, 7, 8-tetrahydro-4-(4-nitro-phenyl)quinazoline (14).

A solution of compound 11 (1 mmol) in aqueous potassium hydroxide (0.73 g in 10 ml of distilled water), was added to a solution of 2, 3, 5-tri-O-acetyl-D-arabinosyl bromide (1.0 mmol) in acetone (30 ml). The reaction mixture was stirred at room temperature for 7 h. until the reaction was completed, then evaporated under reduce pressure and the residue was dissolved in water to remove the potassium bromide formed and extracted with chloroform and evaporated to afford pale yellow crystals. Yield 80% m.p. 192-196°C. IR (KBr): 1748, 1602, 1516, 1345 cm^{-1}; ^{1}H NMR δ 7.47-8.25 (d,d, 4H, C_6H_4), 6.21(d, 1H, H-1‘), 4.78-4.98 (m, 3H, H-2‘,3‘,4‘), 3.51(d, 2H, H-5‘), 2.48(m, 4H, $2CH_2$), 2.98 (t, 4H, $2CH_2$), 1.96-2.02 (3s, 9H, 3OAc). M.S: ($M^{+\cdot}$ 545.15) which is corresponding to molecular formula $C_{25}H_{27}N_3O_9S$. Anal. Calcd for $C_{25}H_{27}N_3O_9S$: C, 55.04; H, 4.94; N, 7.70. Found: C, 55.14; H, 5.01; N, 7.79.

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