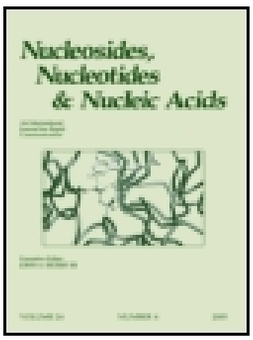

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Novel dihydropyridine thioglycosides and their corresponding dehydrogenated forms as potent anti-hepatocellular carcinoma agents

Galal H. Elgemeie[a] and Dina H. El-Naggar[b]

[a]Department of Chemistry, Faculty of Science, Helwan University, Helwan, Cairo, Egypt; [b]Department of Applied Organic Chemistry, National Research Center, Giza, Egypt

ABSTRACT

A novel method for preparation of a new class of dihydropyridine thioglycosides and their corresponding dehydrogenated forms, via reaction of piperidinium salts of dihydropyridinethiones with 2,3,4,6-tetra-*O*-acetyl-α-D-gluco- and galactopyranosyl bromides has been studied. The evaluation of antiproliferative activity against HepG-2 cell lines (liver carcinoma cell lines) of the dihydropyridine thioglycosides and pyridine thioglycosides revealed that many of the thioglycosides have interesting antitumor activities specifically **5c, 5g, 5l, 5o, 5p, 7a, 7i, 7p, 8b, 8f, 8s**, and **8v**.

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Introduction

In recent years nucleoside analogues have occupied a significant position in the search for effective antiviral agents, owing to the fact that a large number of unnatural nucleoside derivatives have been shown to inhibit infection caused by viruses.[1,2] The heterocyclic thioglycosides constitute a class of analogues with potential biological activity.[3–12] In recent reports from our laboratory, we described the preparation of different novel functionalized pyridinethione glycosides, which revealed antagonistic activity against human carcinoma cells and HIV.[13] In an earlier brief communication we had reported the use of dihydropyridinethione glycosides as *P*-glycoprotein (Pgp) substrates or inhibitors in the protein glycosylation process.[14] These common features encouraged us to develop a new straightforward route for the synthesis of these compounds. In the present report, we describe the synthesis of dihydropyridine thioglycosides through reaction of piperidinium salts of dihydropyridinethiolates with δ-acetylated α-glycosyl halides.

Results and discussion

It has been found that arylmethylidenecyanothioacetamide **1** reacted with 3-oxo-*N*-arylbutanamides **2** in ethanol containing piperidine at 0°C to give the

CONTACT Galal Elgemeie elgemeie@yahoo.com Chemistry Department, Faculty of Science, Helwan University, Helwan, Cairo, Egypt.
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Table 1. Cytotoxcity of the synthesized compounds 5a-v, 7a-v and 8a-v on liver (HEPG2) cancer cell lines.

Compound no.	IC_{50} (μg/mL)	Compound no.	IC_{50} (μg/mL)	Compound no.	IC_{50} (μg/mL)
5a	18.2 ± 0.3	**7a**	9.0 ± 0.5	**8a**	20.0 ± 0.5
5b	21.6 ± 0.9	**7b**	20.2 ± 0.7	**8b**	10.2 ± 0.7
5c	10.2 ± 0.4	**7c**	16.1 ± 0.5	**8c**	15.2 ± 0.9
5d	17.5 ± 0.5	**7d**	21.5 ± 1.2	**8d**	20.0 ± 1.2
5e	20.7 ± 0.8	**7e**	17.3 ± 2.1	**8e**	18.2 ± 0.7
5f	19.4 ± 1.3	**7f**	16.1 ± 0.3	**8f**	8.7 ± 0.8
5g	10.4 ± 0.9	**7g**	19.0 ± 0.5	**8g**	16.0 ± 0.4
5h	19.3 ± 0.5	**7h**	14.3 ± 0.7	**8h**	17.9 ± 0.4
5i	15.1 ± 3.0	**7i**	8.1 ± 0.6	**8i**	19.2 ± 0.6
5j	16.1 ± 1.7	**7j**	22.1 ± 0.3	**8j**	15.5 ± 0.6
5k	10.1 ± 0.3	**7k**	16.1 ± 0.9	**8k**	19.1 ± 0.5
5l	9.5 ± 0.3	**7l**	20.1 ± 0.5	**8l**	16.4 ± 0.6
5m	16.5 ± 0.7	**7m**	17.1 ± 2.2	**8m**	15.5 ± 0.3
5n	20.5 ± 1.2	**7n**	18.3 ± 1.3	**8n**	14.2 ± 0.5
5o	12.5 ± 0.5	**7o**	15.0 ± 0.5	**8o**	21.5 ± 0.2
5p	8.5 ± 0.6	**7p**	8.9 ± 0.2	**8p**	18.0 ± 0.4
5q	20.0 ± 0.8	**7q**	14.2 ± 0.5	**8q**	15.7 ± 0.3
5r	22.5 ± 0.8	**7r**	17.4 ± 0.6	**8r**	22.5 ± 0.3
5s	17.7 ± 0.3	**7s**	19.1 ± 0.7	**8s**	11.8 ± 2.1
5t	15.3 ± 2.0	**7t**	18.0 ± 0.4	**8t**	17.1 ± 0.5
5u	19.5 ± 1.4	**7u**	15.2 ± 2.0	**8u**	16.8 ± 1.3
5v	16.1 ± 0.7	**7v**	21.0 ± 0.3	**8v**	8.9 ± 0.7
(5FU)[b]	25	**(5FU)[b]**	25	**(5FU)[b]**	25

corresponding piperidinium salts of 1,4-dihydropyridine-2-thiones **3** (Chart 1). The structures of salts **3** was established on the basis of their elemental analysis and spectral data. Compounds **3** react with 2,3,4,6-tetra-*O*-acetyl-α-D-gluco- and galacto-pyranosyl bromides in acetone at 0°C to give in a high yield the corresponding *S*-glucosides **5a-p** or *S*-galactosides **5q-v**, respectively. The structures of the reaction products **5a-v** were established by their elemental analyses and spectral data (IR, ^{1}H NMR, ^{13}C NMR). As an example, the analytical data for **5l** revealed a

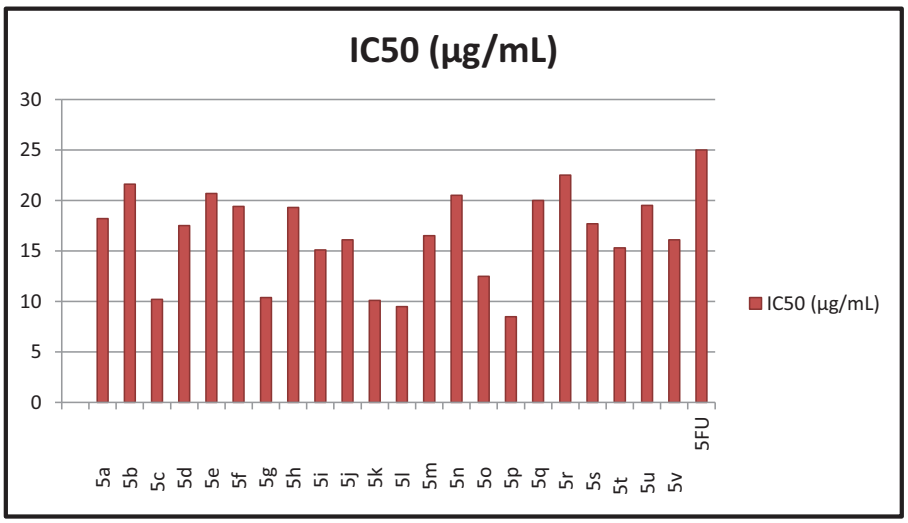


Figure 1. Cytotoxcity of the synthesized acetyled dihydropyridine thioglycosides **5a-v** on liver (HEPG2) cancer cell line.

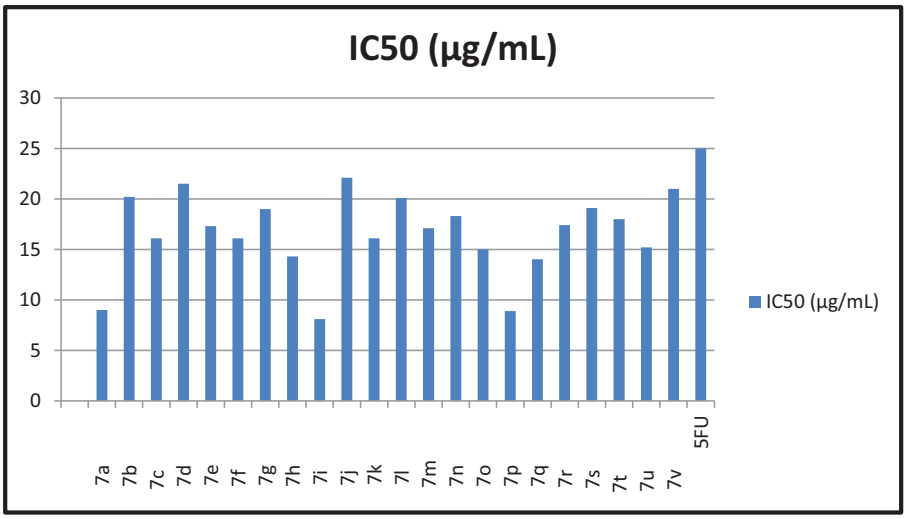


Figure 2. Cytotoxcity of the synthesized acetylated pyridine thioglycosides **7a-v** on liver (HEPG2) cancer cell line.

molecular formula $C_{35}H_{36}ClN_3O_{11}S$. The ^{1}H NMR spectrum showed the anomeric proton as a doublet at δ 5.34 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 3.69–4.92 ppm and the four acetyl groups appeared as four singlets at δ 1.93–2.05 ppm. The ^{13}C NMR spectrum of **5l** contained a signal at δ 82.8 corresponding to the C-1′ atom and five signals appearing at δ 61.9, 67.8, 70.2, 72.7 and 75.0 that were assigned to C-6′, C-4′, C-2′, C-3′ and C-5′, respectively. The formation of *S*-glycosides **5** and not the corresponding *N*-glycosides **6** (chart 1) were proved using ^{13}C NMR spectroscopy, which revealed the absence of the thione carbon at δ 178 ppm and the appearance of a signal at δ 160 ppm corresponding to the C-2 carbon, whose

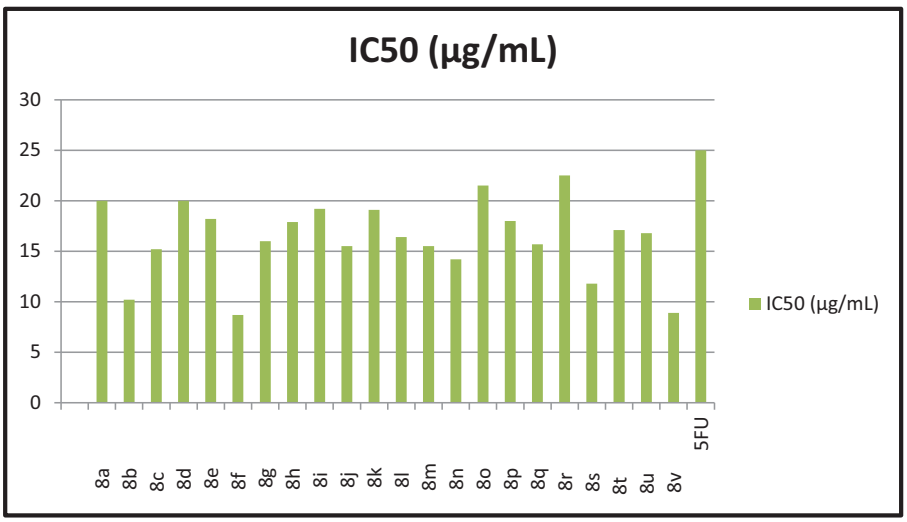
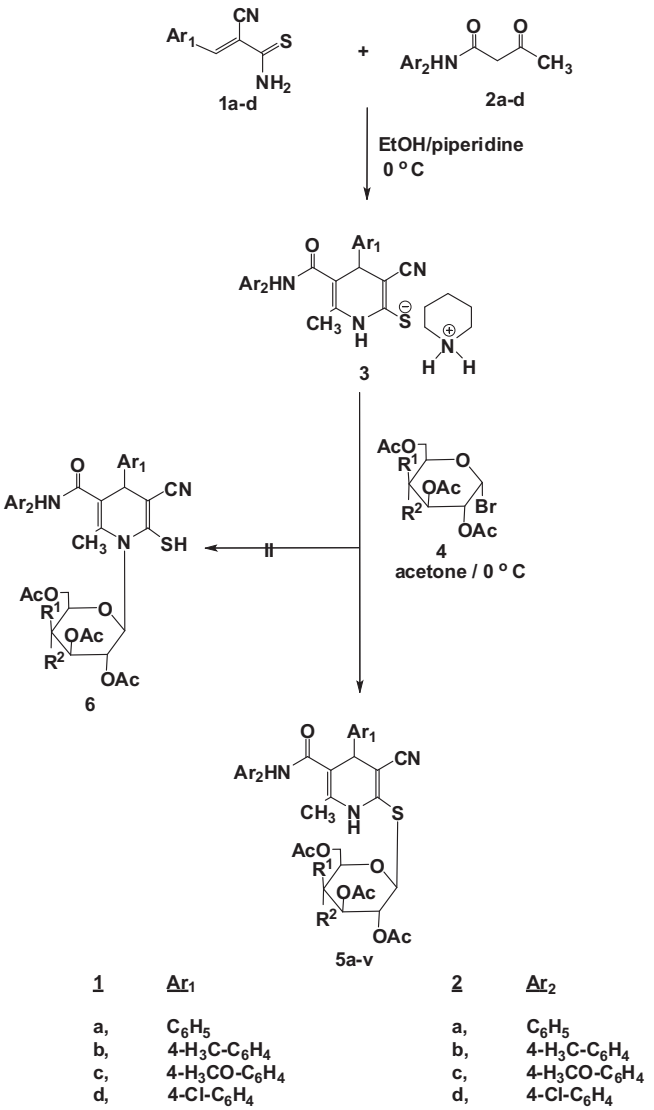
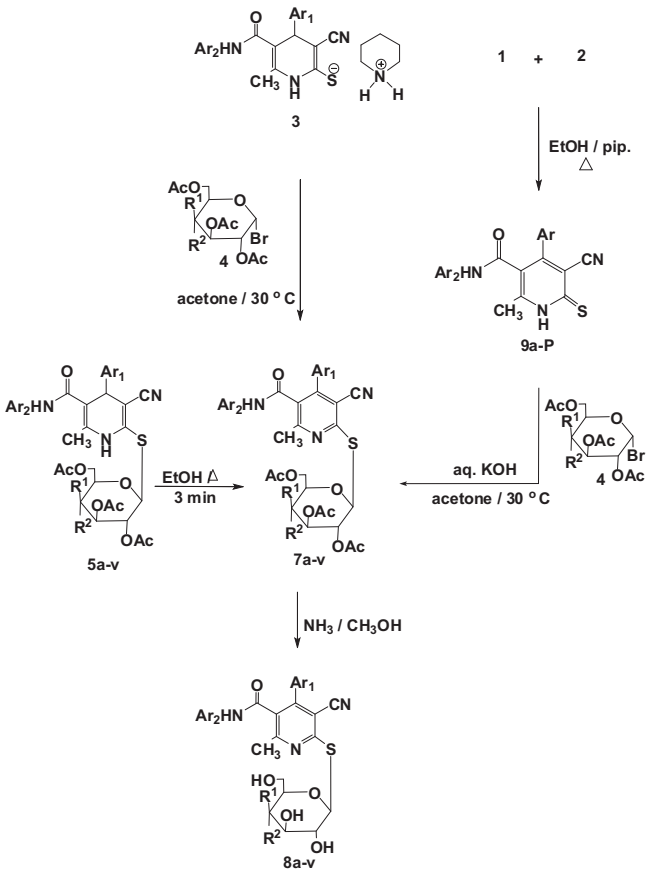


Figure 3. Cytotoxcity of the synthesized free pyridine thioglycosides 8a-v on liver (HEPG2) cancer cell line.



Scheme 1. A synthetic pathway for dihydropyridine thioglycosides 5a-v.

chemical shift is the same as that of the corresponding *S*-methyl derivative.[15] In another experiment, the piperidinium salts **3** reacted with 2,3,4,6-tetra-*O*-acetyl-α-D-gluco- and galactopyranosyl bromides in acetone at 30°C (chart 2) to afford the corresponding aromatized pyridine thioglucosides **7a-p** and thiogalactosides **7q-v**. Compounds **7** could also be prepared by heating dihydro thioglycosides **5** in ethanol for 3 minutes, which underwent spontaneous auto-oxidation to afford the aromatized thioglycosides **7**. The structures of the reaction products **7a-v** were established and confirmed on the basis of their elemental analyses and spectral data (IR, ^{1}H NMR, ^{13}C NMR). When glycosides **7** were treated with methanolic ammonia at room temperature, the free glycoside derivatives **8a-v** were obtained, the structures of which were established on the basis of elemental analysis and spectral data.



Scheme 2. Synthetic pathways for pyridine thioglycosides 5a-v, 8a-v.

Thus, the analytical data for **8e** revealed a molecular formula $C_{27}H_{27}N_3O_6S$. The ^{1}H NMR spectrum showed the anomeric proton as a doublet at δ 5.58 ppm ($J_{1',2'} =$ 9.8 Hz) indicating a β-D-configuration. The signals of the other six glucose protons appeared as a multiplet at $\delta = 3.17–3.66$ ppm, while the signals that are disappeared on rapid exchange with D_2O and are observed at δ 4.47–5.23 ppm were assigned as the four hydroxy groups, the ^{13}C NMR spectrum of **8e** is characterized by a signal at $\delta = 82.3$ ppm corresponding to the C-1′ and five other signals at δ 59.4, 68.4, 70.4, 77.3 and 80.4 ppm that are assigned to C-6′, C-4′, C-2′, C-3′ and C-5′, respectively. Encouraged by these results, we decided to synthesize the pyridine thioglycosides **7** using the silylation method and comparing the resulting products for stereochemical considerations. Thus, in a simple experimental procedure (Chart 4) treatment of the piperidinium salts **3** with dilute hydrochloric acid at 30°C converted them to the corresponding pyridine-2(1*H*)thiones **9**. The latter compounds were reacted with hexamethyldisilazane (HMDS) in the presence of ammonium sulfate to give the corresponding 2-trimethylsilylthiopyridines **11**, which were subsequently treated with peracetylated sugars **13** in the presence of redistilled $SnCl_4$ to afford the *S*-glycosyl compounds **7** (Chart 5). The latter were shown to be the same as those obtained from the reaction of **3** with **4** by comparison of their melting points and spectral data.

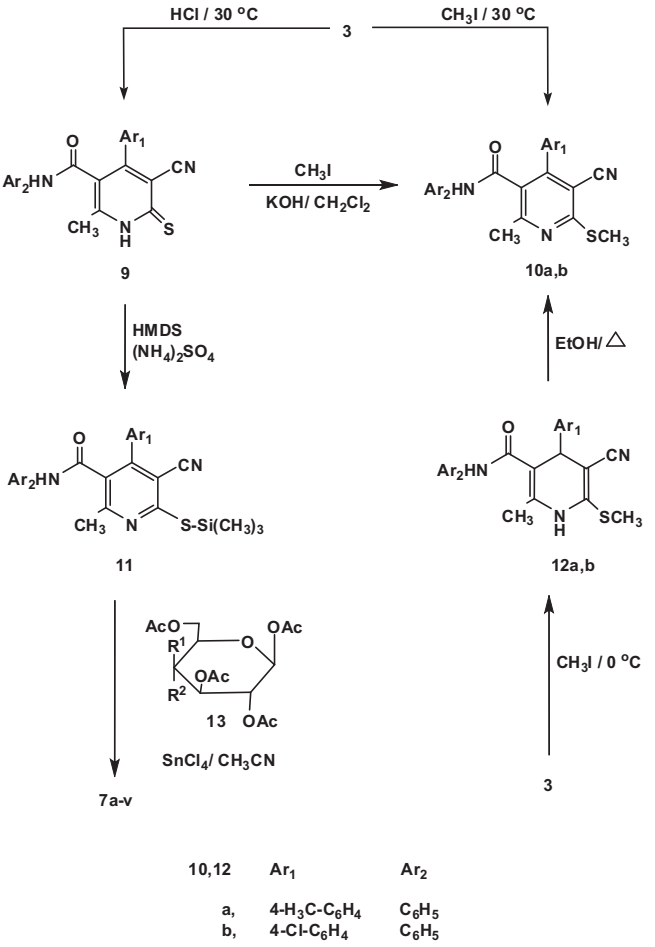
5,7	Ar_1	Ar_2	R^1	R^2
a,	C_6H_5	C_6H_5	H	OAc
b,	C_6H_5	4-H_3C-C_6H_4	H	OAc
c,	C_6H_5	4-H_3CO-C_6H_4	H	OAc
d,	C_6H_5	4-Cl-C_6H_4	H	OAc
e,	4-H_3C-C_6H_4	C_6H_5	H	OAc
f,	4-H_3C-C_6H_4	4-H_3C-C_6H_4	H	OAc
g,	4-H_3C-C_6H_4	4-H_3CO-C_6H_4	H	OAc
h,	4-H_3C-C_6H_4	4-Cl-C_6H_4	H	OAc
i,	4-H_3CO-C_6H_4	C_6H_5	H	OAc
j,	4-H_3CO-C_6H_4	4-H_3C-C_6H_4	H	OAc
k,	4-H_3CO-C_6H_4	4-H_3CO-C_6H_4	H	OAc
l,	4-H_3CO-C_6H_4	4-Cl-C_6H_4	H	OAc
m,	4-Cl-C_6H_4	C_6H_5	H	OAc
n,	4-Cl-C_6H_4	4-H_3C-C_6H_4	H	OAc
o,	4-Cl-C_6H_4	4-H_3CO-C_6H_4	H	OAc
p,	4-Cl-C_6H_4	4-Cl-C_6H_4	H	OAc
q,	C_6H_5	C_6H_5	OAc	H
r,	4-H_3C-C_6H_4	4-H_3C-C_6H_4	OAc	H
s,	4-H_3C-C_6H_4	4-H_3CO-C_6H_4	OAc	H
t,	4-H_3CO-C_6H_4	C_6H_5	OAc	H
u,	4-H_3CO-C_6H_4	4-H_3CO-C_6H_4	OAc	H
v,	4-H_3CO-C_6H_4	4-Cl-C_6H_4	OAc	H

8	Ar_1	Ar_2	R^1	R^2
a,	C_6H_5	C_6H_5	H	OH
b,	C_6H_5	4-H_3C-C_6H_4	H	OH
c,	C_6H_5	4-H_3CO-C_6H_4	H	OH
d,	C_6H_5	4-Cl-C_6H_4	H	OH
e,	4-H_3C-C_6H_4	C_6H_5	H	OH
f,	4-H_3C-C_6H_4	4-H_3C-C_6H_4	H	OH
g,	4-H_3C-C_6H_4	4-H_3CO-C_6H_4	H	OH
h,	4-H_3C-C_6H_4	4-Cl-C_6H_4	H	OH
i,	4-H_3CO-C_6H_4	C_6H_5	H	OH
j,	4-H_3CO-C_6H_4	4-H_3C-C_6H_4	H	OH
k,	4-H_3CO-C_6H_4	4-H_3CO-C_6H_4	H	OH
l,	4-H_3CO-C_6H_4	4-Cl-C_6H_4	H	OH
m,	4-Cl-C_6H_4	C_6H_5	H	OH
n,	4-Cl-C_6H_4	4-H_3C-C_6H_4	H	OH
o,	4-Cl-C_6H_4	4-H_3CO-C_6H_4	H	OH
p,	4-Cl-C_6H_4	4-Cl-C_6H_4	H	OH
q,	C_6H_5	C_6H_5	OH	H
r,	4-H_3C-C_6H_4	4-H_3C-C_6H_4	OH	H
s,	4-H_3C-C_6H_4	4-H_3CO-C_6H_4	OH	H
t,	4-H_3CO-C_6H_4	C_6H_5	OH	H
u,	4-H_3CO-C_6H_4	4-H_3CO-C_6H_4	OH	H
v,	4-H_3CO-C_6H_4	4-Cl-C_6H_4	OH	H

Scheme 3. List of synthesized derivatives 5a-v, 7a-v, 8a-v.

Anti proliferative activity study

The in vitro anti-hepatocellular carcinoma (anti-HCC) activity of the newly synthesized dihydropyridine thioglycosides and pyridinethioglycosides was examined against the HepG2 cell line using the MTT method.[16] 5FU, which is one of the most effective anticancer agents was used as positive control. The activity was expressed by median growth inhibitory concentration (IC_{50}) and was provided in Table 1. The results revealed that many of the synthesised dihydropyridine and pyridine thioglycosides exhibited antiproliferative activity with IC_{50} values ranging from 8.5–22.5 µg/ml. Thioglycoside compounds **5c, 5g, 5l, 5o, 5p, 7a, 7i, 7p, 8b, 8f, 8s**, and **8v** possessed activity at low microgram levels (IC_{50} = 10.2, 10.4, 9.5, 12.5, 8.5, 9.0, 8.1, 8.9, 10.2, 8.7, 11.8 and 8.9 µg/ml, respectively), which were shown in (Figures 1–3). The different substituents on phenyl and carbamoyl



Scheme 4. Synthetic pathways for 10a,b; 12a,b.

moieties at positions 4 and 5 of pyridine ring were responsible for the variation the cytotoxic activity. The acetylated dihydropyridine thioglycoside series with 4-chlorophenyl and 4-chlorophenyl carbamoyl moieties at positions 4 and 5 **5c** displayed the best inhibitory activity (IC_{50} = 8.5 µg/ml). Compounds of the acetylated pyridine thioglycoside series with 4-methoxyphenyl and phenyl carbamoyl at positions 4 and 5 **7i** showed the higher inhibitory activity (IC_{50} = 8.1 µg/ml). The free pyridine thioglycoside series with 4-methylphenyl and methylphenyl carbamoyl moieties at positions 4 and 5 **8f** displayed the best inhibitory activity (IC_{50} = 8.7 µg/ml).

Conclusion

In summary, we have achieved a novel synthesis of interesting nonclassical dihydropyridine thioglycosides and their corresponding aromatized forms by the reaction of the piperidinium salts of dihydropyridinethiones with *δ*-acetylated

α-glycosyl halides, the nature of the products depending upon thermodynamic factors. These glycosides can be utilized as excellent starting materials for the synthesis of other carbohydrate derivatives and for further biological evaluation studies.

Experimental

All evaporations were carried out under reduced pressure at 40°C M.p.s are uncorrected. Aluminum sheets coated with silica gel F_{254} (Merck) were used for TLC. Detection was effected by viewing under a short-wavelength UV lamp. IR spectra were obtained (KBr disk) on a Pye Unicam spectra 1000. ^{1}H NMR and ^{13}C NMR spectra were measured on a Wilmad 270 MHz or on a Vairan 400 MHz spectrometer for solution in $CDCl_3$ or $(CD_3)_2SO$ with $SiMe_4$ as internal standard. J Values are given in Hz. Analytical data were obtained from the Microanalytical Data Center at Cairo University.

4-Aryl-3-cyano-6-methyl-2-(2,3,4,6-tetra-O-acetyl-β-D-gluco- and galacto-pyranosylthio)-1,4-dihydropyridines (5a-v)

General procedures

A mixture of **1a-d** (0.01 mol) and **2a-d** (0.01 mol) was dissolved in dry ethanol (5 ml), and then piperidine (0.01 mol) was added. The reaction mixture was stirred at 0°C for 1 h and then left to stand to room temperature. The solvent was removed at reduced pressure and the resulting piperidinium salt of 1,4-dihydropyridine-2-thione **3** was dissolved in dry acetone (5 ml) and a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-gluco- or galactopyranosyl bromide (0.01 mol) in dry acetone (20 ml) was then added at 0°C. The reaction mixture was stirred until the reaction was judged complete by TLC, using chloroform:ether 4:1 (Rf, 0.68–0.72), then concentrated under reduced pressure and the residue crystallized from chloroform-petroleum ether at 0°C.

5a: yellow, mp: 158°C; yield: (81%). Anal. Calcd. for ($C_{34}H_{35}N_3O_{10}S$): C, 60.26; H, 5.16; N, 6.20. Found: C, 60.71; H, 5.02; N, 6.35.

5b: yellow, mp: 155°C; yield: (80%); IR.: ν_{max} /Cm^{-1} (KBr) 3312 (NH); 2191(CN); 1750 (CO); 1655 (CO). Anal.: Calcd. for ($C_{35}H_{37}N_3O_{10}S$): C, 60.78; H, 5.35; N, 6.07. Found: C, 60.42; H, 5.72; N, 6.38.

5c: yellow, mp: 153°C; yield: (86%); IR.: ν_{max} /Cm^{-1} (KBr) 3412 (NH); 2185 (CN); 1750 (CO). Anal. Calcd. for ($C_{35}H_{37}N_3O_{11}S$): C, 59.40; H, 5.23; N, 5.94.

Found: C, 59.83; H, 5.65; N, 6.01.

5d: yellow, mp: 222°C; yield: (80%); IR.: ν_{max} /Cm^{-1} (KBr) 3372 (NH); 2186(CN); 1748 (CO); 1671 (CO). Anal. Calcd. for ($C_{34}H_{34}ClN_3O_{10}S$): C, 57.34; H, 4.77; N, 5.90. Found: C, 57.79; H, 4.93; N, 5.79.

5e: pale yellow, mp: 197°C; yield: (85%); IR.: ν_{max} /Cm^{-1} (KBr) 3370 (NH); 2202 (CN); 1752 (CO); 1669 (CO). Anal. Calcd. for ($C_{35}H_{37}N_3O_{10}S$): C, 60.78; H, 5.35; N, 6.07. Found: C, 61.15; H, 5.02; N, 6.39.

5f: pale yellow, mp: 160°C; yield: (81%); IR.: ν_{max} /Cm^{-1} (KBr) 3370 (NH); 2202 (CN); 1752 (CO); 1667 (CO). Anal. Calcd. for ($C_{36}H_{39}N_3O_{10}S$): C, 61.27; H, 5.53; N, 5.95. Found: C, 61.11; H, 5.49; N, 6.15.

5g: yellow, mp: 191°C; yield: (83%); IR.: ν_{max} /Cm^{-1} (KBr) 3422 (NH); 2226 (CN); 1752 (CO); 1642 (CO). Anal. Calcd. for ($C_{36}H_{39}N_3O_{11}S$): C, 59.91; H, 5.40; N, 5.82. Found: C, 60.25; H, 5.56; N, 5.41.

5h: pale yellow, mp: 180°C; yield: (81%); IR.: ν_{max} /Cm^{-1} (KBr) 3361 (NH); 2202 (CN); 1753 (CO); 1671 (CO); ^{1}H NMR: δ_H 1.96–2.08 (4s, 12H, $4CH_3CO$); 2.20 (s, 3H, CH_3); 2.41 (s, 3H, CH_3); 4.01 (m, 2H, 2H-6′); 4.17 (m, 2H, H-5′ and pyridine H-4); 4.79 (d, 1H, H-4′); 4.91 (m, 2H, H-3′ and H-2′); 5.34 (d, $J_{1',2'} = 9.8$ Hz, 1H, H-1′); 7.10–7.61 (m, 8H, $2C_6H_4$); 8.70 (bs, 1H, NH); 9.76 (bs, 1H, NH). Anal. Calcd. for ($C_{35}H_{36}ClN_3O_{10}S$): C, 57.89; H, 4.96; N, 5.78. Found: C, 57.99; H, 5.17; N, 5.66.

5i: yellow, mp: 170°C; yield: (82%); IR.: ν_{max} /Cm^{-1} (KBr) 3388 (NH); 2200 (CN); 1752 (CO). Anal. Calcd. for ($C_{35}H_{37}N_3O_{11}S$): C, 59.40; H, 5.23; N, 5.94.

Found: C, 59.67; H, 5.38; N, 6.01.

5j: yellow, mp: 175°C; yield: (85%); IR.: ν_{max} /Cm^{-1} (KBr) 3370 (NH); 2201 (CN); 1752 (CO); 1665 (CO). Anal. Calcd. for ($C_{36}H_{39}N_3O_{11}S$): C, 59.91; H, 5.40; N, 5.82. Found: C, 59.68; H, 5.57; N, 5.49.

5k: yellow, mp: 214°C; yield: (80%); IR.: ν_{max} /Cm^{-1} (KBr) 3386 (NH); 2203 (CN); 1752 (CO). Anal. Calcd. for ($C_{36}H_{39}N_3O_{12}S$): C, 58.61; H, 5.29; N, 5.69. Found: C, 58.85; H, 5.18; N, 5.83.

5l: yellow, mp: 165°C; yield: (82%); IR.: ν_{max} /Cm^{-1} (KBr) 3378 (NH); 2201 (CN); 1752 (CO); ^{1}H NMR: δ_H 1.93–2.05 (4s, 12H, $4CH_3CO$); 2.20 (s, 3H, CH_3); 3.55 (s, 3H, OCH_3); 3.69 (m, 2H, 2H-6′); 4.0 (m, 2H, H-5′ and pyridine H-4); 4.78 (d, 1H, H-4′); 4.92 (m, 2H, H-3′ and H-2′); 5.34 (d, $J_{1',2'} = 9.6$ Hz, 1H, H-1′); 6.80–7.63 (m, 8H, 2 C_6H_4); 8.60 (bs, 1H, NH); 9.74 (bs, 1H, NH);^{13}C NMR: δ_C 20.3–21.6 ($4CH_3CO$); 22.2 (CH_3); 55.0 (OCH_3); 61.9 (C-6′); 67.8 (C-4′); 70.2 (C-2′); 72.7 (C-3′); 75.0 (C-5′); 82.8 (C-1′); 90.9 (C-4); 107.3 (C-3); 114.0 (CN); 120.2–137.9 (2Ar-C); 139.4 (C-5); 140.1 (C-6); 158.4 (C-2); 169.0–170.1 (4CO of glucose); 182.0 (CO of pyridine). Anal. Calcd. for ($C_{35}H_{36}ClN_3O_{11}S$): C, 56.64; H, 4.85; N, 5.66. Found: C, 56.48; H, 4.63; N, 5.98.

5m: yellow, mp: 175°C; yield: (80%); IR.: ν_{max} /Cm^{-1} (KBr) 3370 (NH); 2202(CN); 1752 (CO); 1666 (CO); ^{1}H NMR: δ_H 1.93–2.05 (4s, 12H, $4CH_3CO$); 2.52 (s, 3H, CH_3); 4.02 (m, 2H, 2H-6′); 4.14 (m, 2H, H-5′ and pyridine H-4); 4.87 (d, 1H, H-4′); 5.00 (m, 2H, H-3′ and H-2′); 5.37 (d, $J_{1',2'} = 9.7$ Hz, 1H, H-1′); 6.99–7.54 (m, 9H, C_6H_4 and C_6H_5); 8.82 (bs, 1H, NH); 9.71 (bs, 1H, NH). Anal. Calcd. for ($C_{34}H_{34}ClN_3O_{10}S$): C, 57.34; H, 4.77; N, 5.90.

Found: C, 57.73; H, 4.93; N, 5.77.

5n: yellow, mp: 241°C; yield: (85%); IR.: ν_{max} /Cm^{-1} (KBr) 3381 (NH); 2202 (CN); 1752 (CO); 1669 (CO); ^{1}H NMR: δ_H 1.98–2.04 (4s, 12H, $4CH_3CO$); 2.23 (s, 3H, CH_3); 2.51 (s, 3H, CH_3); 3.96 (m, 2H, 2H-6′); 4.17 (m, 2H, H-5′ and pyridine H-4); 4.85 (d, 1H, H-4′); 4.99 (m, 2H, H-3′ and H-2′); 5.36 (d, $J_{1',2'} = 9.6$ Hz, 1H, H-1′); 7.04–7.44 (m, 8H, $2C_6H_4$); 8.84 (bs, 1H, NH); 9.61 (bs, 1H, NH). Anal. Calcd. for ($C_{35}H_{36}ClN_3O_{10}S$): C, 57.89; H, 4.96; N, 5.78. Found: C, 58.11; H, 4.72; N, 5.91.

5o: yellow, mp: 195°C; yield: (81%); IR.: ν_{max} /Cm^{-1} (KBr) 3321 (NH); 2202 (CN); 1752 (CO). Anal. Calcd. for ($C_{35}H_{36}ClN_3O_{11}S$): C, 56.64; H, 4.85; N, 5.66. Found: C, 56.97; H, 4.59; N, 5.87.

5p: yellow, mp: 173°C; yield: (84%); IR.: ν_{max} /Cm^{-1} (KBr) 3371 (NH); 2202 (CN); 1752 (CO); 1647 (CO);^{1}H NMR: δ_H 1.89–2.04 (4s, 12H, $4CH_3CO$); 2.20 (s, 3H, CH_3); 3.96 (m, 2H, 2H-6′); 4.22 (m, 2H, H-5′ and pyridine H-4); 4.85 (d, 1H, H-4′); 4.89 (m, 2H, H-3′ and H-2′); 5.35 (d, $J_{1',2'} = 9.8$ Hz, 1H, H-1′); 6.99–7.64 (m, 8H, $2C_6H_4$); 8.80 (bs, 1H, NH); 9.80 (bs, 1H, NH); ^{13}C NMR: δ_C 20.4–21.6 ($4CH_3CO$); 30.6 (CH_3); 60.7 (C-6′); 68.1 (C-4′); 71.8 (C-2′); 73.8 (C-3′); 75.5 (C-5′); 89.8 (C-1′); 94.0 (C-4); 108.8 (C-3); 115.0 (CN); 121.2–131.9 (2Ar-C); 137.7 (C-5); 142.2 (C-6); 157.0 (C-2); 166.0–170.0 (4CO of glucose); 182.0 (CO of pyridine). Anal. Calcd. for ($C_{34}H_{33}Cl_2N_3O_{10}S$): C, 54.69; H, 4.42; N, 5.63. Found: C, 54.22; H, 4.87; N, 5.38.

5q: yellow, mp: 215°C; yield: (80%). Anal. Calcd. for ($C_{34}H_{35}N_3O_{10}S$): C, 60.26; H, 5.16; N, 6.20. Found: C, 60.07; H, 5.42; N, 6.37.

5r: yellow, mp: 249°C; yield: (80%); IR.: ν_{max} /Cm^{-1} (KBr) 3386 (NH); 2201 (CN); 1752 (CO). Anal. Calcd. for ($C_{36}H_{39}N_3O_{10}S$): C, 61.27; H, 5.53; N, 5.95.

Found: C, 61.39; H, 5.71; N, 5.58.

5s: yellow, mp: 189°C; yield: (80%); IR.: ν_{max} /Cm^{-1} (KBr) 3366 (NH); 2201 (CN); 1752 (CO); ^{1}H NMR: δ_H 1.91–2.07 (4s, 12H, $4CH_3CO$); 2.15 (s, 3H, CH_3); 2.24 (s, 3H, CH_3); 3.61 (s, 3H, OCH_3); 3.69 (m, 2H, 2H-6′); 4.01 (m, 2H, H-5′ and pyridine H-4); 4.78 (d, 1H, H-4′); 5.00 (m, 2H, H-3′ and H-2′); 5.31 (d, $J_{1',2'} = 9.7$ Hz, 1H, H-1′); 6.80–7.45 (m, 8H, $2C_6H_4$); 8.72 (bs, 1H, NH); 9.48 (bs, 1H, NH). Anal. Calcd. for ($C_{36}H_{39}N_3O_{11}S$): C, 59.91; H, 5.40; N, 5.82. Found: C, 60.22; H, 5.29; N, 6.03.

5t: yellow, mp: 233°C; yield: (84%); IR.: ν_{max} /Cm^{-1} (KBr) 3437 (NH); 2200 (CN); 1752 (CO); 1664 (CO); ^{1}H NMR: δ_H 1.97–2.07 (4s, 12H, $4CH_3CO$); 2.42 (s, 3H, CH_3); 3.65 (s, 3H, OCH_3); 3.70 (m, 2H, 2H-6′); 4.33 (m, 2H, H-5′ and pyridine H-4); 4.83 (d, 1H, H-4′); 5.05 (m, 2H, H-3′ and H-2′); 5.36 (d, $J_{1',2'} = 9.8$ Hz, 1H, H-1′); 6.78–7.64 (m, 9H, C_6H_4 and C_6H_5); 8.68 (bs, 1H, NH); 9.51 (bs, 1H, NH). Anal. Calcd. for ($C_{35}H_{37}N_3O_{11}S$): C, 59.40; H, 5.23; N, 5.94. Found: C, 59.09; H, 5.38; N, 6.03.

5u: yellow, mp: 190°C; yield: (83%); IR.: ν_{max} /Cm^{-1} (KBr) 3375 (NH); 2200 (CN); 1752 (CO); ^{1}H NMR: δ_H 1.64–2.09 (4s, 12H, $4CH_3CO$); 2.44 (s, 3H, CH_3); 3.65 (s, 3H, OCH_3); 3.67 (s, 3H, OCH_3); 3.68 (m, 2H, 2H-6′); 3.70 (m, 2H, H-5′ and pyridine H-4); 4.77 (d, 1H, H-4′); 5.00 (m, 2H, H-3′ and H-2′); 5.36 (d, $J_{1',2'} = 10.0$ Hz, 1H, H-1′); 6.80–7.45 (m, 8H, $2C_6H_4$); 8.75 (bs, 1H, NH); 9.48 (bs, 1H, NH). Anal. Calcd. for ($C_{36}H_{39}N_3O_{12}S$): C, 58.61; H, 5.29; N, 5.69. Found: C, 58.86; H, 5.05; N, 5.81.

5v: pale yellow, mp: 185°C; yield: (80%); IR.: ν_{max} /Cm^{-1} (KBr) 3384 (NH); 2201 (CN); 1752 (CO); 1668 (CO); ^{1}H NMR: δ_H 1.93–2.07 (4s, 12H, $4CH_3CO$); 2.14 (s, 3H, CH_3); 3.65 (s, 3H, OCH_3); 3.69 (m, 2H, 2H-6′); 4.34 (m, 2H, H-5′ and pyridine H-4); 4.84 (d, 1H, H-4′); 5.04 (m, 2H, H-3′ and H-2′); 5.29 (d, $J_{1',2'} = 9.7$ Hz, 1H, H-1′); 6.81–7.64 (m, 8H, $2C_6H_4$); 8.86 (bs, 1H, NH); 9.51 (bs, 1H, NH). Anal. Calcd. for ($C_{35}H_{36}ClN_3O_{11}S$): C, 56.64; H, 4.85; N, 5.66. Found: C, 56.92; H, 5.13; N, 5.75.

4-Aryl-3-cyano-6-methyl-2-(2,3,4,6-tetra-O-acetyl-β-D-gluco- and galacto-pyranosylthio)pyridines (7a-v).

General procedures

Method (A). The piperidinium salt of dihydropyridinethiones **3** (0.01 mol) was dissolved in dry acetone (5 ml) and a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-gluco- or galactopyranosyl bromide (0.01 mol) in dry acetone (20 ml) was then added at 30°C. The reaction mixture was stirred until the reaction was judged complete by TLC, using chloroform:petroleum ether 9:1 (Rf, 0.62–0.66), then concentrated under reduced pressure at 40°C. The resulting product was crystallized from ethanol.

Method (B). A suspension of pyridine thioglycosides **5** (0.01 mol) in ethanol were refluxed with stirring for 3 min. The resulting products were collected by filtration and crystallized from ethanol.

Method (C). To a solution of condensed pyridine-2(1*H*)thiones **9a-p** (0.01 mol) in aqueous potassium hydroxide [0.569, 0.01 mol, in distilled water (6 ml)] was added a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-gluco or galacto-pyranosyl bromide **4** (4.521 g, 0.011 mol) in acetone (30 ml). The reaction mixture was stirred at room temperature until reaction judged to be complete by TLC (30 min to 2 h). The mixture was evaporated under reduced pressure at 40°C and the residue was washed with distilled water to remove the formed potassium bromide. The product was dried, and crystallized from ethanol.

Method (D). A mixture of pyridine-2(1*H*)-thiones **9** (0.01 mol), hexamethyldisilazane (25 ml) and ammonium sulphate (0.02 g) were boiled under reflux, with stirring for 48 h. The excess of hexamethyldisilazane was removed under diminished pressure, providing the silylated bases **11** as a colorless oil. The latter was added to a solution of 1,2,3,4,6-penta-*O*-acetyl-α-D-gluco- or galactopyranose (0.011 mol) in dry acetonitrile (20 ml) and $SnCl_4$ (1.6 ml). The reaction mixture was stirred at room temperature for (6 h), poured into saturated $NaHCO_3$ solution and then extracted with $CHCl_3$. The organic layers were dried over $MgSO_4$, filtered and then concentrated to give the crude nucleosides which were purified by recrystallization from ethanol.

7a: pale yellow, mp: 258°C; yield: (72%);^{1}H NMR: δ_H 2.00–2.05 (4s, 12H, $4CH_3CO$); 2.68 (s, 3H, CH_3); 4.06 (m, 3H, 2H-6′ and H-5′); 5.06 (t, 1H, H-4′); 5.22 (t, 1H, H-3′); 5.60 (t, 1H, H-2′); 6.22 (d, $J_{1',2'} = 10.1$ Hz, 1H, H-1′); 7.08–7.48 (m, 10H, $2C_6H_5$); 10.43 (bs, 1H, NH). Anal. Calcd. for ($C_{34}H_{33}N_3O_{10}S$): C, 60.44; H, 4.88; N, 6.22. Found: C, 60.40; H, 4.90; N, 6.05.

7b: pale yellow, mp: 266°C; yield: (76%); IR.: ν_{max} /Cm^{-1} (KBr) 3449 (NH); 2223 (CN); 1752 (CO); 1650 (CO). Anal. Calcd. for ($C_{35}H_{35}N_3O_{10}S$): C, 60.95; H, 5.07; N, 6.09. Found: C, 60.98; H, 5.11; N, 6.01.

7c: yellow, mp: 232°C; yield: (77%); IR.: ν_{max} /Cm^{-1} (KBr) 3447 (NH); 2223 (CN); 1752 (CO); 1647 (CO). Anal. Calcd. for ($C_{35}H_{35}N_3O_{11}S$): C, 59.57; H, 4.96; N, 5.95. Found: C, 59.42; H, 4.81; N, 5.88.

7d: pale yellow, mp: 261°C; yield: (79%); IR.: ν_{max} /Cm^{-1} (KBr) 3440 (NH); 2224 (CN); 1751 (CO); 1652 (CO). Anal. Calcd. for ($C_{34}H_{32}ClN_3O_{10}S$): C, 57.50; H, 4.51; N, 5.91. Found: C, 57.62; H, 4.49; N, 5.87.

7e: white, mp: 265°C; yield: (75%); IR.: ν_{max} /Cm^{-1} (KBr) 3472 (NH); 2226 (CN); 1748 (CO); 1650 (CO). Anal. Calcd. for ($C_{35}H_{35}N_3O_{10}S$): C, 60.95; H, 5.07; N, 6.09. Found: C, 60.68; H, 5.13; N, 6.25.

7f: pale yellow, mp: 228°C; yield: (73%); IR.: ν_{max} /Cm^{-1} (KBr) 3464 (NH); 2224 (CN); 1750 (CO); 1649(CO). Anal. Calcd. for ($C_{36}H_{37}N_3O_{10}S$): C, 61.45; H, 5.26; N, 5.97. Found: C, 61.40; H, 5.38; N, 5.89.

7g: yellow, mp: 176°C; yield: (76%); Anal. Calcd. for ($C_{36}H_{37}N_3O_{11}S$): C, 60.08; H, 5.14; N, 5.84. Found: C, 60.27; H, 5.32; N, 5.59.

7h: white, mp: 282°C; yield: (79%); IR.: ν_{max} /Cm^{-1} (KBr) 3613 (NH); 2227 (CN); 1751 (CO); 1652 (CO). Anal. Calcd. for ($C_{35}H_{34}ClN_3O_{10}S$): C, 58.05; H, 4.69; N, 5.80. Found: C, 58.50; H, 4.28; N, 5.71.

7i: yellow, mp: 238°C; yield: (78%); IR.: ν_{max} /Cm^{-1} (KBr) 3289 (NH); 2224 (CN); 1749 (CO); 1655 (CO); ^{1}H NMR: δ_H 1.99–2.04 (4s, 12H, 4CH_3CO); 2.65 (s, 3H, CH_3); 3.75 (s, 3H, OCH_3); 4.00 (m, 3H, 2H-6′ and H-5′); 5.04 (t, 1H, H-4′); 5.20 (t, 1H, H-3′); 5.59 (t, 1H, H-2′); 6.20 (d, $J_{1',2'}$ = 9.5 Hz, 1H, H-1′); 7.01–7.45 (m, 9H, C_6H_4 and C_6H_5); 10.45 (bs, 1H, NH). Anal. Calcd. for ($C_{35}H_{35}N_3O_{11}S$): C, 59.57; H, 4.96; N, 5.95. Found: C, 59.48; H, 4.99; N, 5.88.

7j: yellow, mp: 159°C; yield: (74%); IR.: ν_{max} /Cm^{-1} (KBr) 3419 (NH); 2225 (CN); 1750(CO); 1654 (CO); ^{1}H NMR: δ_H 1.83–2.16 (4s, 12H, 4CH_3CO); 2.22 (s, 3H, CH_3); 2.65 (s, 3H, CH_3); 3.76 (s, 3H, OCH_3); 4.01 (m, 3H, 2H-6′ and H-5′); 5.01 (t, 1H, H-4′); 5.17 (t, 1H, H-3′); 5.54 (t, 1H, H-2′); 6.19 (d, $J_{1',2'}$ = 10.8 Hz, 1H, H-1′); 7.00–7.45 (m, 8H, 2C_6H_4); 10.30 (bs, 1H, NH);^{13}C NMR: δ_C 20.3–20.4 (4CH_3CO); 22.8 (CH_3); 30.6 (CH_3); 55.2 (OCH_3); 61.8 (C-6′); 68.1 (C-4′); 68.8 (C-2′); 73.2 (C-3′); 75.1 (C-5′); 80.2 (C-1′); 104.9 (C-3); 113.9 (CN); 114.7–135.0 (2Ar-C); 151.2 (C-5); 157.9 (C-4); 158.5 (C-6); 160.2 (C-2); 163.5–169.9 (4CO of glucose); 182.3 (CO of pyridine). Anal. Calcd. for ($C_{36}H_{37}N_3O_{11}S$): C, 60.08; H, 5.14; N, 5.84. Found: C, 60.35; H, 5.08; N, 5.88.

7k: yellow, mp: 267°C; yield: (72%); IR.: ν_{max} /Cm^{-1} (KBr) 3444 (NH); 2223 (CN); 1751 (CO); 1648 (CO); ^{1}H NMR: δ_H 1.98–2.08 (4s, 12H, 4CH_3CO); 2.64 (s, 3H, CH_3); 3.69 (s, 3H, OCH_3); 3.76 (s, 3H, OCH_3); 3.99 (m, 3H, 2H-6′ and H-5′); 5.00 (t, 1H, H-4′); 5.16 (t, 1H, H-3′); 5.54 (t, 1H, H-2′); 6.19 (d, $J_{1',2'}$ = 9.8 Hz, 1H, H-1′); 6.86–7.45 (m, 8H, 2C_6H_4); 10.25 (bs, 1H, NH);^{13}C NMR: δ_C 20.3–20.4 (4CH_3CO); 22.8 (CH_3); 55.2 (OCH_3); 61.8 (C-6′); 68.1 (C-4′); 68.8 (C-2′); 73.1 (C-3′); 75.0 (C-5′); 80.2 (C-1′); 104.9 (C-3); 114.7 (CN); 121.4–157.8 (2Ar-C); 158.0 (C-5); 160.3 (C-4); 160.3 (C-6); 163.3 (C-2); 169.3–169.9 (4CO of glucose); 179.8 (CO of pyridine). Anal. Calcd. for ($C_{36}H_{37}N_3O_{12}S$): C, 58.77; H, 5.03; N, 5.71. Found: C, 58.89; H, 5.24; N, 5.59.

7l: yellow, mp: 298°C; yield: (73%); IR.: ν_{max} /Cm^{-1} (KBr) 3438 (NH); 2226 (CN); 1752 (CO); 1650 (CO); ^{1}H NMR: δ_H 1.98–2.13 (4s, 12H, 4CH_3CO); 2.56 (s, 3H, CH_3); 3.76 (s, 3H, OCH_3); 4.00 (m, 3H, 2H-6′ and H-5′); 5.00 (t, 1H, H-4′); 5.08 (t, 1H, H-3′); 5.19 (t, 1H, H-2′); 6.02 (d, $J_{1',2'}$ = 10.6 Hz, 1H, H-1′); 6.96–7.44 (m, 8H, 2Ar-H); 10.50 (bs, 1H, NH);^{13}C NMR: δ_C 20.3–20.4 (4CH_3CO); 22.8 (CH_3); 55.1 (OCH_3); 61.7 (C-6′); 68.0 (C-4′); 68.7 (C-2′); 73.2 (C-3′); 75.1 (C-5′); 80.2 (C-1′); 104.8 (C-3); 114.5 (CN); 121.0–158.4 (2Ar-C); 160.2 (C-5); 160.3 (C-4); 162.5 (C-6); 163.9 (C-2); 169.1–169.8 (4CO of glucose); 178.1 (CO of pyridine). Anal. Calcd. for ($C_{35}H_{34}ClN_3O_{11}S$): C, 56.79; H, 4.59; N, 5.67. Found: C, 56.56; H, 4.68; N, 5.75.

7m: white, mp: 280°C; yield: (77%); IR.: ν_{max} /Cm^{-1} (KBr) 3465 (NH); 2225 (CN); 1750 (CO); 1649 (CO); ^{13}C NMR: δ_C 20.3–20.4 (4CH_3CO); 22.8 (CH_3); 61.8 (C-6′); 68.0 (C-4′); 68.8 (C-2′); 73.1 (C-3′); 75.0 (C-5′); 80.2 (C-1′); 104.7 (C-3); 114.3 (CN); 119.7–137.9 (2Ar-C); 150.2 (C-5); 158.0 (C-4); 158.7 (C-6); 163.3 (C-2); 169.0–170.0 (4CO of glucose); 181.2 (CO of pyridine). Anal. Calcd. for ($C_{34}H_{32}ClN_3O_{10}S$): C, 57.50; H, 4.51; N, 5.91. Found: C, 57.59; H, 4.77; N, 5.62.

7n: yellow, mp: 251°C; yield: (78%); IR.: ν_{max} /Cm^{-1} (KBr) 3452 (NH); 2220 (CN); 1750 (CO); 1648 (CO); ^{13}C NMR: δ_C 25.6–25.7 (4CH_3CO); 28.1 (CH_3); 67.0 (C-6′); 73.34 (C-4′); 74.10 (C-2′); 78.3 (C-3′); 80.3 (C-5′); 85.49 (C-1′); 110.0 (C-3); 119.6 (CN); 124.9–140.6 (2Ar-C); 155.4 (C-5); 163.2 (C-4); 164.0 (C-6); 168.3 (C-2); 174.3–175.0 (4CO of glucose); 182.5 (CO of pyridine). Anal. Calcd. for ($C_{35}H_{34}ClN_3O_{10}S$): C, 58.05; H, 4.69; N, 5.80. Found: C, 58.43; H, 4.52; N, 5.86.

7o: yellow, mp: 209°C; yield: (74%); IR.: ν_{max} /Cm^{-1} (KBr) 3440 (NH); 2225 (CN); 1751 (CO); 1644 (CO);^{1}H NMR: δ_H 1.99–2.03 (4s, 12H, 4CH_3CO); 2.66 (s, 3H, CH_3); 3.69 (s, 3H, OCH_3); 3.99 (m, 3H, 2H-6′ and H-5′); 5.00 (t, 1H, H-4′); 5.16 (t, 1H, H-3′); 5.55 (t, 1H, H-2′); 6.20 (d, $J_{1',2'}$ = 10.2 Hz, 1H, H-1′); 6.83–7.59 (m, 8H, 2C_6H_4); 10.31 (bs, 1H, NH);^{13}C NMR: δ_C 20.3–20.4 (4CH_3CO); 22.8 (CH_3); 55.2 (OCH_3); 61.8 (C-6′); 68.0 (C-4′); 68.8 (C-2′); 73.1 (C-3′); 75.0 (C-5′); 80.2 (C-1′); 104.7 (C-3); 114.0 (CN); 114.4–150.2 (2Ar-C); 156.0 (C-5); 157.0 (C-4); 158.9 (C-6); 162.9 (C-2); 169.3–170.0 (4CO of glucose); 181.5 (CO of pyridine). Anal. Calcd. for ($C_{35}H_{34}ClN_3O_{11}S$): C, 56.79; H, 4.59; N, 5.67. Found: C, 56.85; H, 4.49; N, 5.71.

7p: yellow, mp: 235°C; yield: (76%); IR.: ν_{max} /Cm^{-1} (KBr) 3450 (NH); 2226 (CN); 1751 (CO); 1648 (CO); ^{13}C NMR: δ_C 20.3–20.4 (4CH_3CO); 22.8 (CH_3); 61.7 (C-6′); 68.0 (C-4′); 68.8 (C-2′); 73.1 (C-3′); 75.0 (C-5′); 80.2 (C-1′); 104.3 (C-3); 114.5 (CN); 121.1–136.8 (2Ar-C); 151 (C-5); 157.5 (C-4); 158.7 (C-6); 163.1 (C-2); 169.3–170.0 (4CO of glucose); 180.3 (CO of pyridine). Anal. Calcd. for ($C_{34}H_{31}Cl_2N_3O_{10}S$): C, 54.83; H, 4.16; N, 5.64. Found: C, 55.09; H, 4.21; N, 5.32.

7q: yellow, mp: 206°C; yield: (72%); IR.: ν_{max} /Cm^{-1} (KBr) 3459 (NH); 2225 (CN); 1751 (CO); 1646 (CO). Anal. Calcd. for ($C_{34}H_{33}N_3O_{10}S$): C, 60.44; H, 4.88; N, 6.22. Found: C, 60.41; H, 4.50; N, 5.40.

7r: white, mp: 191°C; yield: (72%); IR.: ν_{max} /Cm^{-1} (KBr) 3475 (NH); 2224 (CN); 1752 (CO); 1645 (CO). Anal. Calcd. for ($C_{36}H_{37}N_3O_{10}S$): C, 61.45; H, 5.26; N, 5.97. Found: C, 61.09; H, 5.53; N, 5.99.

7s: yellow, mp: 186°C; yield: (78%); IR.: ν_{max} /Cm^{-1} (KBr) 3441 (NH); 2222 (CN); 1751 (CO); 1650 (CO). Anal. Calcd. for ($C_{36}H_{37}N_3O_{11}S$): C, 60.08; H, 5.14; N, 5.84. Found: C, 60.01; H, 5.35; N, 5.78.

7t: white, mp: 262°C; yield: (79%); IR.: ν_{max} /Cm^{-1} (KBr) 3390 (NH); 2201 (CN); 1752 (CO); 1668 (CO). Anal. Calcd. for ($C_{35}H_{35}N_3O_{11}S$): C, 59.57; H, 4.96; N, 5.95. Found: C, 59.46; H, 4.87; N, 5.99.

7u: pale yellow, mp: 230°C; yield: (78%); IR.: ν_{max} /Cm^{-1} (KBr) 3382 (NH); 2200 (CN); 1752 (CO); 1669 (CO). Anal. Calcd. for ($C_{36}H_{37}N_3O_{12}S$): C, 58.77; H, 5.03; N, 5.71. Found: C, 58.79; H, 5.42; N, 5.62.

7v: yellow, mp: 203°C; yield: (79%); IR.: ν_{max} /Cm^{-1} (KBr) 3474 (NH); 2201 (CN); 1752 (CO); 1669 (CO). Anal. Calcd. for ($C_{35}H_{34}ClN_3O_{11}S$): C, 56.79; H, 4.59; N, 5.67. Found: C, 56.82; H, 4.57; N, 5.56.

4-Aryl-3-cyano-6-methyl-2-(β-D-gluco- and galactopyranosylthio)-pyridines (8a-v).

General procedures

Dry gaseous ammonia was passed through a solution of protected nucleoside **7** (0.5 g) in dry methanol (20 ml) at room temperature for 10 min. The mixture was stirred until the reaction was judged complete by TLC (10–12h) using $CHCl_3$:MeOH, 9:1 (Rf, 0.66–0.68). The resulting mixture was then concentrated under reduced pressure to afford a solid residue that was crystallized from methanol.

8a: pale yellow, mp: 282°C; yield: (60%). Anal. Calcd. for ($C_{26}H_{25}N_3O_6S$): C, 61.53; H, 4.93; N, 8.28. Found: C, 62.01; H, 4.42; N, 8.39.

8b: pale yellow, mp: 346°C; yield: (58%). Anal. Calcd. for ($C_{27}H_{27}N_3O_6S$): C, 62.18; H, 5.18; N, 8.06. Found: C, 62.53; H, 5.11; N, 8.26.

8c: yellow, mp: 282°C; yield: (62%). Anal. Calcd. for ($C_{27}H_{27}N_3O_7S$): C, 60.33; H, 5.02; N, 7.82. Found: C, 60.75; H, 5.29; N, 7.34.

8d: white, mp: 290°C; yield: (60%). Anal. Calcd. ($C_{26}H_{24}ClN_3O_6S$): C, 57.61; H, 4.43; N, 7.75. Found: C, 57.29; H, 4.67; N, 7.93.

8e: white, mp: 224°C; yield: (61%); ^{1}H NMR: δ_H 2.28 (s, 3H, CH_3); 2.60 (s, 3H, CH_3); 3.17–3.66 (m, 6H, 2H-6′, H-5′, H-4′, H-3′ and H-2′); 4.47 (t, 2H, 2′-OH and 3′-OH); 5.05 (d,1H, 4′-OH); 5.23 (d,1H, 6′-OH); 5.58 (d, $J_{1',2'} = 9.8$ Hz, 1H, H-1′); 7.04–7.59 (m, 9H, C_6H_4 and C_6H_5); 10.57 (bs, 1H, NH);^{13}C NMR: δ_C 19.5 (CH_3); 21.7 (CH_3); 59.4 (C-6′); 68.4 (C-4′); 70.4 (C-2′); 77.3 (C-3′); 80.4 (C-5′); 82.3 (C-1′); 103.1 (C-3); 113.6 (CN); 118.4–138.0 (2Ar-C); 150.0 (C-5); 157.0 (C-4); 159.1 (C-6); 162.6 (C-2); 178.0 (CO). Anal. Calcd. for ($C_{27}H_{27}N_3O_6S$): C, 62.18; H, 5.18; N, 8.06. Found: C, 62.46; H, 5.53; N, 8.27.

8f: white, mp: 217°C; yield: (59%); ^{1}H NMR: δ_H 2.22 (s, 3H, CH_3); 2.29 (s, 3H, CH_3); 2.58 (s, 3H, CH_3); 3.21–3.48 (m, 6H, 2H-6′, H-5′, H-4′, H-3′ and H-2′); 4.45 (t, 2H, 2′-OH and 3′-OH); 5.03 (d,1H, 4′-OH); 5.22 (d,1H, 6′-OH); 5.56 (d, $J_{1',2'} = 10.0$ Hz, 1H, H-1′); 7.06–8.44 (m, 8H, $2C_6H_4$); 10.26 (bs, 1H, NH);^{13}C NMR: δ_C 20.7 (CH_3); 21.1 (CH_3); 23.3 (CH_3); 61.0 (C-6′); 70.0 (C-4′); 72.0

(C-2′); 78.9 (C-3′); 82.0 (C-5′); 83.9 (C-1′); 104.7 (C-3); 115.2 (CN); 120.0–139.6 (2Ar-C); 155.5 (C-5); 158.6 (C-4); 160.6 (C-6); 164.0 (C-2); 179.1 (CO). Anal. Calcd. for ($C_{28}H_{29}N_3O_6S$): C, 62.80; H, 5.42; N, 7.85. Found: C, 62.37; H, 5.83; N, 7.52.

8g: pale yellow, mp: 230°C; yield: (60%); ^{1}H NMR: δ_H 1.77 (s, 3H, CH_3); 2.33 (s, 3H, CH_3); 3.23–3.26 (m, 6H, 2H-6′, H-5′, H-4′, H-3′ and H-2′); 3.71 (s, 3H, OCH_3); 4.47 (t, 2H, 2′-OH and 3′-OH); 5.03 (d,1H, 4′-OH); 5.22 (d,1H, 6′-OH); 5.56 (d, $J_{1',2'} = 9.9$ Hz, 1H, H-1′); 6.83–7.40 (m, 8H, $2C_6H_4$); 10.29 (bs, 1H, NH);^{13}C NMR: δ_C 21.4 (CH_3); 23.5 (CH_3); 55.7 (OCH_3); 61.3 (C-6′); 70.2 (C-4′); 72.3 (C-2′); 79.1 (C-3′); 82.3 (C-5′); 84.2 (C-1′); 104.9 (C-3); 114.5 (CN); 115.5–151.8 (2Ar-C); 156.5 (C-5); 158.8 (C-4); 160.8 (C-6); 164.0 (C-2); 172.1 (CO). Anal. Calcd. for ($C_{28}H_{29}N_3O_7S$): C, 60.98; H, 5.26; N, 7.62.

Found: C, 61.20; H, 5.47; N, 7.21.

8h: yellow, mp: 259°C; yield: (61%). Anal. Calcd. for ($C_{27}H_{26}ClN_3O_6S$): C, 58.32; H, 4.68; N, 7.56. Found: C, 58.73; H, 4.32; N, 7.75.

8i: yellow, mp: 272°C; yield: (59%). Anal. Calcd. for ($C_{27}H_{27}N_3O_7S$): C, 60.33; H, 5.02; N, 7.82. Found: C, 60.64; H, 5.50; N, 7.47.

8j: yellow, mp: 214°C; yield: (60%); ^{1}H NMR: δ_H 2.23 (s, 3H, CH_3); 2.59 (s, 3H, CH_3); 3.26–3.43 (m, 6H, 2H-6′, H-5′, H-4′, H-3′ and H-2′); 3.76 (s, 3H, OCH_3); 4.52 (t, 2H, 2′-OH and 3′-OH); 5.10 (d,1H, 4′-OH); 5.29 (d,1H, 6′-OH); 5.62 (d, $J_{1',2'} = 10.3$ Hz, 1H, H-1′); 7.01–7.45 (m, 8H, $2C_6H_4$); 10.38 (bs, 1H, NH);^{13}C NMR: δ_C 18.7 (CH_3); 21.2 (CH_3); 53.5 (OCH_3); 59.0 (C-6′); 68.0 (C-4′); 70.0 (C-2′); 76.8 (C-3′); 80.0 (C-5′); 81.9 (C-1′); 102.7 (C-3); 112.0 (CN); 117.9–149.3 (2Ar-C); 156.5 (C-5); 158.4 (C-4); 158.6 (C-6); 162.1 (C-2); 173.0 (CO). Anal. Calcd. for ($C_{28}H_{29}N_3O_7S$): C, 60.98; H, 5.26; N, 7.62.

Found: C, 61.22; H, 5.43; N, 7.18.

8k: yellow, mp: 236°C; yield: (61%); ^{1}H NMR: δ_H 2.08 (s, 3H, CH_3); 3.21–3.66 (m, 6H, 2H-6′, H-5′, H-4′, H-3′ and H-2′); 3.76 (s, 3H, OCH_3); 3.83 (s,3H, OCH_3); 4.45 (t, 2H, 2′-OH and 3′-OH); 5.04 (d,1H, 4′-OH); 5.22 (d,1H, 6′-OH); 5.57 (d, $J_{1',2'} = 10.7$ Hz, 1H, H-1′); 6.83–7.43 (m, 8H, $2C_6H_4$); 10.26 (bs, 1H, NH);^{13}C NMR: δ_C 22.0 (CH_3); 54.2 (OCH_3); 54.3 (OCH_3); 59.8 (C-6′); 68.7 (C-4′); 70.8 (C-2′); 77.6 (C-3′); 80.7 (C-5′); 82.6 (C-1′); 103.5 (C-3); 114.1 (CN); 120.4–150.1 (2Ar-C); 155.0 (C-5); 157.3 (C-4); 159.2 (C-6); 162.6 (C-2); 175.0 (CO). Anal. Calcd. for ($C_{28}H_{29}N_3O_8S$): C, 59.25; H, 5.11; N, 7.40.

Found: C, 59.32; H, 5.45; N, 7.49.

8l: yellow, mp: 195°C; yield: (62%); ^{1}H NMR: δ_H 2.08 (s, 3H, CH_3); 3.25–3.47 (m, 6H, 2H-6′, H-5′, H-4′, H-3′ and H-2′); 3.75 (s, 3H, OCH_3); 4.44 (t, 2H, 2′-OH and 3′-OH); 5.03 (d,1H, 4′-OH); 5.21 (d,1H, 6′-OH); 5.56 (d, $J_{1',2'} = 9.7$ Hz, 1H, H-1′); 7.00–8.61 (m, 8H, $2C_6H_4$); 10.66 (bs, 1H, NH). Anal. Calcd. for ($C_{27}H_{26}ClN_3O_7S$): C, 56.69; H, 4.54; N, 7.34. Found: C, 56.33; H, 4.34; N, 7.53.

8m: white, mp: 211°C; yield: (60%); ^{1}H NMR: δ_H 2.61 (s, 3H, CH_3); 3.21–3.48 (m, 6H, 2H-6′, H-5′, H-4′, H-3′ and H-2′); 4.46 (t, 2H, 2′-OH and 3′-OH); 5.04 (d,1H, 4′-OH); 5.23 (d,1H, 6′-OH); 5.62 (d, $J_{1',2'} = 10.1$ Hz, 1H, H-1′); 7.05–8.60 (m, 9H, C_6H_4 and C_6H_5); 10.40 (bs, 1H, NH). Anal. Calcd.

for ($C_{26}H_{24}ClN_3O_6S$): C, 57.61; H, 4.43; N, 7.75. Found: C, 58.01; H, 4.75; N, 7.51.

8n: pale yellow, mp: 215°C; yield: (61%). Anal. Calcd. for ($C_{27}H_{26}ClN_3O_6S$): C, 58.32; H, 4.68; N, 7.56. Found: C, 58.59; H, 4.83; N, 7.51.

8o: yellow, mp: 298°C; yield: (60%). Anal. Calcd. for ($C_{27}H_{26}ClN_3O_7S$): C, 56.69; H, 4.54; N, 7.34. Found: C, 56.32; H, 4.76; N, 7.65.

8p: yellow, mp: 217°C; yield: (60%); ^{1}H NMR: δ_H 2.60 (s, 3H, CH_3); 3.20–3.66 (m, 6H, 2H-6′, H-5′, H-4′, H-3′ and H-2′); 4.44 (t, 2H, 2′-OH and 3′-OH); 5.03 (d,1H, 4′-OH); 5.22 (d,1H, 6′-OH); 5.57 (d, $J_{1',2'} = 9.7$ Hz, 1H, H-1′); 7.30–8.61 (m, 8H, $2C_6H_4$); 10.61 (bs, 1H, NH). Anal. Calcd. for ($C_{26}H_{23}Cl_2N_3O_6S$): C, 54.16; H, 3.99; N, 7.29. Found: C, 54.55; H, 4.09; N, 7.35.

8q: yellow, mp: 212°C; yield: (59%). Anal. Calcd. for ($C_{26}H_{25}N_3O_6S$): C, 61.53; H, 4.93; N, 8.28. Found: C, 62.01; H, 4.86; N, 8.47.

8r: white, mp: 288°C; yield: (60%). Anal. Calcd. for ($C_{28}H_{29}N_3O_6S$): C, 62.80; H, 5.42; N, 7.85. Found: C, 63.11; H, 5.21; N, 7.97.

8s: yellow, mp: 284°C; yield: (61%). Anal. Calcd. for ($C_{28}H_{29}N_3O_7S$): C, 60.98; H, 5.26; N, 7.62. Found: C, 60.87; H, 5.19; N, 7.83.

8t: yellow, mp: 256°C; yield: (59%). Anal. Calcd. for ($C_{27}H_{27}N_3O_7S$): C, 60.33; H, 5.02; N, 7.82. Found: C, 60.31; H, 5.45; N, 7.91.

8u: white, mp: 283°C; yield: (60%). Anal. Calcd. for ($C_{28}H_{29}N_3O_8S$): C, 59.25; H, 5.11; N, 7.40. Found: C, 59.32; H, 5.09; N, 7.57.

8v: pale yellow, mp: 240°C; yield: (61%). Anal. Calcd. for ($C_{27}H_{26}ClN_3O_7S$): C, 56.69; H, 4.54; N, 7.34. Found: C, 56.74; H, 4.42; N, 7.57.

4-Aryl-3-cyano-6-methyl-2-(methylthio)-1,4-dihydropyridines (12a,b)

General procedures

A mixture of **1** (0.01 mol) and **2** (0.01 mol) was dissolved in dry ethanol (5 ml), and then piperidine (0.01 mol) was added. The reaction mixture was stirred at 0°C for 1 h and then left to stand to room temperature. The solvent was removed at reduced pressure and the resulting piperidinium salt of 1,4-dihydropyridine-2-thione **(3)** was dissolved in dry acetone (5 ml) and a solution of methyl iodide (0.015 mol) in dry acetone (20 ml) was then added at 0°C. The reaction mixture was stirred until the reaction was judged complete by TLC, then concentrated under reduced pressure and the residue crystallized from chloroform-petroleum ether at 0°C.

12a: pale yellow, mp: 165°C; yield: (80%); IR.: ν_{max} /Cm^{-1} (KBr) 3262 (NH); 2197 (CN); 1632 (CO). Anal. Calcd. for ($C_{22}H_{21}N_3OS$): C, 70.40; H, 5.60; N, 11.20. Found: C, 70.62; H, 5.41; N, 11.08.

12b: pale yellow, mp: 125°C; yield: (83%); IR.: ν_{max} /Cm^{-1} (KBr) 3438 (NH); 2195 (CN); 1644 (CO); ^{1}H NMR: δ_H 2.09 (s, 3H, CH_3); 3.38 (s, 3H, CH_3); 4.78 (s, 1H, pyridine H-4); 6.98-7.56 (m, 9H, C_6H_4 and C_6H_5); 9.10 (bs, 1H, NH); 9.71 (bs, 1H, NH). Anal. Calcd. for ($C_{21}H_{18}ClN_3OS$): C, 63.71; H, 4.55; N, 10.60. Found: C, 63.95; H, 4.79; N, 10.81.

4-Aryl-3-cyano-6-methyl-2-(methylthio)-pyridines (10a,b)

General procedures

Method (A). The piperidinium salt of dihydro-pyridinethiones **3** (0.01 mol) was dissolved in dry acetone (5 ml) and a solution of methyl iodide (0.015 mol) in dry acetone (20 ml) was then added at 30°C. The reaction mixture was stirred until the reaction was judged complete by TLC, then evaporated under reduced pressure at 40°C and the resulting product was crystallized from ethanol.

Method (B). pyridine-2(1*H*)thiones **9** (0.01 mole) was stirred with methyl idodie (0.015 mol) and potassium hydroxide (0.01 mol) in dichloromethame (30 ml) for 6 hours. The resulting product was collected by filtration and purified by recrystallization from ethanol.

10a: pale yellow, mp: 233°C; yield: (79%); IR.: ν_{max} /Cm^{-1} (KBr) 3439 (NH); 2222 (CN); 1642 (CO); ^{1}H NMR: δ_H 2.06 (s, 3H, CH_3); 2.40 (s, 3H, CH_3); 2.60 (s, 3H, SCH_3); 7.08-8.0 (m, 9H, C_6H_5 and C_6H_4); 10.44 (bs, 1H, NH); ^{13}C NMR: δ_C 16.00 (CH_3); 20.00 (CH_3); 30.5 (SCH_3); 104.0 (C3); 110.0 (CN); 121.3–129.0 (2Ar-C); 130 (C-5); 146 (C-4); 150.0 (C6); 168.0 (C2); 177.0 (CO). Anal. Calcd. for ($C_{22}H_{19}N_3OS$): C, 70.47; H, 5.69; N, 11.26. Found: C, 70.85; H, 5.71; N, 11.01.

10b: pale yellow, mp: 273°C; yield: (78%); IR.: ν_{max} /Cm^{-1} (KBr) 3435 (NH); 2220 (CN); 1648 (CO); ^{1}H NMR: δ_H 2.50 (s, 3H, CH_3); 2.69 (s, 3H, CH_3); 7.08–7.60 (m, 9H, C_6H_4 and C_6H_5); 10.44 (bs, 1H, NH). Anal. Calcd. for ($C_{21}H_{16}ClN_3OS$): C, 64.04; H, 4.06; N, 10.67. Found: C, 64.37; H, 4.31; N, 10.77.

Anti proliferative activity

Cells were plated in 96-multiwell plate (104 cells /well) for 24 hr before treatment with the compounds to allow attachment of cell to the wall of plate. For each tested compound, a solution of the compound was prepared by dissolving 1μM of each in dimethyl sulfoxide. After being incubated for 24 hr, cells were treated with 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 48 h at 37°C and in atmosphere of 5% CO_2. Control cells were treated with vehicle alone. After 48 hr, cells were fixed, washed and stained with MTT Stain (1.2 mM) dissolved in 1% hydrochloric acid for 2 hr. Excess stain was washed with sodium dodecyl sulfate in hydrochloric acid. Color intensity was measured in an ELISA reader at wave length of 550 nm.

References

[1] Kren, V.; Martinkova, L. Glycosides in Medicine: the Role of Glycosidic Residue in Biological Activity. *Curr. Med. Chem. 8, 1303–1328. Curr. Med. Chem.* **2001**, *8*, 1303–1328.

[2] Tiwari, N. A.; Shortancy-Fowler, A. T.; Parker, W. B.; Woud, W. R.; Secrist, J. A. Synthesis and Anticancer Evaluation of 4′-*C*-Methyl-2′-Fluoro *Arabino* Nucleosides. *Nucleosides Nucleotides Nucleic Acids* **2009**, *28*, 657–677.
[3] Elgemeie, G. H.; Masoud, D. Microwave-Assisted Synthesis of Heterocyclic Nucleosides. *Book, Publisher: Lap Lampert Academic Publishing*; Saarbrücken, Germany, ISBN: 978-3-659-78816-1. **2015**; pp 1–220.
[4] Elgemeie, G. H.; Elsayed, S. H.; Hassan, A. S. Direct Route to a New Class of Acrylamide Thioglycosides and their Conversions to Pyrazole Derivatives *Synth. Commun.* **2008**, *38*, 2700–2706.
[5] Elgemeie, G. H.; Kamal, E. A. Pyrimidinethione Nucleosides and their Deaza Analogues. *Nucleosides Nucleotides Nucleic Acids* **2002**, *21*, 287–325.
[6] Elgemeie, G. H.; Farag, A. B.; Amin, K. M.; El-Badry, O. M.; Hassan, G. S. Design, Synthesis and Cytotoxic Evaluation of Novel Heterocyclic Thioglycosides. *Med. Chem. (omics)* **2014**, *4*, 814–820.
[7] Elgemeie, G. H.; Saber, N. M. Synthesis and in Vitro Anti-tumor Activity of a New Class of Acyclic Thioglycosides. *Nucleosides Nucleotides* **2015**, *34*, 463–474.
[8] Elgemeie, G. H.; Abu-Zaied, M.; Alsaid, S.; Hebishy, A.; Essa, H. Novel Nucleoside Analogues: First Synthesis of Pyridine 4-Thioglycosides and their Cytotoxic Evaluation. *Nucleosides Nucleotides Nucleic Acids* **2015**, *34*, 659–673.
[9] Elgemeie, G. H.; Abou-Zeid, M. Purine and Guanine Thioglycoside Analogs: Novel Synthesis of a New Class of Pyrazolo[1,5-A][1,3,5]Triazine-4-Thioglycoside Derivatives Under Microwave Activation. *Nucleosides Nucleotides Nucleic Acids* **2015**, *34*, 834–847.
[10] Yoshimura, Y.; Kitano, K.; Yamada, K.; Sakata, S.; Miura, S.; Ashida, N.; Machida, H. Synthesis and Biological Activities of 2′-Deoxy-2′-Fluoro-4′-Thioarabinofuranosylpyrimidine and -Purine Nucleosides. *Bioorg. Med. Chem.* **2000**, *8*, 1545–58.
[11] Komor, R.; Pastuch-Gawołek, G.; Sobania, A.; Jadwi´nski, M.; Szeja, W. Application of Different Alpha-1-Thioglycosides Preparation Methods in Synthesis of 5-nitro-2-Pyridyl-1-Thioglycosides Substrates in Synthesis of Conjugates with Uridine Moiety. Part I. *Acta Pol. Pharm.* **2012**, *69*, 1259.
[12] Kananovich, D. G.; Reino, A.; Ilmarinen, K.; Roomusoks, M.; Mati Karelson, M.; Lopp, M. A. General Approach to the Synthesis of 5-S-Functionalized Pyrimidine Nucleosides and their Analogues. *Org. Biomol. Chem.* **2014**, *12*, 5634.
[13] Scala, S.; Akhmed, N.; Rao, U. S.; Paull, K.; Lan, L.; Dickstein, B.; Lee, J.; Elgemeie, G. H.; Stein, W. D.; Bates, S. E. P-Glycoprotein Substrates and Antagonists Cluster into Two Distinct Groups. *Mol. Pharmacol.* **1997**, *51*, 1024–1033.
[14] Elgemeie, G. H.; Mahdy, E. M.; Elgawish, M. A.; Ahmed, M. M.; Shousha, W. G.; Eldin, M. E. A New Class of Antimetabolites: Pyridine Thioglycosides as Potential Anticancer Agents. *Z. Naturforsch.* **2010**, *65*, 577–587.
[15] Elgemeie, G. H.; Hussein, M.; Jones, P. G. 2-(2′,3′,4′,6′-Tetra-O-Cetyl-D-Glucopyranosylthio)-4-Pyridine-4-yl-6,7,8,9-Tetrahedro-5H-Cycloheptapyridine-3-Carbonitril. *Acta Cryst.* **2002**, *E58*, 1244–1245.
[16] Mosmann, T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays *J. Immunol. Methods* **1983**, *65*, 55–63.