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RESEARCH ARTICLE

Eco-friendly, green synthesis and antimicrobial evaluation of 4,6-disubstituted-2-(6`-acetyl-O-β-D-glucopyranosylsulfanyl)-nicotinonitrile

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Abstract

A new and efficient solid phase route to the synthesis of 3-cyano-2(1H) pyridinethione/one derivatives, 4,6-disubstituted-2-(2`,3`,4`,6`-tetra-O-acetyl-β-D-glucopyranosylsulfanyl)-nicotinonitriles and 4,6-disubstituted-2-(6`-acetyl-O-β-D-glucopyranosylsulfanyl)-nicotinonitrile by grinding is described. This protocol has the advantages of excellent yields, short reaction time, easy setup, mild reaction conditions, and environmental friendliness. The tested compounds were found to exhibit remarkably better antibacterial activities against Escherichia coli and Bacillus thuringiensis than the control drug

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1. Introduction

Over the past few years 3-cyano-2(*1H*)pyridinethione and 3-cyano-2-(*1H*)pyridone derivatives have gained considerable interest due to their importance as intermediates for both the synthesis of many biologically active compounds as antiallergic, antibacterial, anticancer, antidepressant as well as their wide range of practical uses in the synthesis of medicinal compounds, vitamins, dyes and intermediate compounds in fine organic synthesis [1-5]. On the other hand, glycosylthio-heterocycles have attracted much attention because of their abilities to function as biological inhibitors, inducers, and ligands for affinity of chromatography of carbohydrate-process enzymes and proteins [6]. At present, developing green chemical protocols is one of the most powerful tools in organic synthesis. Moreover solid state synthesis can be utilized for the generation of structurally diverse molecules. Grinding the reactants together constitutes the simplest green chemistry protocol. It has proven to be an efficient, economical and environmentally benign process [7-11].Thus, in continuation of our interest in developing a green protocol for the synthesis of organic compounds [12], we report herein a convenient synthesis of 3-cyano-2(*1H*)pyridinethione/one, 4,6-disubstituted-2-(2`,3`,4`,6`-tetra-O-acetyl-β-D-glucopyranosylsulfanyl)-nicotinonitrile, 4,6-disubstituted-2-(6`-acetyl-O-β-D-glucopyranosyl-sulfanyl)-nicotinonitrile derivatives and the results of their antimicrobial screening.

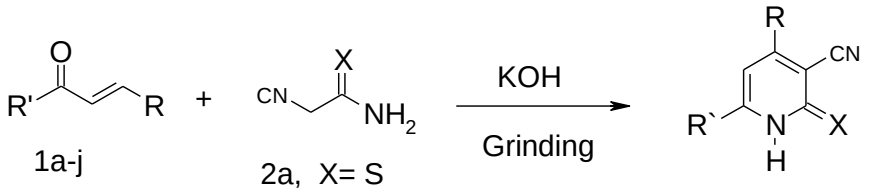
2. Results and discussion

2.1. Chemistry.

Pyridinethione/ones are traditionally synthesized by treatment of cyanothioacetamide/ cyanoacetamide with chalcone derivatives under reflux in alkoxide solution for 4-7 hours[1,13]. In the present work, 3-cyano-2(*1H*) pyridinethiones/ones **3a-j**, **4a-h** were prepared by grinding together equivalent amounts of each of 2-cyanothioacetamide/2-cyanoacetamide **2a,b** with the appropriate chalcones **1a-r** in presence of solid potassium hydroxide in a porcelain mortar under solvent-free condition at room temperature.(Scheme 1). Grinding for about 3-5 minutes led to a colored solid mass and grinding continued for another 5-10 minutes. The crude products were easily separated by washing with cold water, and purified by crystallization from the proper solvent (Table 1).

Table 1: Yields of synthesized pyridinethiones/ones 3a-j, 4a-i by grinding.

Entry	R`	R	X	Time (min)	Yield% Reported/found
3a	2-furyl	C_6H_5	S	15	68[13] / 80
3b	2-furyl	4-FC_6H_4	S	15	32[13] / 75
3c	2-furyl	4-ClC_6H_4	S	10	34[13] / 73
3d	2-furyl	2-thienyl	S	15	66
3e	2-furyl	2-furyl	S	10	48[1] / 77
3f	C_6H_5	C_6H_5	S	15	81
3g	C_6H_5	4-$CH_3C_6H_4$	S	15	70
3h	C_6H_5	4-ClC_6H_4	S	10	82
3i	2-naphthyl	C_6H_5	S	10	96
3j	2-naphthyl	2-thienyl	S	15	60
4a	2-furyl	4-FC_6H_4	O	15	61
4b	2-furyl	2-thienyl	O	15	72
4c	2-furyl	2-furyl	O	15	65
4d	C_6H_5	C_6H_5	O	10	88[14] / 95
4e	C_6H_5	4-$CH_3C_6H_4$	O	15	78
4f	C_6H_5	4-ClC_6H_5	O	10	98
4g	2-naphthyl	C_6H_5	O	10	72
4h	2-naphthyl	2-thienyl	O	15	52



1a-j

2a, X= S
2b, X=O

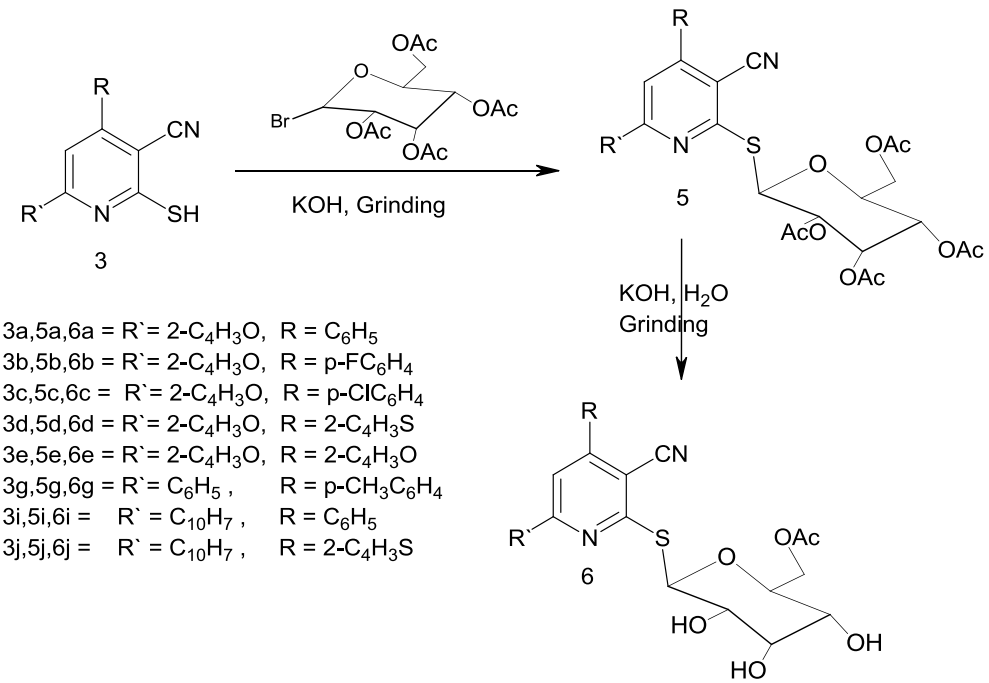
3a-j, X=S
4a-h, X=O

3a, R`= 2-C_4H_3O R= C_6H_5
3b, R`= 2-C_4H_3O R= 4-FC_6H_4
3c, R`= 2-C_4H_3O R= 4-ClC_6H_4
3d, R`= 2-C_4H_3O R= 2-C_4H_3S
3e, R`= 2-C_4H_3O R= 2-C_4H_3O
3f, R`= C_6H_5 R= C_6H_5
3g, R`= C_6H_5 R= 4-$CH_3C_6H_4$
3h, R`= C_6H_5 R= 4-ClC_6H_4
3i, R`= $C_{10}H_7$ R= C_6H_5
3j, R`= $C_{10}H_7$ R= 2-C_4H_3S

4a, R`= 2-C_4H_3O R= 4-FC_6H_5
4b, R`= 2-C_4H_3O R= 2-C_4H_3S
4c, R`= 2-C_4H_3O R= 2-C_4H_3O
4d, R`= C_6H_5 R= C_6H_5
4e, R`= C_6H_5 R=4-$CH_3C_6H_4$
4f, R`= C_6H_5 R= 4-ClC_6H_5
4g, R`= $C_{10}H_7$ R= C_6H_5
4h, R`= $C_{10}H_7$ R= 2-C_4H_3S

Scheme 1. Synthesis of 3-cyano-2(*1H*) pyridinethione/one derivatives

Peracetyl *β*-D-glucopyranosylsulfanyl-nicotinonitriles were synthesized by grinding equimolar amounts of each of compounds **3a-e**, **3g**, **3i,j** and solid potassium hydroxide in a porcelain mortar for 5-10 minutes, then equivalent amounts of 2,3,4,6-tetra-*O*-acetyl-*α*-D-glucopyranosyl bromide were added and grinding continued for further 15 minutes. Triturating with water and crystallization from the ethanol gave the corresponding 4,6-disubstituted-2-(2`,3`,4`,6`-tetra-*O*-acetyl-*β*-D-glucopyranosylsulfanyl)-nicotinonitriles (Scheme 2).



3a,5a,6a = R`= 2-C_4H_3O, R = C_6H_5
3b,5b,6b = R`= 2-C_4H_3O, R = p-FC_6H_4
3c,5c,6c = R`= 2-C_4H_3O, R = p-ClC_6H_4
3d,5d,6d = R`= 2-C_4H_3O, R = 2-C_4H_3S
3e,5e,6e = R`= 2-C_4H_3O, R = 2-C_4H_3O
3g,5g,6g = R`= C_6H_5 , R = p-$CH_3C_6H_4$
3i,5i,6i = R` = $C_{10}H_7$, R = C_6H_5
3j,5j,6j = R` = $C_{10}H_7$, R = 2-C_4H_3S

Scheme 2. Synthesis of β-D-glucopyranosylsulfanyl-nicotinonitriles

The structure of the synthesized peracetyl β-D-glucopyranosylsulfanyl-nicotinonitriles was confirmed on the basis of their elemental analysis and spectral data. Their IR spectra showed bands at 1747-1743 cm^{-1} for the acetoxy carbonyl groups. The ^1H NMR spectrum of 5g showed the presence of signals at δ 1.75, 2.04, 2.06 and 2.10 ppm characteristic for 4 CH_3 of OAc groups and a doublet at δ 6.19 ppm for anomeric proton with $J_{1`,2`}$ = 8.21 Hz, which confirmed the β-configuration. The ^{13}C NMR spectrum of 5g showed peak at δ 86.0 ppm, characteristic for anomeric carbon, and absence of C=S group, which indicates the formation of S-glycoside not N-glycoside.

Deprotection of the previously synthesized S-glucosides 5a-e, 5g, 5i,j was carried out by grinding with KOH in presence of 2-3 drops of water for few minutes; and also by stirring in a methanolic solution containing triethylamine and few drops of water overnight [15] (Scheme 2). Both procedures gave the deprotected glycosides 6a-e, 6g, 6i,j with the grinding procedure providing higher yields[16].

IR spectra of compound 6g showed bands at 3414-3124 cm^{-1} (3OH), 2222 cm^{-1} (CN) and 1747 cm^{-1} (CO). Its ^1H NMR revealed signals at δ = 2.35 (s, 3H, CH_3CO), 3.13 (s, 3H, CH_3), 3.24-3.95 (m, 6 H, H-6`,H-6``,H-5`,H-4`,H-3`and H-2`), 4.81 (d, 1H $J_{1`,2`}$ = 8.22 Hz,, H-1`), 7.90 (s, 1H, H-5 pyridine), 7.12-7.99 (m, 9H, Ar`H), 9.81 (d, J = 4.29 Hz, 1 H, OH-4`), 10.11 (d, 1H, J = 5.24 Hz, OH-3`), 10.38 (d, 1H, J = 4.96 Hz, OH-2`). Its mass spectrum showed a molecular ion peak at m/z 506. Based on the above mentioned spectral data and elemental analysis, the product was assigned structure of 6g.

2.2. Biological Evaluation.

Bacterial resistance that some animal and humans pathogens have recently developed against known antimicrobial agents has caught the attention of the scientific community worldwide. Studying this phenomenon has pushed this community towards working on finding new antimicrobial compounds that may control the spread of these bacterial pathogens especially that cause infectious diseases [17].Measuring the minimum inhibitory concentration (MIC) of new proposed antimicrobial compounds has become a known tool to determine the efficiency of these compounds to resist the growth of the bacterial pathogens [18].

Two bacteria, Gram positive *B. thuringiensis* and Gram negative *E. coli*, were used to assay their sensitivity against the antimicrobial impact of newly synthesized Pyridinethiones/one derivatives. Qualitative test has shown that compounds (**3b, 3d, 3e, 3f, 3h, 3i, 4b, 4c, 4d, 4f, 4g**, **6d & 6g)** have a clear inhibitory effect on the growth on of both *Escherichia coli* and *Bacillus thuringiensis* (Table 2).

In vitro antimicrobial activity of assayed compounds has been assayed according to their minimum inhibitory concentrations (MIC) (Table3). The results clearly show the inhibitory effect of these compounds on both *Escherichia coli* and *Bacillus thuringiensis.* Also, the MIC of the assayed compounds to that of local sample of tetracycline was compared to that of the assayed compounds. This comparison has clearly shown that assayed compounds are 6-8 times more efficient as antimicrobial compounds than tetracycline. Finally the assayed compounds did not show any significant difference in their inhibitory effect on both used bacterial strains. These findings confirm the efficiency of assayed compounds as new antimicrobial agents that have a wide activity range towards different bacterial strains, regardless of the bacterial variation in nature and cell wall type.

Table 2. Antibacterial activities of some of the synthesized compounds.

Compound	R`	R	X	*E. coli*	*B. thuringiensis*
3a	2-furyl	C_6H_5	S	-	-
3b	2-furyl	4-FC_6H_4	S	+	+
3c	2-furyl	4-ClC_6H_4	S	-	-
3d	2-furyl	2-thienyl	S	+	+
3e	2-furyl	2-furyl	S	+	+
3f	C_6H_5	C_6H_5	S	+	+
3g	C_6H_5	4-$CH_3C_6H_4$	*S*	-	-
3h	C_6H_5	4-ClC_6H_4	S	+	+
3i	2-naphthyl	C_6H_5	S	+	+
3j	2-naphthyl	2-thienyl	S	-	-
4a	2-furyl	4-FC_6H_4	O	-	-
4b	2-furyl	2-thienyl	O	+	+
4c	2-furyl	2-furyl	O	+	+
4d	C_6H_5	C_6H_5	O	+	+
4e	C_6H_5	4-$CH_3C_6H_4$	O	-	-
4f	C_6H_5	4-ClC_6H_5	O	+	+
4g	2-naphthyl	C_6H_5	O	+	+
4h	2-naphthyl	2-thienyl	O	-	-
6a	2-furyl	C_6H_5	S	-	-
6b	2-furyl	4-FC_6H_4	S	-	-
6d	2-furyl	2-thienyl	S	+	+
6g	C_6H_5	4-$CH_3C_6H_4$	S	+	-
6i	2-naphthyl	C_6H_5	S	-	-
6j	2-naphthyl	2-thienyl	S	-	-

Table 3. In vitro antimicrobial activity of assayed compounds.

Compound	R`	R	X	MIC (mg/ml DMSO)	
				E. coli	*B. thuringiensis*
3b	2-furyl	4-FC_6H_4	S	≥20	≥20
3d	2-furyl	2-thienyl	S	≥15	≥15
3e	2-furyl	2-furyl	S	≥15	≥15
3f	C_6H_5	C_6H_5	S	≥15	≥15
3h	C_6H_5	4-ClC_6H_4	S	≥15	≥15
3i	2-naphthyl	C_6H_5	S	≥15	≥15
4b	2-furyl	2-thienyl	O	≥15	≥15
4c	2-furyl	2-furyl	O	≥20	≥20
4d	C_6H_5	C_6H_5	O	≥15	≥15
4f	C_6H_5	4-ClC_6H_5	O	≥15	≥15
4g	2-naphthyl	C_6H_5	O	≥15	≥15
6d	2-furyl	2-thienyl	S	≥15	≥15
6g	C_6H_5	4-$CH_3C_6H_4$	S	≥20	-
Tetracycline				***125***	***75***

3. Conclusion

In summary, we have developed a simple and efficient procedure for the synthesis of 3-cyano-2(1H)pyridinethione/ones, peracetyl β-D-glucopyranosylsulfanyl-nicotinonitriles and their deprotected derivatives which proved to be potent wide spectrum antibacterial agents, via simply grinding the reactants together at room temperature in presence of KOH. This protocol offers several advantages such as high yields, short reaction times, environmentally benign and a simple experimental work-up procedure.

4. Experimental

Melting points were measured on an Electrothermal melting point apparatus and are uncorrected. IR spectra were recorded on Shimadzu FT-IR 8101 PC infrared spectrophotometer. The ^{1}H NMR spectra were determined in DMSO-d_6 at 300 MHz on a Varian Mercury VX 300 NMR spectrometer using TMS as an internal standard. Mass spectra were measured on a GCMS-QP1000 EX spectrometer at 70 eV. Elemental analyses were carried out at the Microanalytical Center of Cairo University. Escherichia coli and Bacillus thuringiensis were obtained on slants from the Faculty of Agriculture, Cairo University. Bacterial growth was detected as turbidity at 450 nm using DU 640 Spectrophotometer (BECKMAN, USA).

4.1. Chemistry

4.1.1. General Procedure for the Synthesis of Pyridinthione/one Derivatives 3a-j, 4a-i.

A mixture of the appropriate chalcones 1a-j (3mmol), 2-cyanothioacetamide (2a) or 2-cyanoacetamide (2b) (0.3g/0.25, 3mmol) and KOH (0.22g, 4 mmol), was thoroughly ground with a pestle in an open mortar at room temperature for 3-5 minutes until the mixture turned into a melt. The initial syrupy reaction mixture solidified within 4-6 minutes. Grinding continued for 5-15 minutes and the reaction was monitored by TLC. The solid was washed with water, and recrystallized from the appropriate solvent to give the corresponding pyridinethione/one derivatives 3a-j and 4a-i, respectively.

4.1.1.1. 6-(Furan-2-yl)-1,2-dihydro-4-phenyl-2-thioxopyridine-3-carbonitrile (3a).
Brown powder from dil DMF; m.p. 214-219^{o}C; (lit. [13] 212-214^{o}C). Anal. Calcd. for $C_{16}H_{10}N_2OS$: C, 69.05; H, 3.62; N, 10.06; S, 11.52%; Found: C, 68.99; H, 3.58; N, 10.08; S, 11.35%.

4.1.1.2. 4-(4-Fluorophenyl)-6-(furan-2-yl)-1,2-dihydro-2-thioxopyridine-3-carbonitrile (3b). Orange powder from toluene; m.p. 235-238^{o}C; (lit. [13] 238-245^{o}C). Anal. Calcd. for $C_{16}H_9FN_2OS$: C, 64.85; H, 3.06; N, 9.45; S, 10.82%; Found: C, 64.95; H, 3.11; N, 9.55; S, 11.00%.

4.1.1.3. 4-(4-Chlorophenyl)-6-(furan-2-yl)-1,2-dihydro-2-thioxopyridine-3-carbonitrile (3c).
Brown powder from toluene; m.p. 219-221^{o}C; (lit. [13] 219-221^{o}C). IR (KBr): 3398, 2212 cm^{-1} (NH, CN); ^{1}H NMR: δ = 6.8 (s, 1H, pyridinyl C_5H), 7.13-8.08 (m, 7H, Ar`H), 8.2 (s, 1H, NH). GCMs m/z: 312 (M^+). Anal. Calcd. for $C_{16}H_9ClN_2OS$: C, 61.44; H, 2.90; N, 8.96; S, 10.25%; Found: C, 61.12; H, 3.10; N, 9.01; S, 10.01%.

4.1.1.4. 6-(Furan-2-yl)-1,2-dihydro-4-(thiophen-2-yl)-2-thioxopyridine-3-carbonitrile (3d). Brown powder from dil DMF; m.p. 157-160^{o}C. IR (KBr): 3435, 2215 cm^{-1} (NH, CN); ^{1}H NMR: δ = 6.8 (s, 1H, pyridinyl C_5H), 6.96-7.45 (m, 6H, Ar`H), 8.5 (s, 1H, NH). GCMs m/z: 284 (M^+). Anal. Calcd. for $C_{14}H_8N_2OS_2$: C, 59.13; H, 2.84; N, 9.85; S, 22.55%; Found: C, 59.12; H, 3.10; N, 9.68; S, 22.78%.

4.1.1.5. 4,6-Di(furan-2-yl)-1,2-dihydro2-thioxopyridine-3-carbonitrile (3e).
Orange powder from MeOH; m.p. 232-234^{o}C; (lit. [1] 233-234^{o}C). Anal. Calcd. for $C_{14}H_8N_2O_2S$: C, 62.68; H, 3.01; N, 10.44; S, 11.95%; Found: C, 62.57; H, 3.11; N, 10.53; S, 11.77%.

4.1.1.6. 1,2-Dihydro-4,6-diphenyl-2-thioxopyridine-3-carbonitrile (3f).
Orange powder dil EtOH; m.p. 255-258^{o}C. IR (KBr): 3430, 2215 cm^{-1} (NH, CN); ^{1}H NMR: δ = 6.8 (s, 1H, pyridinyl C_5H), 6.99-8.35 (m, 10H, Ar`H), 9.0 (s, 1H, NH). GCMs m/z: 288 (M^+). Anal. Calcd. for $C_{18}H_{12}N_2S$: C, 74.97; H, 4.19; N, 9.71; S, 11.12%; Found: C, 75.08; H, 4.00; N, 9.68; S, 11.00%.

4.1.1.7. 1,2-Dihydro-6-phenyl-2-thioxo-4-ptolylpyridine-3-carbonitrile (3g).
Yellow crystals from dil. EtOH; m.p. 191-195^{o}C. IR (KBr): 3420, 2220 cm^{-1}(NH, CN); ^{1}H NMR: δ = 2.5 (s, 3H, CH_3); 6.9 (s, 1H, pyridinyl C_5H), 7.38-8.07 (m, 9H, Ar`H), 9.2 (s, 1H, NH). GCMs m/z: 302 (M^+). Anal. Calcd. for $C_{19}H_{14}N_2S$: C, 75.47; H, 4.67; N, 9.26; S, 10.60%; Found: C, 75.25; H, 4.56; N, 9.48; S, 10.50%.

4.1.1.8. 4-(4-Chlorophenyl)-1,2-Dihydro-6-phenyl-2-thioxopyridine-3-carbonitrile (3h).
Black crystals from EtOH); m.p. 195-198^{o}C. IR (KBr): 3398, 2221 cm^{-1} (NH, CN); ^{1}H NMR: δ = 6.84 (s, 1H, pyridinyl C_5H), 6.77-8.10 (m, 9H, Ar`H), 12.63 (s, 1H, NH). GCMs m/z: 322 (M^+). Anal. Calcd. for $C_{18}H_{11}ClN_2S$: C, 66.97; H, 3.43; N, 8.68; S, 9.93%; Found: C, 67.02; H, 3.56; N, 9.78; S, 10.00%.

4.1.1.9. 1,2-Dihydro-6-(naphthalene-2-yl)-4-phenyl-2-thioxopyridine-3-carbonitrile (3i).

Orange crystals from EtOH; m.p. 125-128^{o}C. IR (KBr): 3415, 2194 cm^{-1}(NH, CN); ^{1}H NMR: δ = 6.84 (s, 1H, pyridinyl C_5H), 6.65-8.50 (m, 12H, Ar`H), 10.50 (s, 1H, NH). GCMs m/z: 338 (M^+). Anal. Calcd. for $C_{22}H_{14}N_2S$: C, 78.08; H, 4.17; N, 8.28; S, 9.47%; Found: C, 77.92; H, 3.86; N, 8.18; S, 9.75%.

4.1.1.10. 1,2-Dihydro-6-(naphthalene-2-yl)-4-(thiophen-yl)-2-thioxopyridine-3-carbonitrile (3j).

Brown powder from EtOH; m.p. 125-127^{o}C. IR (KBr): 3396, 2221 cm^{-1}(NH, CN); ^{1}H NMR: δ = 6.84 (s, 1H, pyridinyl C_5H), 7.33-8.45 (m, 10H, Ar`H), 9.26 (s, 1H, NH). GCMs m/z: 344 (M^+). Anal. Calcd. for $C_{20}H_{12}N_2S$: C, 69.74; H, 3.51; N, 8.13; S, 18.62%; Found: C, 69.92; H, 3.75; N, 8.18; S, 18.88%.

4.1.1.11. 4-(4-Fluorophenyl)-6-(furan-2-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile (4a).

Yellow crystals from EtOH/DMF; m.p. 218-222^{o}C. IR (KBr): 3394, 2215, 1655 cm^{-1} (NH, CN, amide CO). ^{1}H NMR: δ = 6.8 (s, 1H, pyridinyl C_5H), 6.94-8.01 (m, 7H, Ar`H), 10.33 (s, 1H, NH), 12.81 (s, 1H, OH). GCMs m/z: 280 (M^+). Anal. Calcd. for $C_{16}H_9FN_2O_2$: C, 68.57; H, 3.24; N, 10.00%; Found: C, 68.52; H, 3.45; N, 9.98%.

4.1.1.12. 6-(furan-2-yl)-1,2-dihydro-2-oxo-4-(thiophen-2-yl)pyridine-3-carbonitrile (4b).

Yellow crystals from EtOH/DMF; m.p. > 300^{o}C. IR (KBr): 3391, 2212, 1645 cm^{-1} (NH, CN, amide CO). ^{1}H NMR: δ = 6.8 (s, 1H, pyridinyl C_5H), 6.99-8.31 (m, 6H, Ar`H), 9.8 (s, 1H, NH), 12.75 (s, 1H, OH). GCMs m/z: 268 (M^+). Anal. Calcd. for $C_{14}H_8N_2O_2S$: C, 62.68; H, 3.01; N, 10.44; S, 11.95%; Found: C, 62.52; H, 2.99; N, 10.14; S, 12.00%.

4.1.1.13. 4,6-(furan-2-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile (4c).

Yellow crystals from EtOH; m.p. > 300^{o}C. IR (KBr): 3339, 2194, 1665 cm^{-1} (NH, CN, amide CO). ^{1}H NMR: δ = 6.8 (s, 1H, pyridinyl C_5H), 7.00-8.45 (m, 6H, Ar`H), 9.88 (s, 1H, NH), 11.96 (s, 1H, OH). GCMs m/z: 252 (M^+). Anal. Calcd. for $C_{14}H_8N_2O_3$: C, 66.67; H, 3.20; N, 11.11%; Found: C, 66.98; H, 3.45; N, 10.90%.

4.1.1.14. 1,2-dihydro-2-oxo-4,6-diphenylpyridine-3-carbonitrile (4d).

Peige crystals from EtOH; m.p. > 300^{o}C; (lit. mp > 300^{o}C [14]) Anal. Calcd. for $C_{18}H_{12}N_2O$: C, 79.40; H, 4.44; N, 10.29%; Found: C, 79.60; H, 4.45; N, 10.43%.

4.1.1.15. 1,2-dihydro-2-oxo-6-phenyl-4-p-tolylpyridine-3-carbonitrile (4e).

Grey crystals from EtOH; m.p. > 300^{o}C. IR (KBr): 3430, 2222, 1645 cm^{-1} (NH, CN, amide CO); ^{1}H NMR: δ = 2.49 (s, 3H, CH_3) 6.77 (s, 1H, pyridinyl C_5H), 7.18-7.90 (m, 9H, Ar`H), 10.24 (s, 1H, NH), 12.74 (s, 1H, OH). GCMs m/z: 286 (M^+). Anal. Calcd. for $C_{19}H_{14}N_2O$: C, 79.70; H, 4.93; N, 9.78%; Found: C, 79.68; H, 4.85; N, 9.58%.

4.1.1.16. 4-(4-Chlorophenyl)-1,2-dihydro-2-oxo-6-phenylpyridine-3-carbonitrile (4f).

White crystals from EtOH; m.p. 95-97^{o}C. IR (KBr): 3434, 2215, 1652 cm^{-1}(NH, CN, amide CO); ^{1}H NMR: δ = 6.8 (s, 1H, pyridinyl C_5H), 6.99-7.90 (m, 9H, Ar`H), 9.88 (s, 1H, NH), 12.75 (s, 1H, OH). GCMs m/z: 306 (M^+). Anal. Calcd. for $C_{18}H_{11}ClN_2O$: C, 70.48; H, 3.61; N, 9.13%; Found: C, 70.68; H, 3.82; N, 9.28%.

4.1.1.17. 1,2-dihydro-6-(naphthalen-2-yl)-2-oxo-4-phenylpyridine-3-carbonitrile (4g).

Pale green from dil. EtOH; m.p. 98-100^{o}C. IR (KBr): 3415, 2220, 1658 cm^{-1} (NH, CN, amide CO). ^{1}H NMR: δ = 6.77 (s, 1H, pyridinyl C_5H), 7.10-8.35 (m, 12H, Ar`H), 10.45 (s, 1H, NH), 12.75 (s, 1H, OH). GCMs m/z: 322 (M^+). Anal. Calcd. for $C_{22}H_{14}N_2O$: C, 81.97; H, 4.38; N, 8.69%; Found: C, 82.00; H, 4.18; N, 8.99%.

4.1.1.18. 1,2-dihydro-6-(naphthalen-2-yl)-2-oxo-4-(thiophen-2-yl)pyridine-3-carbonitrile (4h).

Pale green crystals from dil. EtOH; m.p. 115-117^{o}C. IR (KBr cm^{-1}): 3430, 2194, 1668 cm^{-1} (NH, CN, amide CO); ^{1}H NMR: δ = 6.8 (s, 1H, pyridinyl C_5H), 6.89-8.13 (m, 10H, Ar`H), 9.88 (s, 1H, NH), 11.99 (s, 1H, OH). GCMs m/z: 328 (M^+). Anal. Calcd. for $C_{20}H_{12}N_2OS$: C, 73.15; H, 3.68; N, 8.53; S, 9.76%; Found: C, 73.35; H, 4.10; N, 8.63%; S, 9.99%.

4.1.2. General procedure for synthesis of glycosides 5a-e, 5g, 5i,j.

A mixture of pyridin-2-(1H)-thiones 3a-e, 3g, 3i,j (2 mmol) and (0.112g, 2 mmol) potassium hydroxide were grinding together for 5-10 minutes, then peracetylated glycosyl bromide (2mmol) was added and then grinding continued for further 15 minutes. The reaction mixture was washed with water and crystallized from the proper solvent to afford 5a-e, 5g, 5i,j respectively.

4.1.2.1 .6-(2-Furanyl)-4-phenyl-2-(2`,3`,4`,6`-tetra-O-acetyl-β-D-glucopyranosylsulfanyl)-nicotinonitrile (5a)

Pale brown crystals from ethanol, 70%, mp. 238^{o}C, IR (KBr, cm^{-1}): 2218(CN) and 1747 (CO, acetoxy). ^{1}H NMR: δ =1.87, 2.05, 2.07 and 2.08 (4s, 12H, 4 CH_3CO), 4.09 (dd, 1H, $J_{6``,5`}$ = 4.27, $J_{6``,6`}$ = 12.19, H-6``), 4.18 (dd, 1H, $J_{6`,5`}$ = 5.67, $J_{6`,6``}$ = 12.20, H-6`), 4.38 (m, 1H, H-5`), 5.01 (t, 1H, $J_{4`,3`}$ = 8.92, $J_{4`,5`}$ = 9.25 Hz, H-4`), 5.27 (t, 1H, $J_{2`,1`}$ = 7.92, $J_{2`,3`}$ = 9.53 Hz, H-2`), 5.55 (t, 1H, $J_{3`,2`}$ = 9.30, $J_{3`,4`}$ = 8.79 Hz, H-3`), 6.13 (d, 1H, $J_{1`,2`}$ = 7.89 Hz, H-1`), 7.96 (s, 1H, H-5 pyridine), 6.30-7.48 (m, 8H, Ar`H). $^{13}$C NMR δ = 20.7, 21.0, 21.0 and 21.0 (4 $CH_3CO$), 62.1(C-6`), 68.6(C-4`), 69.8(C-3`), 70.4(C-2`), 73.6(C-5`), (anomeric carbon 85.0(C-1`)), 102, 105, 107.2, 117.0(CN), 117.1, 127.4, 129.3, 138.0, 142.9, 154.2, 157.7 (Ar-C), 161.5, 164.1 (C=N), 170.3 (4CO); GCMs m/z: 608(M^+) Anal. Calcd. for $C_{30}H_{28}N_2O_{10}S$ (608.63) C, 59.20; H,4.64; N, 4.60; S, 5.17%; Found: C, 59.25; H, 4.70; N, 4.69; S, 5.11%.

4. 1.2.2. 6-(2-Furanyl)-4-(4-fluorophenyl)-2-(2`,3`,4`,6`-tetra-O-acetyl-β-D-glucopyranosylsulfanyl)-nicotinonitrile (5b)

Pale brown crystals from ethanol, 63%, mp. 233°C, IR (KBr, cm^{-1}): 2214(CN) and 1747 (CO, acetoxy). ^1H NMR: δ=1.87, 2.01, 2.02 and 2.08 (4s, 12H, 4 CH_3CO), 4.13 (dd, 1H, $J_{6``,5`}$ = 4.23, $J_{6``,6`}$ = 12.20, H-6``), 4.21 (dd, 1H, $J_{6`,5`}$ = 5.67, $J_{6`,6``}$ = 12.20, H-6`), 4.40 (m, 1H, H-5`), 5.05 (t, 1H, $J_{4`,3`}$ = 8.88, $J_{4`,5`}$ = 9.25 Hz, H-4`), 5.33 (t, 1H, $J_{2`,1`}$ = 7.98, $J_{2`,3`}$ = 9.55 Hz, H-2`), 5.65 (t, 1H, $J_{3`,2`}$ = 9.30, $J_{3`,4`}$ = 8.89 Hz, H-3`), 6.15 (d, 1H, $J_{1`,2`}$ = 8.19 Hz, H-1`), 7.96 (s, 1H, H-5 pyridine), 6.30-7.48 (m, 8H, Ar`H). GCMs m/z: 626(M^+) Anal. Calcd. for $C_{30}H_{27}fN_2O_{10}S$ (626.62) C, 57.50; H,4.34; N, 4.47; S, 5.12%; Found: C, 57.78; H, 4.36; N, 4.49; S, 5.10%.

4.1.2.3. .6-(2-Furanyl)-4(4-chlorophenyl)-2-(2`,3`,4`,6`-tetra-O-acetyl-β-D-glucopyranosylsulfanyl)-nicotinonitrile (5c)

Pale yellow crystals from ethanol, 65%, mp. 226°C, IR (KBr, cm^{-1}): 2214(CN) and 1743 (CO, acetoxy). ^1H NMR: δ = 2.00, 2.01, 2.03 and 2.05 (4s, 12H, 4 CH_3CO), 4.11 (dd, 1H, $J_{6``,5`}$ = 4.23, $J_{6``,6`}$ = 12.20, H-6``), 4.19 (dd, 1H, $J_{6`,5`}$ = 5.67, $J_{6`,6``}$ = 12.20, H-6`), 4.35 (m, 1H, H-5`), 4.98 (t, 1H, $J_{4`,3`}$ = 8.88, $J_{4`,5`}$ = 9.25 Hz, H-4`), 5.31 (t, 1H, $J_{2`,1`}$ = 7.99, $J_{2`,3`}$ = 9.55 Hz, H-2`), 5.63 (t, 1H, $J_{3`,2`}$ = 9.30, $J_{3`,4`}$ = 8.89 Hz, H-3`), 6.10 (d, 1H, $J_{1`,2`}$ = 8.19 Hz, H-1`), 7.96 (s, 1H, H-5 pyridine), 6.30-7.48 (m, 7H, Ar`H). GCMs m/z: 642(M^+). Anal. Calcd. for $C_{30}H_{27}ClN_2O_{10}S$ (643.07) C, 56.03; H,4.23; N, 4.36; S, 4.99%; Found: C, 56.06; H, 4.25; N, 4.34; S, 5.00%.

4.1.2.4. 6-(2-Furanyl)-4-(2-thienyl)-2-(2`,3`,4`,6`-tetra-O-acetyl- β -D-glucopyranosylsulfanyl)-nicotinonitrile (5d)

White crystals from ethanol, 68%, mp. 228-230°C, IR (KBr, cm^{-1}): 2218(CN) and 1747 (CO, acetoxy). ^1H NMR: δ = 1.90, 2.05, 2.06 and 2.08 (4s, 12H, 4 CH_3CO), 4.10 (dd, 1H, $J_{6``,5`}$ = 4.24, $J_{6``,6`}$ = 12.22, H-6``), 4.22 (dd, 1H, $J_{6`,5`}$ = 5.62, $J_{6`,6``}$ = 12.22, H-6`), 4.38 (m, 1H, H-5`), 5.04 (t, 1H, $J_{4`,3`}$ = 8.98, $J_{4`,5`}$ = 9.25 Hz, H-4`), 5.29 (t, 1H, $J_{2`,1`}$ = 8.09, $J_{2`,3`}$ = 9.55 Hz, H-2`), 5.66 (t, 1H, $J_{3`,2`}$ = 9.30, $J_{3`,4`}$ = 8.89 Hz, H-3`), 6.12 (d, 1H, $J_{1`,2`}$ = 8.21 Hz, H-1`), 7.96 (s, 1H, H-5 pyridine), 6.30-7.40 (m, 6H, Ar`H). GCMs m/z: 614(M^+) Anal. Calcd. for $C_{28}H_{26}N_2O_{10}S_2$ (614.65) C, 54.72; H,4.26; N, 4.56; S, 10.43%; Found: C, 54.75; H, 4.30; N, 4.59; S, 10.40%.

4.1.2.5. 6,4-Difuranyl-2-(2`,3`,4`,6`-tetra-O-acetyl-β-D-glucopyranosylsulfanyl)-nicotinonitrile (5e)

Pale yellow crystals from ethanol, 80%, mp. 235°C, IR (KBr, cm^{-1}): 2210(CN) and 1746 (CO, acetoxy). ^1H NMR: 1.87, 2.05, 2.07 and 2.08 (4s, 12H, 4 CH_3CO), 4.13 (dd, 1H, $J_{6``,5`}$ = 4.24, $J_{6``,6`}$ = 12.22, H-6``), 4.20 (dd, 1H, $J_{6`,5`}$ = 5.62, $J_{6`,6``}$ = 12.22, H-6`), 4.32 (m, 1H, H-5`), 5.0 (t, 1H, $J_{4`,3`}$ = 8.98, $J_{4`,5`}$ = 9.25 Hz, H-4`), 5.30 (t, 1H, $J_{2`,1`}$ = 8.09, $J_{2`,3`}$ = 9.55 Hz, H-2`), 5.61 (t, 1H, $J_{3`,2`}$ = 9.30, $J_{3`,4`}$ = 8.89 Hz, H-3`), 6.09 (d, 1H, $J_{1`,2`}$ = 8.21 Hz, H-1`), 7.61 (s, 1H, H-5 pyridine), 7.27-7.91 (m, 6H, Ar`H). GCMs m/z: 598(M^+) Anal. Calcd. for $C_{28}H_{26}N_2O_{11}S$ (598.59) C, 56.18; H,4.38; N, 4.68; S, 5.36%; Found: C, 56.20; H, 4.42; N, 4.70; S, 5.50%.

4.1.2.6. 6-phenyl-4(4-methylphenyl)-2-(2`,3`,4`,6`-tetra-O-acetyl-β-D-glucopyranosylsulfanyl)-nicotinonitrile (5g)

White crystals from ethanol, 60%, mp. 210°C, IR (KBr, cm^{-1}): 2218 (CN) and 1747 (CO, acetoxy). ^1H NMR: δ = 1.75, 2.04, 2.06 and 2.10 (4s, 12H, 4 CH_3CO), 3.2 (s, 3H, CH_3), 4.09 (dd, 1H, $J_{6``,5`}$ = 4.22, $J_{6``,6`}$ = 12.20, H-6``), 4.21 (dd, 1H, $J_{6`,5`}$ = 5.64, $J_{6`,6``}$ = 12.20, H-6`), 4.30 (m, 1H, H-5`), 5.06 (t, 1H, $J_{4`,3`}$ = 8.99, $J_{4`,5`}$ = 9.15 Hz, H-4`), 5.37 (t, 1H, $J_{2`,1`}$ = 8.11, $J_{2`,3`}$ = 9.55 Hz, H-2`), 5.67 (t, 1H, $J_{3`,2`}$ = 9.30, $J_{3`,4`}$ = 8.89 Hz, H-3`), 6.19 (d, 1H, $J_{1`,2`}$ = 8.21 Hz, H-1`), 7.90 (s, 1H, H-5 pyridine), 7.12-7.99 (m, 9H, Ar`H). $^{13}C$ NMR δ = 20.7, 21.0, 21.0 and 21.0 (4 $CH_3CO$), 24.3 ($CH_3$), 65.1(C-6`), 70.6(C-4`), 72.4 (C-2`), 73.8 (C-3`), 74.6(C-5`), (anomeric carbon 86.0(C-1`)), 102.5, 117.0(CN), 117.1, 127.4, 127.3, 129.3, 129.6, 135.0, 136.3, 138.9, 142.9, 154.2, 157.7 (Ar-C), 161.5, 164.1 (C=N), 170.3 (4CO); GCMs m/z: 632(M^+) Anal. Calcd. for $C_{33}H_{32}N_2O_9S$ (632.69) C, 62.65; H, 5.10; N, 4.43; S, 5.07%; Found: C, 62.63; H, 5.00; N, 4.40; S, 5.05%.

4.1.2.7. 6-Naphthyl-4-phenyl-2-(2`,3`,4`,6`-tetra-O-acetyl-β-D-glucopyranosylsulfanyl)-nicotinonitrile (5i)

Pale yellow crystals from ethanol, 76%, mp.163-165°C, IR (KBr, cm^{-1}): 2210(CN) and 1747 (CO, acetoxy). ^1H NMR: δ = 1.75, 2.04, 2.06 and 2.10 (4s, 12H, 4 CH_3CO), 4.0 (dd, 1H, $J_{6``,5`}$ = 4.24, $J_{6``,6`}$ = 12.20, H-6``), 4.24 (dd, 1H, $J_{6`,5`}$ = 5.64, $J_{6`,6``}$ = 12.20, H-6`), 4.35 (m, 1H, H-5`), 4.99 (t, 1H, $J_{4`,3`}$ = 8.79, $J_{4`,5`}$ = 9.20 Hz, H-4`), 5.32 (t, 1H, $J_{2`,1`}$ = 8.18, $J_{2`,3`}$ = 9.55 Hz, H-2`), 5.59 (t, 1H, $J_{3`,2`}$ = 9.30, $J_{3`,4`}$ = 8.89 Hz, H-3`), 6.15 (d, 1H, $J_{1`,2`}$ = 8.21 Hz, H-1`), 7.56 (s, 1H, H-5 pyridine), 7.27-8.55 (m, 12H, Ar`H). GCMs m/z: 668(M^+) Anal. Calcd. for $C_{36}H_{32}N_2O_9S$ (668.73) C, 64.66; H,4.82; N, 4.19; S, 4.80%; Found: C, 64.69; H, 4.88; N, 4.20; S, 5.00%.

4.1.2.8. 6-naphthyl-4-(2-thienyl)-2-(2`,3`,4`,6`-tetra-O-acetyl-β-D-glucopyranosylsulfanyl)-nicotinonitrile (5j)

Yellow crystals from ethanol, 60%, mp. 220°C, IR (KBr, cm^{-1}): 2218(CN) and 1747 (CO, acetoxy). ^1H NMR: δ = 1.85, 2.04, 2.06 and 2.10 (4s, 12H, 4 CH_3CO), 4.12 (dd, 1H, $J_{6``,5`}$ = 4.26, $J_{6``,6`}$ = 12.22, H-6``), 4.28 (dd, 1H, $J_{6`,5`}$ = 5.60, $J_{6`,6``}$ = 12.22, H-6`), 4.39 (m, 1H, H-5`), 5.11 (t, 1H, $J_{4`,3`}$ = 8.98, $J_{4`,5`}$ = 9.20 Hz, H-4`), 5.35 (t, 1H, $J_{2`,1`}$ = 7.98, $J_{2`,3`}$ = 9.55 Hz, H-2`), 5.69 (t, 1H, $J_{3`,2`}$ = 9.30, $J_{3`,4`}$ = 8.89 Hz, H-3`), 6.21 (d, 1H, $J_{1`,2`}$ = 8.04 Hz, H-1`), 7.90 (s, 1H, H-5 pyridine), 7.20-8.05 (m, 10H, Ar`H). $^{13}C$ NMR δ = 20.7, 21.0, 21.5, 21.7(4 $CH_3CO$), 62.1(C-6`), 68.8(C-4`), 69.8(C-3`), 70.4(C-2`), 73.6(C-5`), (anomeric carbon 85.0(C-1`), 103, 117.0 (CN), 117.7, 124.6, 125.5, 125.9, 126.2, 127.6, 127.9, 128.1, 128.6, 132.4, 134.3, 135.8, 138.3, 146.4, 158.9 (Ar-C), 164.2 (C=N), 170.3

(4CO); GCMs m/z: 674(M^+) Anal. Calcd. for $C_{34}H_{30}N_2O_9S_2$ (674.75) C, 60.52; H,4.48; N, 4.15; S, 9.50%; Found: C, 60.50; H, 4.46; N, 4.14; S, 9.30%.

4.1.3 General procedure for deprotection

Method A. A mixture of 5a-e, 5g, 5i,j (2 mmole), and KOH (3 mmole) was thoroughly grounded with a pestle in an open mortar at 25 ^oC for 3-5 min until the mixture turned into a melt. 2-3 Drops of water were added and the initial syrupy reaction mixture solidified within 4-6 min. Grinding continued for further 5-15 mintues and the reaction was monitored by TLC. The solid washed with water and recrystallized from ethanol to give 6a-e 6g, 6i,j respectively.

Method B. Triethylamine (0.5 ml) was added to a solution of glycoside 5a-e, 5g, 5i,j (2 mmol) in MeOH (7-10 ml) and 2 drops of water. The mixture was stirred overnight at room temperature, evaporated under reduced pressure and the residue was crystallized from ethanol to give 6a-e 6g, 6i,j respectively.

4.1.3.1. 6-(2-Furanyl)-4-phenyl-2-(6`-acetyl- β -O-D-glucopyranosylsulfanyl)-nicotinonitrile (6a).

Yellow crystals from ethanol, (73% method A, 60% method B) mp. 230^oC, IR (KBr, cm^{-1}): 3414 (broad, 3OH), 2218(CN) and 1747 (CO, acetoxy). ^1H NMR: δ = 2.10 (s, 3H, CH_3CO), 3.49-4.34 (m, 6 H, H-6`,H-6``,H-5`,H-4`,H-3`and H-2`), 4.72 (d, 1H $J_{1`,2`}$ = 8.22 Hz,, H-1`), 7.96 (s, 1H, H-5 pyridine), 6.3-7.48 (m, 8H, Ar`H), 10.98-11.39 (m, 3H, 3OH). ^{13}C NMR δ = 20.7(CH_3), 62.1(C-6`), 71.0(C-4`), 74.2(C-2`), 75.7(C-3`), 77.0(C-5`), 88.4(C-1`), 102, 105, 107.2, 117.0, 117.1, 127.4, 129.3, 138.0, 142.9, 154.2, 157.7 (Ar-C), 161, 164.2 (C=N), 170 (CO). GCMs m/z: 482(M^+) Anal. Calcd. for $C_{24}H_{22}N_2O_7S$ (482.52) C, 59.74; H,4.60; N, 5.81; S, 6.65%; Found: C, 59.60; H, 4.56; N, 5.79; S, 6.60%.

4.1.3.2. 6-(2-Furanyl)-4(4-fluorophenyl)-2-(6`-acetyl-O-β-D-glucopyranosylsulfanyl)-nicotinonitrile (6b).

Yellow crystals from ethanol, (68% method A, 55% method B), mp. 247^oC, IR (KBr, cm^{-1}): 3124 (broad, 3OH) 2214(CN) and 1723(CO). ^1H NMR: δ = 2.11 (s, 3H, CH_3CO), 3.38-3.98 (m, 6 H, H-6`,H-6``,H-5`,H-4`,H-3`and H-2`), 4.68 (d, 1H $J_{1`,2`}$ = 8.22 Hz,, H-1`), 7.96 (s, 1H, H-5 pyridine), 6.3-7.46 (m, 7H, Ar`H), 11.37-11.45 (m, 3H, 3OH). ^{13}C NMR δ = 20.7(CH_3), 62.2(C-6`), 71.0(C-4`), 74.2(C-2`), 75.7(C-3`), 77.0(C-5`), 88.4(C-1`), 102.5, 105, 107.2, 116.0, 117.0 (CN), 117.1, 129.0, 133.6, 142.9, 154.2, 157.7, 163.4 (Ar-C), 161.5, 164.2 (C=N), 170.3 (CO). GCMs m/z: 500(M^+) Anal. Calcd. for $C_{24}H_{21}FN_2O_7S$ (500.51) C, 57.60; H,4.23; N, 5.60; S, 6.41%; Found: C, 57.55; H, 4.30; N, 5.59; S, 6.45%.

4.1.3.3. 6-(2-Furanyl)-4(4chlorophenyl)-2-(6`-acetyl-O-β-D-glucopyranosylsulfanyl)-nicotinonitrile (6c).

Yellow crystals from ethanol, (69% method A, 55% method B), mp. 226-230^oC, IR (KBr, cm^{-1}): 3433 (3OH), 2214(CN) and 1697 (CO). GCMs m/z: 517(M^+) Anal. Calcd. for $C_{24}H_{22}N_2O_7S$ (516.96) C, 55.76; H,4.09; N, 5.42; S, 6.20%; Found: C, 55.60; H, 4.10; N, 5.40; S, 6.00%.

4.1.3.4. 6-(2-Thienyl)-4-(2-furanyl)-2-(6`-acetyl-O-β-D-glucopyranosylsulfanyl)-nicotinonitrile (6d).

Yellow crystals from ethanol, (75% method A, 65% method B) mp. 215^oC, IR (KBr, cm^{-1}): 3415 (broad, 3OH), 2210(CN) and 1737 (CO). ^1H NMR: δ = 2.17 (s, 3H, CH_3CO),), 3.54-4.18 (m, 6 H, H-6`,H-6``,H-5`,H-4`,H-3`and H-2`), 4.61 (d, 1H $J_{1`,2`}$ = 8.22 Hz,, H-1`), 7.96 (s, 1H, H-5 pyridine), 6.30-7.40 (m, 6H, Ar`H), 9.11(d, J = 4.39 Hz, 1 H, OH-4`), 9.24(d, 1H, J = 5.26 Hz, OH-3`), 9.51(d, 1H, J = 4.96 Hz, OH-2`). GCMs m/z: 488(M^+) Anal. Calcd. for $C_{22}H_{20}N_2O_7S_2$ (488.54) C, 54.09; H,4.13; N, 5.73; S, 13.13%; Found: C, 54.10; H, 4.15; N, 5.75; S, 12.90%.

4.1.3.5. 6,4-Difuranyl-2-(6` acetyl-O- β -D-glucopyranosylsulfanyl)-nicotinonitrile (6e)

Yellow crystals from ethanol, (90% method A, 80% method B), mp. >300^oC, IR (KBr, cm^{-1}):
3166(Broad, 3OH), 22220 (CN) and 1743 (CO). GCMs m/z: 472(M^+) Anal. Calcd. for $C_{22}H_{20}N_2O_8S$ (472.48) C, 55.93; H,4.27; N, 5.93; S, 6.79%; Found: C, 56.00; H, 4.25; N, 5.89; S, 6.80%.

4.1.3.6. 6-phenyl-4(4-methylphenyl)-2-(6`-acetyl-O- β -D-glucopyranosylsulfanyl)-nicotinonitrile (6g)

White crystals from ethanol, (90% method A, 85% method B), mp. 220-223^oC, IR (KBr, cm^{-1}): 3414 (Broad, 3OH), 2222 (CN) and 1747 (CO). ^1H NMR: δ = 2.35 (s, 3H, CH_3CO), 3.13 (s, 3H, CH_3), 3.24-3.95 (m, 6 H, H-6`,H-6``,H-5`,H-4`,H-3`and H-2`), 4.81 (d, 1H $J_{1`,2`}$ = 8.22 Hz,, H-1`), 7.90 (s, 1H, H-5 pyridine), 7.12-7.99 (m, 9H, Ar`H), 9.81 (d, J = 4.29 Hz, 1 H, OH-4`), 10.11 (d, 1H, J = 5.24 Hz, OH-3`), 10.38 (d, 1H, J = 4.96 Hz, OH-2`). $^{13}C$ NMR δ = 20.7($CH_3$), 24.3 ($CH_3$), 62.2(C-6`), 71.0(C-4`), 74.2(C-2`), 75.7(C-3`), 77.0(C-5`), 88.4(C-1`), 102.5, 117.0, 117.1, 127.5, 129.6, 135.0, 136.3, 138.9, 154.2, 158.8 (Ar-C), 164.1 (C=N), 170(CO). GCMs m/z: 506 Anal. Calcd. for $C_{27}H_{26}N_2O_6S$ (506.58) C, 64.62; H, 5.17; N, 5.53; S, 6.33%; Found: C, 64.63; H, 5.10; N, 5.40; S, 6.25%.

4.1.3.7. 6-Naphthyl-4-phenyl-2-(6`-acetyl-O- β -D-glucopyranosylsulfanyl)-nicotinonitrile (6i)

Pale yellow crystals from ethanol, (65% method A, 54% method B), mp.205-208^oC, IR (KBr, cm^{-1}): 3136(3OH), 2214(CN) and 1739 (CO, acetoxy). ^1H NMR: δ = 2.26 (s, 3H, CH_3CO), 3.30-3.95 (m, 6 H, H-6`,H-6``,H-5`,H-4`,H-3`and H-2`), 4.77 (d, 1H $J_{1`,2`}$ = 8.20 Hz,, H-1`), 7.90 (s, 1H, H-5 pyridine), 7.12-7.99 (m, 9H, Ar`H), 9.38 (d, J = 4.27 Hz, 1 H, OH-4`), 9.83 (d, 1H, J = 5.31 Hz, OH-3`), 10.17 (d, 1H, J = 4.96 Hz, OH-2`). GCMs m/z: 542(M^+) Anal. Calcd. for $C_{30}H_{26}N_2O_6S$ (542.62) C, 66.41; H,4.83; N, 5.16; S, 5.91%; Found: C, 66.54; H, 4.84; N, 5.10; S, 5.80%.

4.1.3.8. 6-naphthyl-4-(2-thienyl)-2-(6`-acetyl-O- β -D-glucopyranosylsulfanyl)-nicotinonitrile (6j)

Yellow crystals from ethanol, (88% method A 79% method B), mp. 237-238^{o}C, IR (KBr, cm^{-1}): 3386 (Broad, 3OH), 2214(CN) and 1743 (CO). ^{1}H NMR: δ = 2.26 (s, 3H, CH_3CO), 3.49-4.11 (m, 6 H, H-6`,H-6``,H-5`,H-4`,H-3`and H-2`), 4.69 (d, 1H $J_{1`,2`}$ = 8.22 Hz,, H-1`), 7.90 (s, 1H, H-5 pyridine), 7.12-7.99 (m, 9H, Ar`H), 7.90 (s, 1H, H-5 pyridine), 7.20-8.01 (m, 10H, Ar`H), 10.1 (d, J = 4.27 Hz, 1 H, OH-4`), 10.37 (d, 1H, J = 5.36 Hz, OH-3`), 10.83 (d, 1H, J = 4.98 Hz, OH-2`).

4.2. Antimicrobial activities

We determined the minimum inhibitory concentration (MIC) of the synthesized compounds by using measuring the turbidimetry of bacterial growth present in liquid culture using spectrophotometer [19]. We prepared the pre-cultures of the tested bacteria by inoculating 10 mL of Luria-Bertani (LB) and incubating for 24 h at 37^{o} C. Then cultures were kept in fridge at 4^{o} C for preservation. 1 ml DMSO was used to dissolve the assayed compounds. The stock was prepared with concentration 20 mg/ml DMSO. All required different concentrations were obtained from the stock that was prepared as mentioned. The used concentrations were as following: 1, 5, 10, 15 and 20 mg/ml DMSO. For each assayed compound, three LB agar plates (for each bacterial strain) were inoculated with 10 µl of the bacterial suspension. The bacterial suspension was spread on the medium using an autoclaved L-shaped glass rod. A well was made using an autoclaved cork poorer. The following concentrations were used: 0.1, 0.25, 0.5, 0.75, 1, 5, 10, 15 and 20 mg/ml DMSO, were used to determine the preliminary concentration that could be used in the qualitative test. 100 µl of the assayed compounds was poured into the well. The concentration of the compounds used was 20 mg/ml DMSO. Then, the plates were incubated at 37^{o} C for 24 hours. Inhibition zone around wells were recorded whenever occurred. Compounds that proved antimicrobial effect on both or at least one of the used bacterial strains were used in this assay. Dilutions of 1, 5, 10, 15 and 20 mg/ml DMSO were prepared from the stock. The diluted tubes were completed up to 10 ml with LB liquid medium. 10 µl of the bacterial suspension was added to diluted tubes. Three tubes were used for each chemical against one bacterial strain as replicates. The tubes were then incubated at 37^{o} C for 24 hours and the bacterial growth was measured.

5. References

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