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Original article

Synthesis, antitumor and antimicrobial activities of 4-(4-chlorophenyl)-3-cyano-2-(β-*O*-glycosyloxy)-6-(thien-2-yl)-nicotinonitrile

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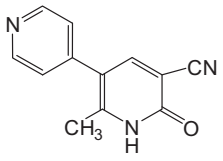
ABSTRACT

4-(4-Chlorophenyl)-3-cyano-6-(thien-2-yl)-1*H*-pyridin-2-one (**2**) was obtained by reaction of 2-acetyl thiophene with 4-chlorobenzaldehyde and ethyl cyanoacetate in presence of ammonium acetate or by the reaction of α,β-unsaturated compound **1** with ethyl cyanoacetate in the presence of ammonium acetate. 4-(4-Chlorophenyl)-2-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-gluco/galactopyranosyloxy)-6-(thien-2-yl) nicotinonitrile (**5a** and **5b**), riboside **11**, xyloside **12** and lactoside **16** were prepared by the reaction of **2** with glycosyl/galactosyl/xylosyl/lactosyl bromide and peracetylated xylose/ribose under the conventional and microwave irradiation methods. The reaction has regioselectively gave the *O*-glycosides and not the *N*-glycosides. The glycosides **5a**,**b**, riboside **11**, xyloside **12** and lactoside **16** were deacetylated in the presence of Et_3N/MeOH and few drops of water to give **7a**,**b**, **13**, **14** and **17**. The structure of the new synthesized compounds was confirmed by using IR, ^1H, ^{13}C NMR spectra and microanalysis. Selected members of these compounds were screened for antitumor and antibacterial activity.

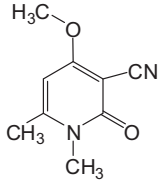
1. Introduction

Pyridine derivatives have shown a broad spectrum of biological activities [1–3]. Pyridine derivatives and their nucleoside analogs showed strong cytotoxicity against several human cancer cell [4,5], potent and selective farnesyltransferase inhibitions [6] and inhibitors of HCV NS5B polymerase inhibitions [7], as well as antimicrobial [8], and antimycobacterial activities [9]. The pharmacological and physiological activity of 3-cyanopyridin-2(1*H*)-ones has attracted much attention in recent years. Thus the non-glycosidic cardiotonic agent milrinone (**I**) (Fig. 1) [10,11] and other pyridine derivatives proved to be active against herpes and the human immunodeficiency virus [12–15]. The 3-cyanopyridin-2-(1*H*)-one nucleus is also the structural basis of the alkaloid ricinine (**II**) (Fig. 1) [16]. Moreover, the thiophene nucleus has constituted the active part of several biologically active compounds [17–21].

Several glycosides have exhibited good biological inhibitions [22–29], inducers and ligands [30], in addition of having excellent chemoselectivity in glycosylation processes as donors and acceptors [31]. Having the above aspects in mind and a continuation of our work on the synthesis of glycosides and nucleosides with having biological activity [32–34], glycosylative have planned to target a group of pyridine compounds functionalized with cyano group, thiophene and *p*-chlorophenyl rings. The presence of glycosyl residue is expected to enhance the biological activity. The antitumor and antimicrobial activities of the namely synthesized compounds have been established.



milrinone
(I)



ricinine
(II)

Fig. 1.

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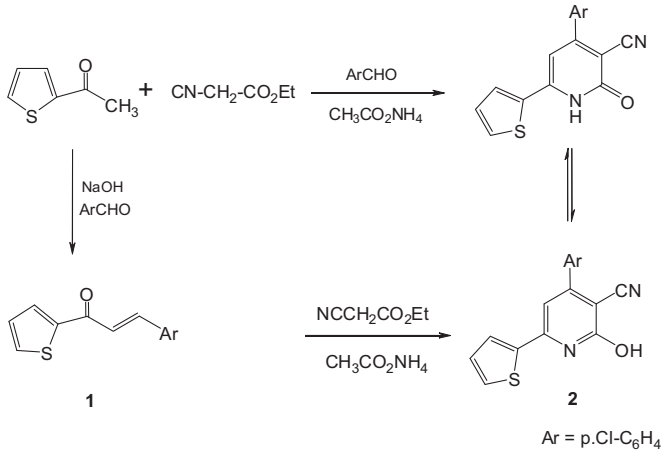
2. Chemistry

4-(4-Chlorophenyl)-3-cyano-6-(thien-2-yl)-pyridin-2(1*H*)-one (**2**) selected as a starting material for this study. It was obtained from the condensation of the 2-acetyl thiophene and 4-chlorobenzaldehyde in presence of sodium hydroxide to give **1** whose heterocyclization with ethyl cyanoacetate in presence of ammonium acetate gave **2** (Scheme 1) [35]. Alternatively, **2** was obtained in a better yield by an one pot synthesis of the same reactants [35].



Scheme 1. Synthesis of pyridin-2(1H)-one.

The IR spectrum of **2** showed bands at 1687, 2216 and 3212 cm^{-1} indicating the presence of C=O, C≡N and NH groups respectively. Glycosylation of pyridin-2(1*H*)-one **2** with 1.1 M equivalent of 2,3,4,6-tetra-*O*-acetyl-α-D-gluco- or galactopyranosyl bromide (**3** and **4**) in anhydrous DMF/acetone and potassium carbonate afforded 4-(4-chlorophenyl)-2-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-6-(thien-2-yl)nicotinonitrile (**5a**) and 4-(4-chlorophenyl)-2-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-6-(thien-2-yl)nicotinonitrile (**5b**), respectively (Scheme 2).

The structure of compounds **5a** and **5b** were based on the spectroscopic data. Thus, the β-configuration of compounds **5a** and **5b** were supported by their ^{1}H NMR spectra, which revealed the anomeric proton as doublet at δ 6.47 and 6.36 ppm with coupling constant J = 8.20 and 8.40 Hz, respectively, corresponding to a diaxial orientation of H-1′and H-2′. The formation of *O*-glycosides **5a** and **5b** and not the corresponding *N*-glycosides **6a** and **6b** (Scheme 2) were proved by ^{13}C NMR spectroscopy, which revealed the presence of the anomeric carbons at δ 93.8 and 94.7 ppm. The absence of a carbonyl amide band at 1687 cm^{-1} indicated that glycosylation had taken place on the oxygen to give **5a** and **5b** and not on the nitrogen to give **6a** and **6b**, respectively. This preference can be due to the formation of the respective mesomeric anion of **2** that promote the reaction with electrophiles reagent to give the *O*-glycosides.

4-(4-Chlorophenyl)-2-(2′,3′,5′-tri-*O*-acetyl-β-D-xylofuranosyloxy)-6-(thien-2-yl)nicotinonitrile (**11**) was obtained by the reaction of 2,3,5-tri-*O*-α-D-xylofuranosyl bromide (**8**) with pyridin-2(1*H*)-one **2** under the conditions or with peracetylated xylose (**9**) under microwave irradiation using silica gel as a solid support [32,36] (Scheme 3). Similarly pyridin-2(1*H*)-one **2** was reacted with peracetylated ribose **10** under microwave irradiation [32,36] to give the ribofuranosyl derivative **12** (Scheme 3).

The ^{1}H NMR spectra of **11** and **12** showed signals at δ 2.10–2.25 ppm for the acetoxy groups and a doublet in the range at δ 6.79–6.63 ppm characteristic for the anomeric protons with coupling constant J = 3.6–3.8 Hz, which confirmed the β-configuration. Their IR spectra showed the absence of the amide carbonyl band which indicated the formation of the *O*-glycoside and not the *N*-glycoside. ^{13}C NMR spectrum of **11** showed signal at δ 99.9 ppm consistent with the anomeric carbon.

Deacetylation of compounds **5a**, **5b**, **11** and **12** (Schemes 2 and 3), in the presence of methanol/Et_3N and few drops of water, led to the formation of the free glycosides **7a**, **7b**, **13** and **14**. The ^{1}H NMR data of these latter compounds revealed the absence of the acetyl groups at δ 1.96–2.25 ppm and the appearance of the D_2O exchangeable OH protons at δ 3.99–5.44 ppm. Their IR spectra indicated the presence of broad band at 3423 cm^{-1} for OH groups.

Furthermore reaction of pyridin-2-(1*H*)-one **2** with 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl bromide (**15**) in the presence of potassium carbonate gave the respective *O*-lactoside **16**, which was deacetylated in the presence of methanol/Et_3N and few drops of water to give the corresponding deprotected lactoside **17** (Scheme 4).

The ^{1}H NMR spectrum of **16** showed signals at δ 1.90–2.10 ppm for the seven acetoxy groups and a doublet at δ 6.35 ppm characteristic for the anomeric proton with coupling constant $J_{1'a, 2'a}$ = 8.7 Hz, which confirmed the β-configuration. Its IR spectrum showed the absence of the amide carbonyl group which characteristic the formation of the *O*-glycoside and not *N*-glycoside. ^{1}H NMR spectrum of compound **17** revealed the absence of the acetyl groups and the appearance of OH protons at δ 4.40–5.29 ppm. Its ^{13}C NMR spectrum indicated the absence the acetoxy groups and the presence of the two anomeric carbons (C-1′b) and (C-1′a) at δ 97.3 and 99.3 ppm, respectively.

3. Pharmacological studies

3.1. Anticancer activity

3.1.1. Cell culture

All cells were routinely cultured in DMEM (Dulbeco's Modified Eagle's Medium) at 37 °C in humidified air containing 5% CO_2. Media were supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 units/mL penicillin G sodium, 100 units/mL streptomycin sulfate, and 250 mg/mL amphotericin B. Monolayer cells were harvested by trypsin/EDTA treatment, while leukemia cells were harvested by centrifugation. All experiments were repeated four times, unless mentioned, and the data was represented as (mean ± S.D.). Cell culture material was obtained from Cambrex BioScience (Copenhagen, Denmark) and all chemicals were from Sigma (USA).

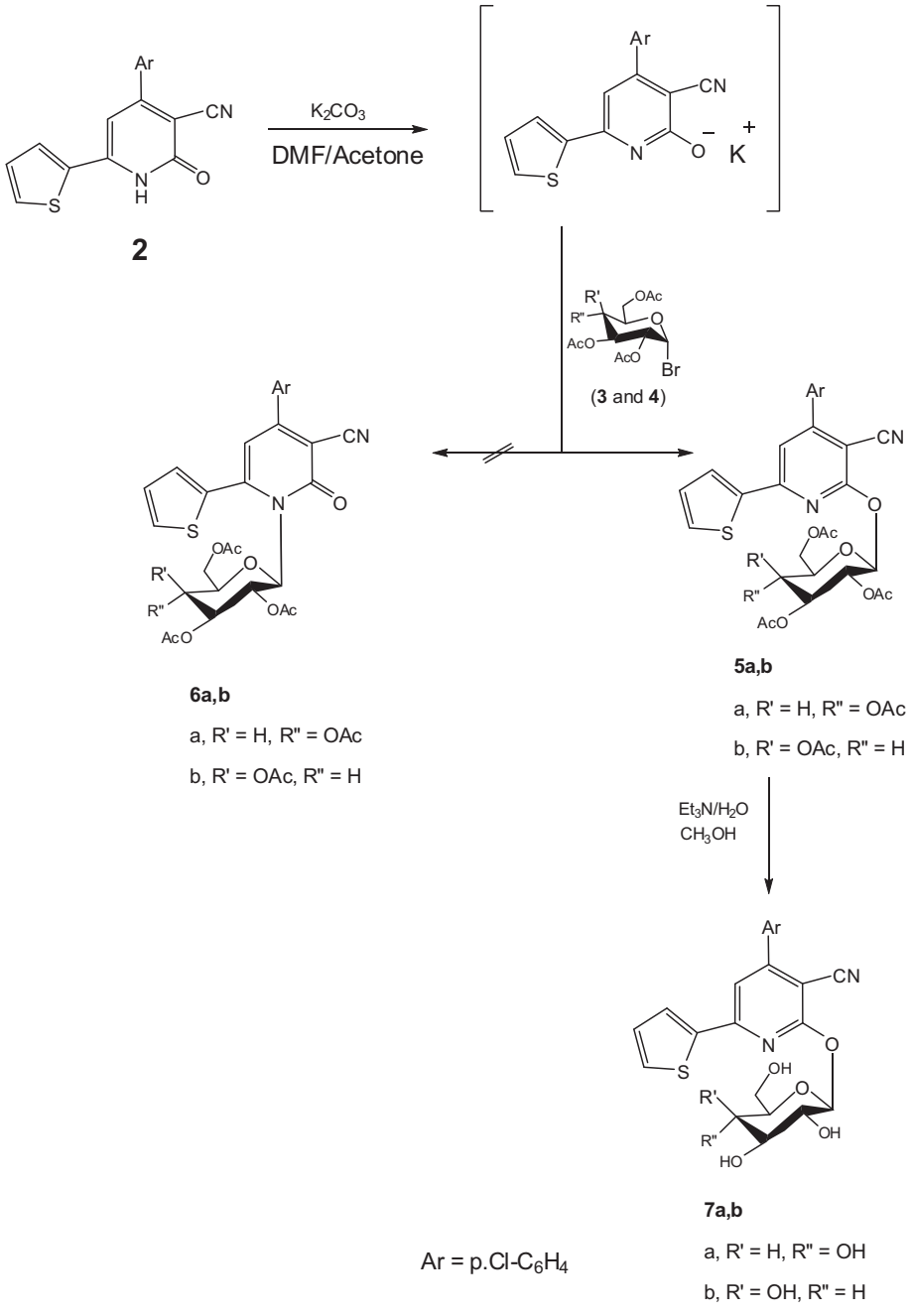
3.1.2. Cytotoxicity assay

Cytotoxicity of tested samples against different types of cells was measured using the MTT Cell Viability Assay. MTT (3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay is based on the ability of active mitochondrial dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT and form a dark blue insoluble formazan crystals which is largely impermeable to cell membranes, resulting in its accumulation within healthy cells. Solubilization of the cells results in the liberation of crystals, which are then solubilized. The number of viable cells is directly proportional to the level of soluble formazan dark blue color. The extent of the reduction of MTT was quantified by measuring the absorbance at 570 nm [37].

3.1.3. Reagents preparation

MTT solution: 5 mg/mL of MTT in 0.9% NaCl.

Acidified isopropanol: 0.04 N HCl in absolute isopropanol.



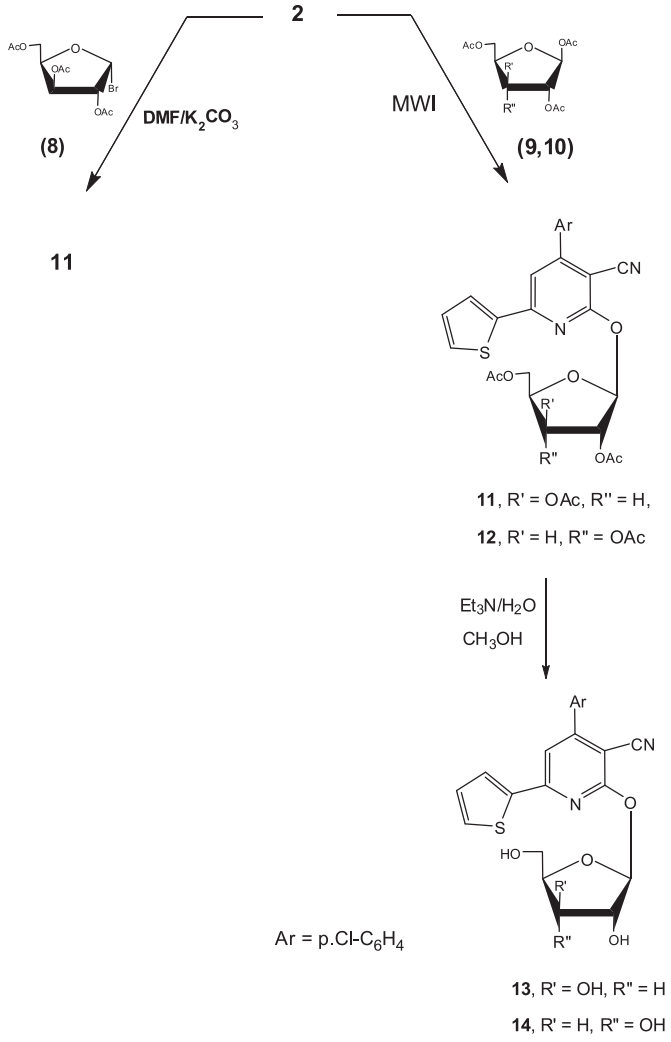
Scheme 2. Glycosylated and galactosylated analogs.

3.1.4. Procedure

Cells (0.5×10^5 cells/well) in serum-free media were plated in a flat bottom 96-well microplate, and treated with 20 μl of different concentrations of each tested compound for 48 h at 37 °C, in a humidified 5% CO_2 atmosphere. After incubation, media were removed and 40 μl MTT solution/well were added and incubated for an additional 4 h. MTT crystals were solubilized by adding 180 μl of acidified isopropanol/well and plate was shacked at room temperature. This has been followed by photometric determination of the absorbance at 570 nm using microplate ELISA reader. Triplicate measures were performed for each concentration and the average was calculated. Data were expressed as the percentage of relative viability compared with the untreated cells.

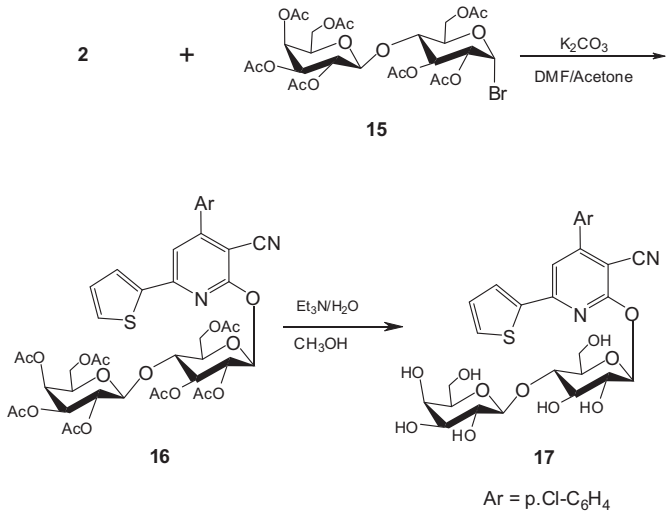
3.1.5. Results

Chemotherapy is a major therapeutic approach for the both localized and metastasized cancers. Seven selected newly synthesized compounds **5a**, **5b**, **7a**, **11**, **12**, **14** and **16** were tested for cytotoxic activity against the MCF_7 (breast carcinoma cell line) in comparison to the known anticancer drugs: 5-Fluorouracil and Doxorubicin as reference drugs. All tested new compounds dissolved in DMSO in different concentrations (25, 50 and 100 μg/mL). The tested compounds **7a** and **14** were proven to have no cytotoxic activity against the MCF_7 at all drug concentrations (see Table 1). The 4-(4-chlorophenyl)-2-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-6-(thien-2-yl)nicotinonitrile (**5a**), 4-(4-chlorophenyl)-2-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyloxy)-6-(thien-2-yl)nicotinonitrile (**12**) and 4-(4-chlorophenyl)-2-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-



Scheme 3. Ribosyl and xylosyl derivatives.

galactopyranosyl-(1 → 4)-(2′,3′,4′,6′-tetra-*O*-acetyl-*β*-D-glucopyranosyloxy-6-(thiophen-2-yl)nicotinonitrile (**16**), have showed higher antitumor activities compared to the reference drug (5-Fluorouracil) while moderate compared with (Doxorubicin). This



Scheme 4. Lactosyl derivatives.

Table 1
Effect of selected 2-*O*-glycosides nicotinonitrile on MCF_7 tumor cell lines.

Compound	%Inhibition of cell viability (MCF_7) (breast carcinoma cell line)		
	100 μg/mL	50 μg/mL	25 μg/mL
5a	10%	5%	0%
5b	20%	5%	0%
7a	0%	0%	0%
11	8%	0%	0%
12	12%	7%	2%
14	0%	0%	0%
16	10%	5%	0%
5-Fluorouracil (**5-Fu**)	7%	0%	0%
Doxorubicin (**Dox**)	50%	15%	6%

activity explained by the presence of the 4-chlorophenyl and acetoxy groups in sugar moiety provided a good affinity toward the enzyme on account of force of electrostatic attraction between the planar 4-chlorophenyl and electronegativity of acetoxy groups with the target site pocket of the tumor cells. The antitumor activity of 4-(4-chlorophenyl)-2-(2′,3′,5′-tri-*O*-acetyl-*β*-D-xylofuranosyloxy)-6-(thien-2-yl)nicotinonitrile (**11**) against MCF_7 has higher activity at high concentration (100 μg/mL) compared to 5-Fluorouracil and low with Doxorubicin, but lower activity at low concentrations (25 and 50 μg/mL). Compound 4-(4-chlorophenyl)-2-(2′,3′,4′,6′-tetra-*O*-acetyl-*β*-D-galactopyranosyloxy)-6-(thien-2-yl)nicotinonitrile (**5b**) exhibited increase in the antitumor activity against MCF_7 at high concentration (100 μg/mL) compared to 5-Fluorouracil and significant activities compared to Doxorubicin and very low effect at concentration (50 μg/mL). All of the tested derivatives showed a significant and higher antitumor activity at high concentration (100 μg/mL) against MCF_7 (breast carcinoma cell line) compared to 5-Fluorouracil and have a significant and moderate activity compared to Doxorubicin.

3.2. Antimicrobial activity

The antimicrobial activities of some synthesized compounds were screened for their antibacterial activity against four species of bacteria, namely *Staphylococcus aureus* and *Bacillus subtilis* as Gram-positive bacteria as well as *Escherichia coli* and *Pseudomonas aeuroginosa* as Gram-negative bacteria, using a cup plate agar diffusion method [38]. The tested compounds were dissolved in dimethyl sulfoxide to get of 1 μg/mL concentration. The inhibition zone were measured in mm at the end of an incubation period of 48 h at 37 °C. Dimethyl sulfoxide showed no inhibition zones. Ampecillin was used as a reference to evaluate the potency of tested compounds.

The newly synthesized glycosides **5a**,**b**, **7a**,**b**, **12**, **13**, **14** and **17** were tested for their *in vitro* antibacterial activity against a panel of standard strains of the Gram-positive bacteria (*S. aureus* and *B. subtilis*) and the Gram-negative bacteria (*E. coli* and *P. aeuroginosa*). Compounds **5b**, **12** and **14** showed higher antibacterial activity than

Table 2
Antimicrobial activity of tested compounds (Inhibition zones mm, Minimum Inhibitory Concentration μg/mL).

Compound No.	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeuroginosa*
5a	8	11	3	13
5b	12	10	5	26
7a	3	1	0	0
7b	0	0	0	0
12	9	8	3	25
13	3	3.5	2	9
14	10	7	6	28
17	5	3	0	10
Ampecillin	7	6	2	23

the standard drug (ampecillin). Compound **5a** showed moderate antibacterial activity against Gram (+ve) bacteria and lower activity against Gram (−ve) bacteria comparing to ampecillin. While compounds **7a** and **17** showed lower activity against Gram (+ve) bacteria and inactive against Gram (−ve) bacteria. Compound **13** showed lower activity against Gram (+ve) and Gram (−ve) bacteria compared to standard drug. Compound **7b** did not show any activity against tested micro-organisms as shown in Table 2.

4. Conclusions

The glycosylation of pyridin-2-(1*H*)-one **2** gave the respective glycosyloxy derivatives **5a**, **5b**, **11**, **12**, **16** and not the respective nucleosides. The *in vitro* growth inhibitory activities of **5a**, **5b**, **7a**, **11**, **12**, **14** and **16** against (MCF_7) cell lines revealed significant potential antitumor activity. Best results were gained by compound **5b** at higher used concentration (100 μg/mL), compared to 5-Fluorouracil and Doxorubicin as known anticancer reference drugs. Although significant results were obtained when evaluation the antibacterial activity of glycosides **5a**,**b**, **7a**,**b**, **12**, **13**, **14** and **17**.

5. Experimental

5.1. Chemistry

All melting points are uncorrected and were measured using an Electro thermal IA 9100 apparatus. TLC was performed on Merck Silica Gel $60F_{254}$ with detection by UV light and by charring after seperating with 10% EtOH solution of H_2SO_4. The IR spectra (KBr disc) were recorded on a Pye Unicam Sp-3-300 or a Shimadzu FTIR 8101 PC infrared spectrophotometer. The ^1H and ^{13}C NMR spectra were determined with JEOL-JNM-LA 300 MHz spectrometer. The chemical shifts are expressed on the δ (ppm) scale using TMS as the standard reference. Elemental analysis determined on a Perkin Elmer 240 (microanalysis). The antitumor activity was performed at Pharmacology unit, National Cancer Institute, Cairo University, Cairo, Egypt. Antibacterial activity was carried out in Microbiological Center at Faculty of Science, Zagazig University, Egypt.

5.2. 4-(4-Chlorophenyl)-3-cyano-6-(thien-2-yl)-1H-pyridin-2-one (*2*)

General procedure: **Method A**: A mixture of chalcone **1** (0.01 mol), ethyl cyanoacetate (0.01 mol) and ammonium acetate (0.08 mol) in ethanol (40 mL) was refluxed for 10 h. The precipitate was filtered off and crystallized from absolute ethanol or acetic acid.

Method B: A mixture of 2-acetyl thiophene (0.01 mol), 4-chlorobenzaldehyde (0.01 mol), ethyl cyanoacetate (0.01 mol), and ammonium acetate (0.08 mol), in ethanol (40 mL) was refluxed for 3 h. The obtained precipitate was filtered off, dried and crystallized as above. **Method A**: yield 30%, **method B**: yield 52%, as yellow crystals, mp 295–297 °C. Anal. Calcd. for $C_{16}H_9ClN_2OS$ (312.77): C, 61.44; H, 2.90; N, 8.96. Found: C, 61.23; H, 3.06; N, 8.95. IR (KBr, cm^{-1}): 3212 (NH), 2216 (C≡N) and 1687 (C=O, amide). ^1H NMR (DMSO-d_6, δ ppm): 7.25 (t, 1H, J = 4.50, 4.2 Hz, thiophene-H), 7.60 (d, 2H, J = 8.70 Hz, Ar-H), 7.65 (d, 2H, J = 8.70 Hz, Ar-H), 7.73 (s, 1H, pyridone-H-5), 7.88 (d, 1H, J = 4.50 Hz, thiophene-H), 8.06 (d, 1H, J = 4.20 Hz, thiophene-H), 12.85 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 116.6 (C≡N), 128.1, 128.9, 129.0, 129.3, 129.4, 130.2, 130.5, 131.0, 132.3, 132.6, 132.7, 135.6 (Ar-C) and 160.4 (C=O).

5.3. General procedure for synthesis of glycosides

Method A: A mixture of pyridin-2-(1*H*)-one **2** (0.01 mol) and (0.01 mol) potassium carbonate was stirred in dry acetone/DMF (15 mL) for 1 h, then glycosyl, galactosyl, xylosyl and lactosyl bromide (0.011 mol) was added. The reaction mixture was stirred at room temperature overnight then refluxed for 3–5 h, filtered off and the solvent was then evaporated under reduced pressure. The product was dried and crystallized from the proper solvent or chromatographed on silica gel column.

Method B: A mixture of pyridin-2-(1*H*)-one (**2**) (0.01 mol) and (0.01 mol) of peracetylated sugar, were dissolved in ethanol/$CHCl_3$ (70/30) then 1.0 g of silica gel (200–400 mesh) was added. The solvent was removed by evaporation. The dried residue was transferred into a glass beaker and irradiated (5 min) in a domestic microwave oven. The product was chromatographed on a silica gel column.

5.3.1. 4-(4-Chlorophenyl)-2-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranos-yloxy)-6-(thien-2-yl)nicotinonitrile (*5a*)

Pale yellow crystals from ethanol, **Method A**: yield 59%, mp 180–182 °C. Anal. Calcd. for $C_{30}H_{27}ClN_2O_{10}S$ (643.06): C, 56.03; H, 4.23; N, 4.36; Found: C, 55.89; H, 4.16; N, 4.40. IR (KBr, cm^{-1}): 2229 (C≡N) and 1750 (C=O, acetoxy). ^1H NMR (DMSO-d_6, δ ppm): 1.96, 1.99, 2.00 and 2.03 (4s, 12H, 4 CH_3CO), 4.05 (dd, 1H, $J_{5',6'}$ = 4.67, $J_{6',6''}$ = 12.22 Hz, H-6′), 4.20 (dd, 1H, $J_{5',6''}$ = 4.94, $J_{6',6''}$ = 12.22 Hz, H-6″), 4.38 (m, 1 H, H-5′), 5.03 (t, 1H, $J_{3',4'}$ = 9.40 Hz, H-4′), 5.22 (t, 1H, $J_{1',2'}$ = 8.23, $J_{2',3'}$ = 9.60 Hz, H-2′), 5.66 (dd, 1H, $J_{2',3'}$ = 9.60, $J_{3',4'}$ = 9.30 Hz, H-3′), 6.47 (d, 1H, $J_{1',2'}$ = 8.20 Hz, H-1′), 7.26 (t, 1H, J = 5.10, 3.60 Hz, thiophene-H), 7.69 (s, 1H, pyridine-H5), 7.75 (d, 2H, J = 7.8 Hz, Ar-H), 7.79 (d, 2H, J = 7.8 Hz, Ar-H), 7.91 (d, 1H, J = 5.10 Hz, thiophene-H), 8.18 (d, 1H, J = 3.60 Hz, thiophene-H). ^{13}C NMR (DMSO-d_6, δ ppm): 20.25, 20.28, 20.3 and 20.4 (4 CH_3CO), 61.5 (C-6′), 68.0 (C-4′), 68.9 (C-3′), 71.4 (C-2′), 72.2 (C-5′), 93.8 (C-1′), 114.0 (C≡N), 128.3, 128.9, 129.0, 129.3, 130.5, 131.0, 131.9, 134.1, 135.1, 141.9, 152.8, 155.3, 161.5 (Ar-C) and 169.0, 169.3, 170.0, 170.1 (4 C=O).

5.3.2. 4-(4-Chlorophenyl)-2-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyran-osyloxy)-6-(thien-2-yl)nicotinonitrile (*5b*)

Method A: As pale yellow crystals chromatographed by using (CH_2Cl_2/MeOH, 9.9: 0.1) as eluent, yield 67%, mp 139–140 °C. Anal. Calcd. for $C_{30}H_{27}ClN_2O_{10}S$ (643.06): C, 56.03; H, 4.23; N, 4.36; Found: C, 56.24; H, 4.20; N, 4.38. IR (KBr, cm^{-1}): 2226 (C≡N) and 1752 (C=O, acetoxy). ^1H NMR (DMSO-d_6, δ ppm): 1.87, 1.96, 2.01 and 2.17 (4s, 12 H, 4 CH_3CO), 4.03 (dd, 1 H, $J_{5',6'}$ = 6.0, $J_{6',6''}$ = 11.06 Hz, H-6′), 4.16 (dd, 1 H, $J_{5',6'}$ = 6.30, $J_{6',6''}$ = 11.06 Hz, H-6″), 4.59 (m, 1 H, H-5′), 5.32 (t, 1H, $J_{3',2'}$ = 10.23, $J_{3',4'}$ = 2.80 Hz, H-3′), 5.40 (t, 1H, $J_{2',1'}$ = 8.40, $J_{2',3'}$ = 10.30 Hz, H-2′), 5.59 (t, 1H, $J_{4',3'}$ = 2.81, $J_{4',5'}$ = 2.78 Hz, H-4′), 6.36 (d, 1H, $J_{1',2'}$ = 8.40 Hz, H-1′), 7.26 (t, 1H, J = 5.7, 4.80 Hz, thiophene-H), 7.67 (d, 2H, J = 8.47 Hz, Ar-H), 7.75 (d, 2H, J = 8.47 Hz, Ar-H), 7.82 (s, 1H, pyridine-H5), 7.89 (d, 1H, J = 5.7 Hz, thiophene-H), 8.15 (d, 1H, J = 4.80 Hz, thiophene-H). ^{13}C NMR (DMSO-d_6, δ ppm): 20.23, 20.31, 20.84 and 20.88 (4 CH_3CO), 61.9 (C-6′), 68.0 (C-4′), 68.1 (C-2′), 70.7 (C-3′), 71.8 (C-5′), 94.7 (C-1′), 114.5 (C≡N), 128.9, 129.3, 129.6, 129.8, 130.5, 131.0, 132.3, 134.6, 135.6, 142.4, 152.3, 155.9, 162.1 (Ar-C) and 169.4, 169.9, 170.3, 170.5 (4 C=O).

5.3.3. 4-(4-Chlorophenyl)-2-(2′,3′,5′-tri-O-acetyl-β-D-xylofurano-syloxy)-6-(thien-2-yl)nicotinonitrile (*11*)

As pale yellow crystals chromatographed by using (CH_2Cl_2/MeOH, 9.9: 0.1), **Method A**: Yield 52%, **method B**: Yield 58%, mp 158–159 °C. Anal. Calcd. for $C_{27}H_{23}ClN_2O_8S$ (571.00): C, 56.79; H, 4.06; N, 4.91. Found: C, 56.82; H, 3.98; N, 4.84. IR (KBr, cm^{-1}): 2224 (C≡N) and 1759 (C=O, acetoxy). ^1H NMR (DMSO-d_6, δ ppm): 2.10, 2.15 and 2.25 (3s, 9H, 3 CH_3CO), 3.76, (dd, 1H, $J_{4',5'}$ = 6.0, $J_{5',5''}$ = 12.0 Hz, H-5′), 3.96 (dd, 1H, $J_{4',5''}$ = 5.80, $J_{5',5''}$ = 12.0 Hz, H-5″), 5.10 (m, 1H, H-4′), 5.25 (t, 1H, $J_{1',2'}$ = 3.0, $J_{2',3'}$ = 9.0 Hz, H-2′), 5.50 (t, 1H, $J_{2',3'}$ = 9.0, $J_{3',4'}$ = 9.0 Hz, H-3′), 6.79 (d, 1H, $J_{1',2'}$ = 3.6 Hz, H-1′), 7.22 (t, 1H, J = 5.32, 4.10 Hz, thiophene-H), 7.70 (d, 2H, J = 8.42 Hz, Ar-H), 7.85 (d, 2H, J = 8.42 Hz, Ar-H), 7.90 (s, 1H, pyridine-H5), 7.94 (d, 1H, J = 5.32 Hz, thiophene-H), 8.13 (d, 1H,

J = 4.10 Hz, thiophene-H). ^{13}C NMR (DMSO-d$_6$, δ ppm): 20.68, 20.91 and 21.04 (3 $\underline{C}H_3CO$), 62.19 (C-5′), 71.20 (C-2′), 73.33 (C-3′), 78.56 (C-4′) and 99.9 (C-1′), 116.0 (C≡N), 129.4, 129.5, 129.9, 130.6, 131.0, 131.4, 132.2, 134.9, 135.3, 140.8, 151.0, 153.1, 161.3 (Ar-C) and 169.9, 170.8, 171.0 (3 C=O).

*5.3.4. 4-(4-Chlorophenyl)-2-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyloxy)-6-(thien-2-yl)nicotinonitrile (**12**)*

Method B: As pale yellow crystals chromatographed by using (CH_2Cl_2/MeOH, 9.9: 0.1) as eluent, yield 55%, mp 155–157 °C. Anal. Calcd. for $C_{27}H_{23}ClN_2O_8S$ (571.00): C, 56.79; H, 4.06; N, 4.91; Found: C, 56.78; H, 4.11; N, 5.01. IR (KBr, cm^{-1}): 2225 (C≡N) and 1751 (C=O, acetoxy). ^{1}H NMR (DMSO-d$_6$, δ ppm): 2.01, 2.06 and 2.18 (3s, 9H, 3 CH_3CO), 4.16, (dd, 1H, $J_{4',5'}$ = 4.5, $J_{5',5''}$ = 11.02 Hz, H-5′), 4.33 (dd, 1H, $J_{4',5''}$ = 4.23, $J_{5',5''}$ = 11.02 Hz, H-5″), 4.49 (m, 1H, H-4′), 5.42 (dd, 1H, $J_{2',3'}$ = 3.10, $J_{3',4'}$ = 6.18 Hz, H-3′), 5.51 (dd, 1H, $J_{1',2'}$ = 3.8, $J_{2',3'}$ = 3.12 Hz, H-2′), 6.63 (d, 1H, $J_{1',2'}$ = 3.8 Hz, H-1′), 7.24 (t, 1H, J = 5.02, 3.90 Hz, thiophene-H), 7.70 (d, 2H, J = 8.42 Hz, Ar-H), 7.72 (d, 2H, J = 8.42 Hz, Ar-H), 7.74 (s, 1H, pyridine-H5), 7.87 (d, 1H, J = 5.02 Hz, thiophene-H), 8.16 (d, 1H, J = 3.90 Hz, thiophene-H).

*5.3.5. 4-(4-Chlorophenyl)-2-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyran-osyl-(1 → 4))-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyloxy)-6-(thien-2-yl)nicotinonitrile (**16**)*

Method A: As pale yellow syrup from ethanol, yield 56%. Anal. Calcd. for $C_{42}H_{43}ClN_2O_{18}S$ (931.31): C, 54.17; H, 4.65; N, 3.01. Found: C, 54.39; H, 4.51; N, 3.18. IR (KBr, cm^{-1}): 2228 (C≡N) and 1742 (C=O, acetoxy). ^{1}H NMR (DMSO-d$_6$, δ ppm): δ 1.90, 1.93, 1.95, 1.97, 2.00, 2.06 and 2.10 (7s, 21H, 7 CH_3CO). 3.98–4.03 (m, 3H, H-2′b, H-6′a, H-6′b), 4.14 (dd, 1H, $J_{6''a,6'a}$ = 11.4, $J_{6''a,5'a}$ = 5.6 Hz, H-6″a), 4.35 (m, 1H, H-5′b), 4.69 (dd, 1H, $J_{6''b,5'b}$ = 6.3 Hz, H-6″b), 4.85 (m, 1H, H-5′a), 4.91 (dd, 1H, $J_{1'b,2'b}$ = 7.6 Hz, H-1′b), 5.03 (1H, $J_{4'b,3'b}$ = 3.1, $J_{4'b,5'b}$ = 3.8 Hz, H-4′b), 5.20 (dd, 1H, $J_{2'a,1'a}$ = 8.7, $J_{2'a,3'a}$ = 8.4 Hz, H-2′a), 5.25 (dd, 1H, $J_{4'a,3'a}$ = 9.1, $J_{4'a,5'a}$ = 9.9 Hz, H-4′a), 5.32 (d, 1H, $J_{3'b,4'b}$ = 3.1 Hz, H-3′b), 5.39 (dd, 1H, $J_{3'a,2'a}$ = 8.4, $J_{3'a,4'a}$ = 9.1 Hz, H-3′a), 6.35 (d, 1H, $J_{1'a,2'a}$ = 8.7 Hz, H-1′a) 7.10 (t, 1H, J = 5.25, 4.11 Hz, thiophene-H), 7.23 (d, 2H, J = 8.12 Hz, Ar-H), 7.65 (d, 2H, J = 8.12 Hz, Ar-H), 7.70 (s, 1H, pyridine-H5), 7.84 (d, 1H, J = 5.25 Hz, thiophene-H), 8.05 (d, 1H, J = 4.11 Hz, thiophene-H).

5.4. General procedure for deprotection

Triethylamine (1 mL) was added to a solution of glycoside (0.01 mol) in MeOH (20 mL) and 3 drops of water. The mixture was stirred overnight at room temperature, evaporated under reduced pressure and the residue was co-evaporated with MeOH until the triethylamine was removed. The residue was crystallized from proper solvent, yield >85%.

*5.4.1. 4-(4-Chlorophenyl)-2-(β-D-glucopyranosyloxy)-6-(thien-2-yl) nico-tinonitrile (**7a**)*

Pale yellow crystals from ethanol, mp 205–206 °C. Anal. Calcd. for $C_{24}H_{21}ClN_2O_7S$ (643.06): C, 55.76; H, 4.09; N, 5.42. Found: C, 55.81; H, 4.23; N, 5.35. IR (KBr, cm^{-1}): 3423 (broad, 4OH) and 2212 (CN). ^{1}H NMR (DMSO-d$_6$, δ ppm): 3.35 (m, 6H, H-6′, H-6″, H-5′, H-4′, H-3′ and H-2′), 3.99 (t, 1H, J = 3.50 Hz, OH-6′, D_2O exchangeable), 4.57 (d, 1H, J = 4.24 Hz, OH-4′, D_2O exchangeable), 5.19 (d, 1H, J = 4.21 Hz, OH-3′, D_2O exchangeable), 5.44 (d, 1H, J = 4.86 Hz, OH-2′, D_2O exchangeable), 6.01 (d, 1H, $J_{1',2'}$ = 8.13 Hz, H-1′), 7.25 (t, 1H, J = 4.99, 3.75 Hz, thiophene-H), 7.70 (d, 2H, J = 8.44 Hz, Ar-H), 7.75 (d, 2H, J = 8.37 Hz, Ar-H), 7.94 (s, 1H, pyridine-H5), 8.09 (d, 1H, J = 4.99 Hz, thiophene-H), 8.38 (d, 1H, J = 3.75 Hz, thiophene-H). ^{13}C NMR (DMSO-d$_6$, δ ppm): 62.7 (C-6′), 62.8 (C-2′), 69.4 (C-3′), 76.7 (C-4′), 79.8 (C-5′), 96.3 (C-1′), 116.4 (C≡N), 128.5, 129.1, 129.4, 130.4, 131.0, 132.9, 133.8, 135.4, 135.8, 140.3, 152.2, 155.6 (Ar-C) and 162.7 (C=N).

*5.4.2. 4-(4-Chlorophenyl)-2-(β-D-galactopyranosyloxy)-6-(thien-2-yl)nic-otinonitrile (**7b**)*

Pale yellow crystals from ethanol, mp 190–191 °C. Anal. Calcd. for $C_{24}H_{21}ClN_2O_7S$ (643.06): C, 55.76; H, 4.09; N, 5.42. Found: C, 55.69; H, 4.10; N, 5.38. IR (KBr, cm^{-1}): 3389 (broad, 4OH) and 2217 (CN). ^{1}H NMR (DMSO-d$_6$, δ ppm): 3.48 (m, 3H, H-3′, H-6′, H-6″), 3.59 (m, 3H, H-2′, H-4′, H-5′), 4.64 (m, 2H, OH-4′, OH-6′), 4.95 (d, 1H, J = 5.10 Hz, OH-3′), 5.28 (d, 1H, J = 4.80 Hz, OH-2′), 5.96 (d, 1H, $J_{1',2'}$ = 8.10 Hz, H-1′), 7.24 (t, 1H, J = 4.99, 3.78 Hz, thiophene-H), 7.64 (d, 2H, J = 8.50 Hz, Ar-H), 7.70 (d, 2H, J = 8.50 Hz, Ar-H), 7.72 (s, 1H, pyridine-H-5), 7.76 (d, 1H, J = 4.99 Hz, thiophene-H), 8.10 (d, 1H, J = 3.78 Hz, thiophene-H). ^{13}C NMR (DMSO-d$_6$, δ ppm): 60.49 (C-6′), 68.40 (C-4′), 70.23 (C-2′), 74.13 (C-3′), 76.80 (C-5′) and 97.89 (C-1′), 115.3 (C≡N), 129.2, 129.3, 129.5, 130.6, 130.9, 131.4, 132.1, 134.9, 135.5, 142.8, 153.3, 155.7 (Ar-C) and 163.3 (C=N).

*5.4.3. 4-(4-Chlorophenyl)-2-(β-D-xylofuranosyloxy)-6-(thien-2-yl) nico-tinonitrile (**13**)*

Pale yellow crystals from ethanol, mp 188–189 °C. Anal. Calcd. for $C_{21}H_{17}ClN_2O_5S$ (444.89): C, 56.69; H, 3.85; N, 6.30. Found: C, 56.68; H, 4.10; N, 6.22. IR (KBr, cm^{-1}): 3423 (broad, 4OH) and 2217 (CN). ^{1}H NMR (DMSO-d$_6$, δ ppm) 3.31 (m, 2H, H-5′, H-5″), 3.37 (m, 1H, H-4′), 3.44–3.51 (2H, H-3′, H-2′), 4.40 (t, 1H, J = 6.9 Hz, OH-5′, D_2O exchangeable), 4.63 (d, 1H, J = 4.5 Hz, OH-3′, D_2O exchangeable), 4.85 (d, 1H, J = 3.9 Hz, OH-2′, D_2O exchangeable), 6.09 (d, 1H, $J_{1',2'}$ = 3.9 Hz, H-1′), 7.23 (t, 1H, J = 5.0, 3.94 Hz, thiophene-H), 7.64 (d, 2H, J = 8.12 Hz, Ar-H), 7.71 (d, 2H, J = 8.12 Hz, Ar-H), 7.78 (s, 1H, pyridine-H5), 7.92 (d, 1H, J = 5.0 Hz, thiophene-H), 8.10 (d, 1H, J = 3.94 Hz, thiophene-H).

*5.4.4. 4-(4-Chlorophenyl)-2-(β-D-ribofuranosyloxy)-6-(thien-2-yl) nicotinonitrile (**14**)*

Pale yellow crystals from ethanol, mp 183–185 °C. Anal. Calcd. for $C_{21}H_{17}ClN_2O_5S$ (444.89): C, 56.69; H, 3.85; N, 6.30. Found: C, 56.65; H, 3.90; N, 6.33. IR (KBr, cm^{-1}): 3424 (broad, 4OH) and 2219 (CN). ^{1}H NMR (DMSO-d$_6$, δ ppm) 3.12 (m, 2H, H-5′, H-5″), 3.25 (m, 1H, H-4′), 3.44–3.5 (2H, H-3′, H-2′), 4.28 (t, 1H, J = 5.4 Hz, OH-5′, D_2O exchangeable), 4.55 (d, 1H, J = 4.81 Hz, OH-3′, D_2O exchangeable), 4.77 (d, 1H, J = 5.1 Hz, OH-2′, D_2O exchangeable), 6.19 (d, 1H, $J_{1',2'}$ = 3.62 Hz, H-1′), 7.24 (t, 1H, J = 4.8, 3.90 Hz, thiophene-H), 7.64 (d, 2H, J = 8.10 Hz, Ar-H), 7.70 (d, 2H, J = 8.12 Hz, Ar-H), 7.72 (s, 1H, pyridine-H5), 7.74 (d, 1H, J = 4.8 Hz, thiophene-H), 8.05 (d, 1 H, J = 3.90 Hz, thiophene-H).

*5.4.5. 4-(4-Chlorophenyl)-2-(β-D-galactopyranosyl-(1 → 4))-(β-D-glucopy ranosyloxy)-6-(thien-2-yl)nicotinonitrile (**17**)*

Pale yellow crystals from ethanol/H_2O, mp 165–167 °C. Anal. Calcd. for $C_{28}H_{29}ClN_2O_{11}S$ (637.05): C, 52.79; H, 4.59; N, 4.40. Found: C, 52.74; H, 4.60; N, 4.38. IR (KBr, cm^{-1}): 3420 (broad, 7 OH) and 2224 (CN). ^{1}H NMR (DMSO-d$_6$, δ ppm): 3.42–3.55 (4 m, 12H, H-2′b, H-3′b, H-4′b, H-5′b, H-6′b, H-6″b, H-2′a, H-3′a, H-4′a, H-5′a, H-6′a, H-6″a), 4.40 (d, 1H, OH-4′b), 4.48 (d, 1H, OH-6′b), 4.80 (d, 1H, J = 4.20 Hz, OH-3′b), 4.92 (d, 1H, OH-2′b), 5.02 (d, 1H, OH-6′a), 5.10 (d, 1H, OH-3′a), 5.29 (d, 1H, OH-2′a), 5.82 (d, 1H, $J_{1'b,2'b}$ = 7.76 Hz, H-1′b), 5.87 (d, 1H, $J_{1'a,2'a}$ = 8.91 Hz, H-1′a), 7.11 (t, 1H, J = 4.60, 4.01 Hz, thiophene-H), 7.54 (d, 2H, J = 8.12 Hz, Ar-H), 7.58 (s, 1H, pyridine-H5), 7.61 (d, 2H, J = 8.12 Hz, Ar-H), 7.70 (d, 1H, J = 4.60 Hz, thiophene-H), 7.97 (d, 1H, J = 4.01 Hz, thiophene-H). ^{13}C NMR (DMSO-d$_6$, δ ppm): 60.7 (C-6′a), 61.3 (C-6′b), 68.1 (C-3′a), 69.8 (C-2′b), 70.0 (C-2′a), 73.0 (C-3′b), 73.1 (C-4′a), 73.5 (C-4′b), 77.6 (C-5′b), 78.5 (C-5′a), 97.3 (C-1′b), 99.3 (C-1′a). 115.2 (C≡N), 128.9, 129.3, 129.5, 130.9, 131.4, 132.1, 133.0, 134.8, 135.6, 142.7, 153.3, 155.7 (Ar-C) and 163.2 (C=N).

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