



# Boosting the antimicrobial performance based on new fused spirothiazolidine framework analogs

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Received: 28 January 2022 / Accepted: 18 May 2022 / Published online: 6 July 2022  
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## Abstract

New spiro[cyclohexane-1,2'-thiazolo[4,5-*b*]pyridine derivatives (**3–23**) were investigated. Then there is in vitro antimicrobial potency against possible organisms *Staphylococcus aureus* ATCC-47,077, *Bacillus cereus* ATCC-12228, *Escherichia coli* ATCC-25922, *Salmonella typhi* ATCC-15566, and *Candida albicans* ATCC-10231 were tested utilizing commercially available antibiotics ampicillin as a reference drug. A preliminary antimicrobial test represented that derivatives: (Aldoses) 3'-(4-fluorophenyl)-5'-(methyleneamino)-7'-(*p*-tolyl)-3'*H*-spiro[cyclohexane-1,2'-thiazolo[4,5-*b*]pyridine]-6'-carbonitrile (**16–19**) and (Acetyl aldoses) 3'-(4-fluorophenyl)-5'-(methyleneamino)-7'-(*p*-tolyl)-3'*H*-spiro [cyclohexane-1,2'-thiazolo[4,5-*b*]pyridine]-6'-carbonitrile (**20–23**) exhibited higher antifungal, and antibacterial performance with minimum inhibitory concentrations values of (39–67 µg/ml) toward all pathogenic strains compared to common reference drug ampicillin.

**Keywords** Spiro-thiazolo[4,5-*b*]pyridines · Spiro-thiazolo[5',4':5,6]pyrido[2,3-*d*]pyrimidines · Antibacterial · Antifungal

## Introduction

The design, synthesis, and manufacture of molecules with therapeutic benefits for humans are one of the fundamental goals of medicinal organic chemistry [1–4]. Chemical libraries based on favored structures have been available thanks to advances in combinatorial chemistry during the last decade [5–7]. Spiro-compounds are a type of naturally founding chemical with significant biological features (Fig. 1) [8–10]. Because of their structure–activity relationship, are a very appealing target for combinatorial library synthesis [11–13]. It is a significant category of nitrogen and sulfur-containing heterocycles that are frequently used as major building blocks in the field of pharmaceuticals and

pharmacological agents [14–17]. Recently; an efficient and additive-free synthesis of heterocycles via green strategy in excellent yields and short reaction time as an environmental procedure [18–22]

The spiro-thiazolidinone nucleus is also recognized as the “wonder nucleus” since it produces a set of derivatives with various biological activities [23–25]. The existence of an N–C–S linkage in the compounds has been found to have antimicrobial and antithyroid properties [24]. The antifungal performance of several 4-thiazolidinones has been evaluated [26]. Antitubercular [27], antioxidant [28], analgesic, [29] anticonvulsant [30], anti-inflammatory [31], antihyperglycemic [32], diuretic [33], antihistaminic [34], antidiabetic [35, 36], cyclooxygenase inhibitors [37], and lipoxygenase inhibitors [37] properties were also discovered. The presence of nitrogen and sulfur atoms is highlighting the importance of thiazolidinone which is widely used as a key building block in the scope of pharmaceutical agents and drugs [38, 39].

Carbohydrates have a unique property: the electrophilic nature of the anomeric core allows the sugar to link with diverse molecules through presenting a functional group that acts as a nucleophile [40, 41]. In nature, the OH; NH<sub>2</sub> group usually performs this linkage; however, sulfur or carbon nucleophile may also be involved, resulting in *S*-glycosides and *C*-glycosides, respectively [42]. The attention to *S*- and *C*-glycosides is attributed to the metabolic stability of the

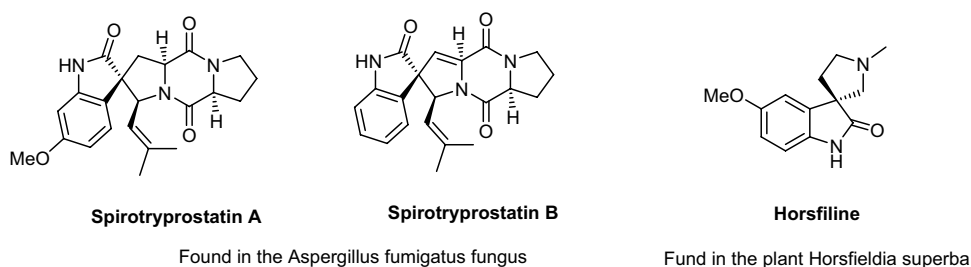
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**Fig. 1** Examples of naturally founding compounds bearing a spirocyclic motif



glycosidic bond, which cannot be hydrolyzed by glycosidases enzyme [43]. The metabolic stability of *S*- and *C*-glycosides is also exploited in the generation of glycomimetic drugs resistant against *in vivo* hydrolysis. [44]

The most popular strategy for treating diseases caused by resistant bacteria is antimicrobial therapy [45]. As a result, there is a lot of demand and benefit in the discovery of novel antimicrobial compounds [46, 47]. Furthermore, some *S*- and *C*-glycosides are significant pharmacophore that is integrated into various bioactive compounds, especially in antimicrobial therapy [48, 49]. In the past decade, many heterocyclic compounds and their corresponding glycosides have been synthesized to obtain a novel antimicrobials drug, which would be able to treat infections caused by resistant bacteria and fungi strains. [50, 51]

Because of the above-mentioned, the main subject of our study is to synthesize the biologically important scaffold based on new spiro(cyclohexane-thiazolidine) derivatives and its corresponding glycosides with the assessment of their antibacterial and antifungal activity.

## Results and discussion

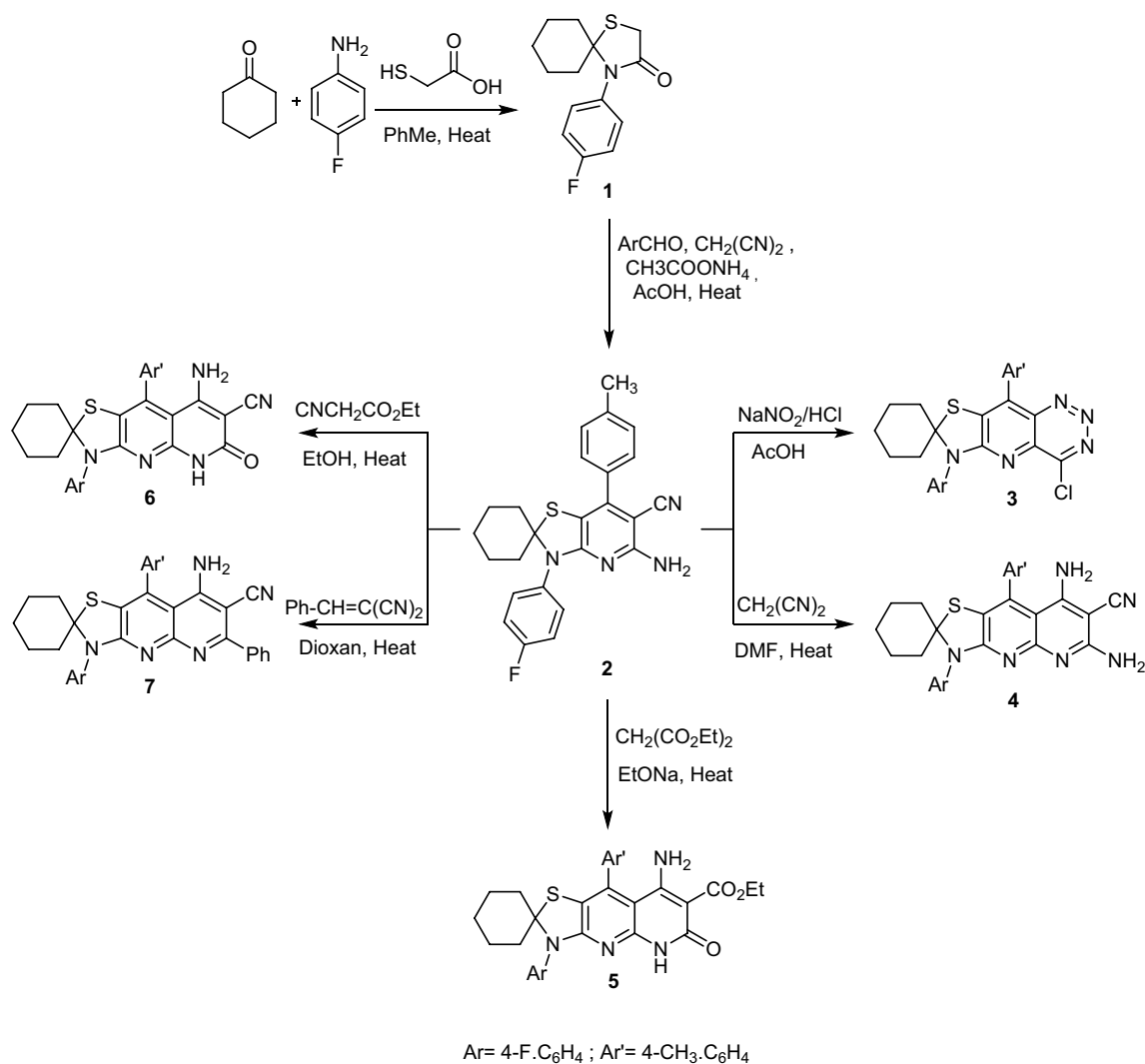
### Chemistry

Continuing our interesting research on the design and investigation of a broad range of applicable heterocyclic derivatives, the 4-(4-Fluorophenyl)-1-thia-4-azaspiro[4.5]decan-3-one **1** was prepared in agreement with our previous method (Fig. 2, *c.f. experimental*). Compound **1** was allowed to react with malononitrile through a one-pot three-component reaction in the presence of both 4-methylbenzaldehyde and ammonium acetate yielded the corresponding 5'-Amino-3'-(4-fluorophenyl)-7'-(*p*-tolyl)-3'*H*-spiro[cyclohexane-1,2'-thiazolo[4,5-*b*]pyridine]-6'-carbonitrile (**2**). The chemical structure of spiro[cyclohexane-1,2'-thiazolo[4,5-*b*]pyridine] carbonitrile derivative **2** is assigned with all spectroscopic data; the new C≡N group displays signals at 2216 cm<sup>-1</sup> and 114.16 ppm in both IR and <sup>13</sup>C-NMR spectrum, respectively. Also, the new NH<sub>2</sub> group shows a peak at 3132 cm<sup>-1</sup> in IR spectra and a broad signal at 8.66 ppm exchangeable with D<sub>2</sub>O in <sup>1</sup>H-NMR spectra. Besides presence signal attributed

to CH<sub>3</sub> group at 2.37 and 21.23 ppm in both <sup>1</sup>H and <sup>13</sup>C-NMR spectrum, respectively (Fig. 2, *c.f. experimental*).

Spiro[cyclohexane-1,2'-thiazolo[4,5-*b*]pyridine]carbonitrile derivative **2** was utilized as an important framework for the preparation of further new fused heterocyclic compounds. It reacted with sodium nitrite in the presence of hydrochloric acid to produce the corresponding spirothiazolo triazine derivative **3**; where its IR and NMR spectrum revealed the disappearance of signal characteristic to both NH<sub>2</sub> and CN groups; besides displaying the molecular weight with isotopic pattern peak; *m/z* at 477 [M<sup>+</sup>], 479 [M<sup>+</sup> + 2] (Fig. 2, *c.f. experimental*). Further treatment of spiro[cyclohexane-1,2'-thiazolo[4,5-*b*]pyridine]carbonitrile derivative **2** with various reagents namely: malononitrile, diethyl malonate, ethyl cyanoacetate and benzylidene malononitrile produced a new spiro[cyclohexane-1,2'-thiazolo[4,5-*b*] [1, 8] naphthyridine]-7'-carbonitrile derivatives **4–7**, respectively (Fig. 2). The IR spectra for previous derivatives **4–7** represented that 6',8'-diamino-3'-(4-fluorophenyl)-9'-(*p*-tolyl)-3'*H*-spiro[cyclohexane-1,2'-thiazolo[4,5-*b*] [1,8]naphthyridine]-7'-carbonitrile(**4**) show the existence of new amino group at 3435 cm<sup>-1</sup> besides the presented amino and cyano groups at 3162, 2219 cm<sup>-1</sup>, respectively, Ethyl 8'-amino-3'-(4-fluorophenyl)-6'-oxo-9'-(*p*-tolyl)-5',6'-dihydro-3'*H*-spiro[cyclohexane-1,2'-thiazolo[4,5-*b*] [1, 8] naphthyridine]-7'-carboxylate (**5**) show the presence of two carbonyl groups at 1734 cm<sup>-1</sup> for ester and 1670 cm<sup>-1</sup> for amide, while 8'-Amino-3'-(4-fluorophenyl)-6'-oxo-9'-(*p*-tolyl)-5',6'-dihydro-3'*H*-spiro[cyclohexane-1,2'-thiazolo[4,5-*b*] [1, 8] naphthyridine]-7'-carbonitrile (**6**) show a peak represented for new amide group at 3116 and 1681 cm<sup>-1</sup> for NH and C=O groups, respectively. The <sup>1</sup>H-NMR spectra for latter derivatives showed that compound **4** showed two broad singlet signals characteristic for two amino groups at 8.40 and 8.65 ppm, while compound **5** showed an ethyl ester group at ppm = 1.31 (t, *J* = 7.49, 3H, CH<sub>2</sub>CH<sub>3</sub>) and 4.26 (q, *J* = 7.50, 2H, CH<sub>2</sub> CH<sub>3</sub>). Also, compound **6** given signal peak characteristic for the amide NH group which is exchangeable with D<sub>2</sub>O involved with aromatic proton peaks at 7.19–7.98 (m, 9H, 8Ar-H + NH amide) (Fig. 2, *c.f. experimental*).

Moreover, the solvent effect in the organic heterocyclization can be illustrated when

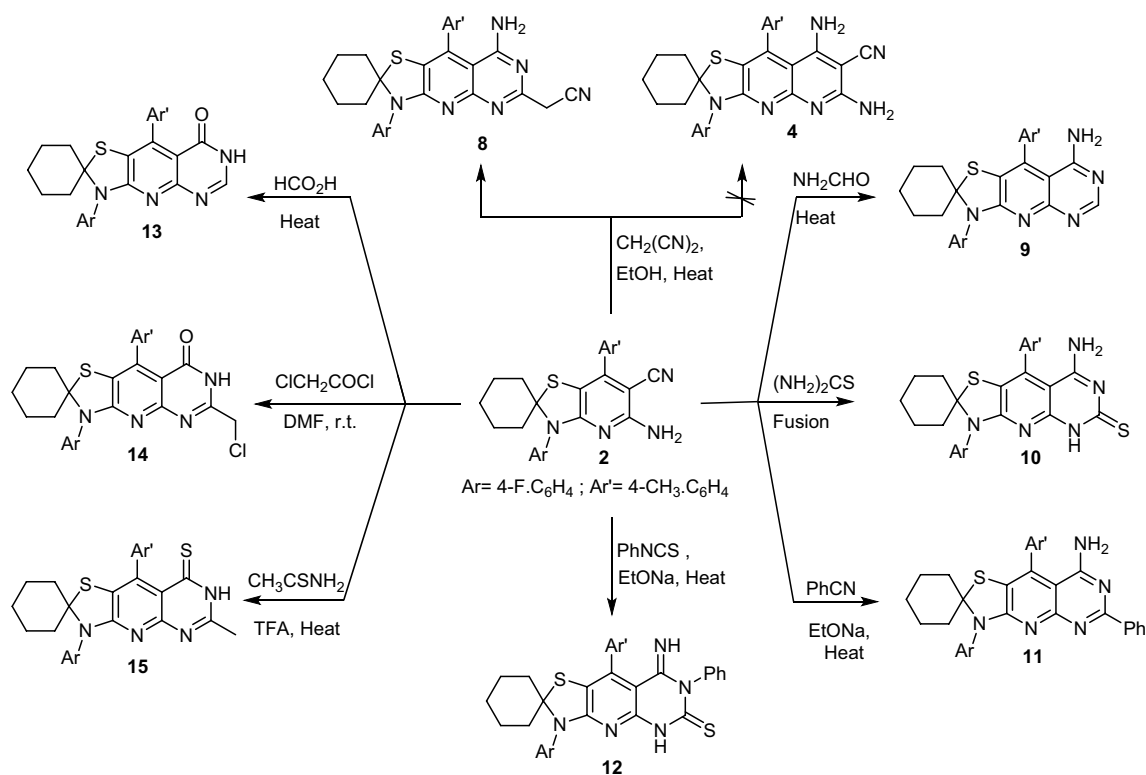


**Fig. 2** Schematic pathway synthesis of derivatives 1–7

spiro[cyclohexane-1,2'-thiazolo[4,5-b]pyridine]carbonitrile derivative **2** is allowed to react with malononitrile in polar protic solvent (EtOH) to produce 2-(8'-Amino-3'-(4-fluorophenyl)-9'-(*p*-tolyl)-3'*H*-spiro[cyclohexane-1,2'thiazolo[5',4':5,6]pyrido[2,3-d]pyrimidin]-6'-yl)acetonitrile (**8**) rather than derivative **4** which previously obtained when the reaction done in a polar aprotic solvent (DMF), where NMR spectra displayed singlet signals in <sup>1</sup>H-NMR at δ 4.56 ppm for new CH<sub>2</sub>-CN group and in <sup>13</sup>C-NMR at δ 27.96 ppm. Additionally; in mass, the spectrum represented the molecular ion peak at *m/z* (%) 496 (M<sup>+</sup>, 59%) and its base peak at *m/z* 456 (M<sup>+</sup>-CH<sub>2</sub>CN, 100%) (Fig. 3, *c.f. experimental*).

Additionally; treatment of spiro[cyclohexane-1,2'-thiazolo[4,5-b]pyridine]carbonitrile derivative **2** with formamide, thiourea, benzonitrile, phenylisothiocyanat, formic acid, chloroacetyl chloride and thioacetamide the

newly synthesized spiro[cyclohexane-1,2'-thiazolo[5',4':5,6]pyrido[2,3-d]pyrimidin]-8'-amine derivatives **9–15** were given, respectively (Fig. 3). The <sup>1</sup>H-NMR spectrum of compounds **9** displayed a new singlet signal related to pyrimidine-H at 8.20 ppm; compound **10** showed a broad singlet signal exchangeable with D<sub>2</sub>O at δ = 7.02 for -NHCS- group in pyrimidine ring; compound **11** showed new signals for substituted phenyl group in pyrimidine ring, compound **12** observe two signals exchangeable with D<sub>2</sub>O specific for two NH groups in pyrimidine ring at 9.19 ppm for C=NH and at aromatic-H range 7.21–8.41 ppm for NHCS; compound **13** showed a characteristic signal for pyrimidine-H at δ 8.28 ppm compound **14** displayed new signal related to CH<sub>2</sub>Cl group substituted in pyrimidine ring at δ 5.17 ppm and compound **15** presented singlet signal at δ 2.32 ppm for new methyl group substituted pyrimidine ring (Fig. 3; *c.f. experimental*). While the IR and <sup>13</sup>C-NMR spectrum



**Fig. 3** Schematic pathway synthesis of derivatives 8–15

of compounds **13** and **14** showed a new characteristic band attributed to the carbonyl group in pyrimidine ring at 1685, 1682  $\text{cm}^{-1}$  and 162.20, 162.19 ppm, respectively; on the other hand, the  $^{13}\text{C}$ -NMR spectra for compounds **10**, **12** and **15** displayed new signals for C=S group in pyrimidine ring at 172.19, 171.21 and 169.32, respectively (Fig. 3; *c.f. experimental*).

Finally, the reaction of spiro[cyclohexane-1,2'-thiazolo[4,5-b]pyridine]carbonitrile derivative **2** with aldoses namely; *D*-xylose, *D*-arabinose, *D*-glucose, and *D*-galactose in presences of Schiff's based reaction condition produced the corresponding sugars amides **16–19**, respectively. The IR and  $^1\text{H}$ -NMR spectrum of latter derivatives represented the disappearance of the characteristic signal of the  $\text{NH}_2$  group besides the appearance of signal characteristics for both OH groups and sugars chain moieties. Acetylating sugars amides derivatives **16–19** leads to the formation of the corresponding acetylated derivatives **20–23**, respectively; where IR and NMR spectrum of last derivatives **20–23** exhibited characteristic signals attributed to acetyl groups (Fig. 4; *c.f. experimental*).

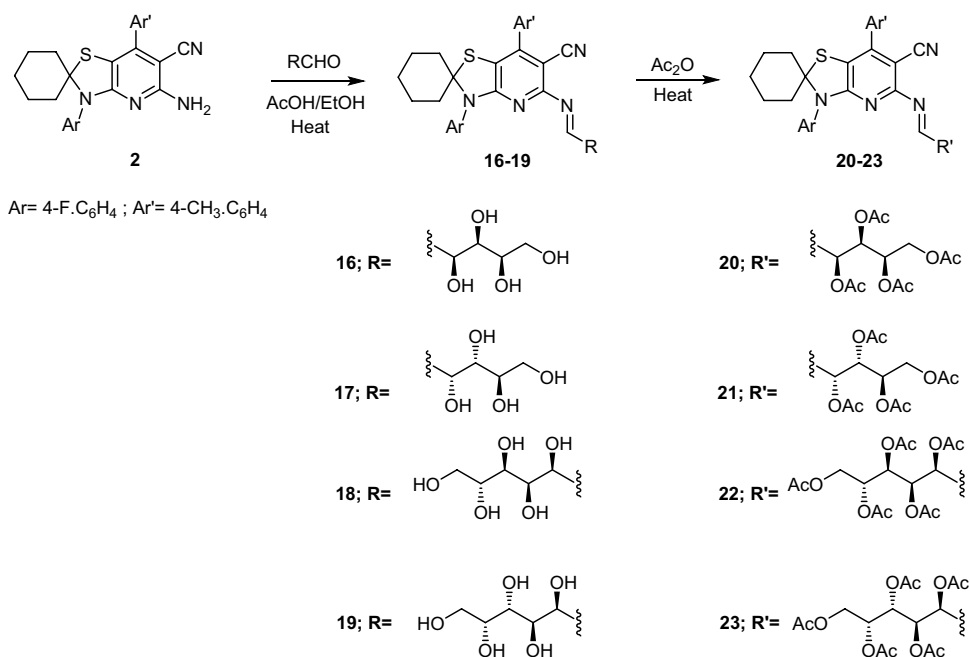
### Anti-microbial activity

The antimicrobial performance for novel derivatives based on spirothiazolidine backbone, which was prepared in

excellent yields, against gram-positive bacteria *Staphylococcus aureus* ATCC- 47,077 (St.), *Bacillus cereus* ATCC-12228 (B.C.), and gram-negative bacteria species *Escherichia coli* ATCC-25922 (E.C.), *Salmonella typhi* ATCC-15566 (Salm.), in addition to fungi strain namely; *Candida albicans* ATCC-10231 (C. Alb.) were tested. The results are shown in Table 1.

As shown in Table 1, compounds **16–23** showed higher performance than the reference drug commonly utilized toward all strains according to the following activity order **16–19** > **20–23** > ampicillin, whereas the potency of derivatives **8–15** against all strains was the same as ampicillin (**8–15**  $\approx$  ampicillin) according to the following activity order; **8–11** > **13–15**  $\geq$  ampicillin. Moreover, the estimation of the potency revealed that compounds **4–7** exhibited moderate performance against all strains when compared with the reference ampicillin drug with potency order; ampicillin  $\geq$  **4–7**. Additionally, all newly prepared compounds were more selective toward fungi strain *Candida albicans* ATCC-10231 (C. Alb.)

The minimal inhibitory concentration (MIC) of all new prepared analogs **1–23** was tested by utilizing different concentrations of each compound and determining the lowest concentration showing pathogen growth inhibition. The results are illustrated in Table 2 based on the type of prepared derivative, and microbe strain. For the

**Fig. 4** Schematic pathway synthesis of derivatives **16–23**

same compound, the MIC values changed depending on the pathogen.

### Structure Activity Relationship (SAR)

The structure–activity relationship (SAR) of spirothiazolopyridine can be established from the data of the antimicrobial potency shown in Table 1. Analysis of the SAR of the spirothiazolopyridine derivatives **1–23** explains the link between the activity and the structure, which offers clues for structural modifications that can enhance antimicrobial performance. SAR analysis is important in understanding antibacterial and antifungal activities for newly prepared derivatives **1–23**. First: Introduce open-chain sugar moiety shows a significant role to increase antimicrobial performance more than the common reference drug; ampicillin and the activity increased by increasing the number of –OH groups rather than –OAc groups in the open-chain sugar nucleus according to the following order: **19**≈**18** (5 free polar hydroxyl groups) > **17**≈**16** (4 free polar hydroxyl groups) > **23**≈**22** (5 free acetylated hydroxyl groups) > **21**≈**20** (4 free acetylated hydroxyl groups) > Ampicillin (Fig. 5a). Second: The presence of pyrimidine ring fused to spirothiazolopyridine backbone leads to an increase in the antimicrobial potency for derivatives **8–15** more than common reference drugs. The substitutions on position 4 and 2 in the pyrimidine nucleus play an essential role to enhance the activity; where the presence of group with high nucleophilicity ( $\text{NH}_2 > \text{C}=\text{S} > \text{C}=\text{O} > \text{C}=\text{NH}$ ) in position 4 is preferred beside presence of electron-donating group in position 2 according to the following derivatives order

(**10** > **9** > **8** > **11**) > (**15** > **14** > **13** > **12**) ≥ Ampicillin (Fig. 5b). Third: The presence of pyridine ring fused to spirothiazolopyridine framework leads to a decrease in the antimicrobial potency for derivatives **4–7** less than common reference drugs. The substitutions on positions 2, 3, and 4 in the pyridine ring effect directly the antimicrobial activity, the existence of an amino group in position 4 is a fundamental group with the presence of an electron-withdrawing group in position 3 ( $\text{CN} > \text{CO}_2\text{C}_2\text{H}_5$ ) and presence of an electron-donating group in position 2 ( $\text{NH}_2 > \text{OH} > \text{C}_6\text{H}_5$ ); the derivatives **4–7** order can be represented as Ampicillin > (**4** > **6** > **5** > **7**) (Fig. 5c).

### Conclusion

In the present work, we discovered a new antimicrobial candidate that might be utilized alone or in combination with research methods for therapeutic, preventive, and growth promoter purposes. A series of novel spirothiazolopyridine derivatives were designed and prepared. Most of the analogs exhibited excellent antibacterial and antifungal activity against all tested species. Some of the compounds displayed lower MIC values than the positive control on some of the tested species. We carried out the first SAR investigation into the antimicrobial activity of all prepared compounds. The SAR results showed that the *N*-nucleoside and *S*-nucleoside produced in spirothiazolopyridine skeleton have significant effects on the activity. All of the results revealed that the compounds are potential antimicrobial agents, which could be further optimized and developed as a new antibiotic

**Table 1** In vitro antimicrobial performance for novel prepared derivatives (200 mg/ml)

Compounds No.	Inhibition zone diameter (mm) *				
	Gram-positive bacteria		Gram-negative bacteria		Fungi
	St	B.C	E.C	Salm	C. Alb
1	7	7	5	6	3
2	9	8	7	6	5
3	6	4	3	3	4
4	16	14	10	11	8
5	12	10	10	9	7
6	13	11	11	10	7
7	10	10	9	8	6
8	20	18	15	19	10
9	21	19	14	18	9
10	23	20	16	17	9
11	19	17	14	19	8
12	13	10	11	11	11
13	17	13	14	19	11
14	17	14	14	21	10
15	19	17	15	17	9
16	33	36	35	37	18
17	33	36	35	37	18
18	35	34	37	39	20
19	35	34	37	39	20
20	24	25	22	24	13
21	24	25	22	24	13
22	26	27	25	27	15
23	26	27	25	27	15
DMSO (Control)	00	00	00	000	00
Ampicillin	23	20	16	19	9

\*The diameter of the well (6 mm) is included

against pathogenic that cause serious infections in public health.

## Experimental

### Chemistry

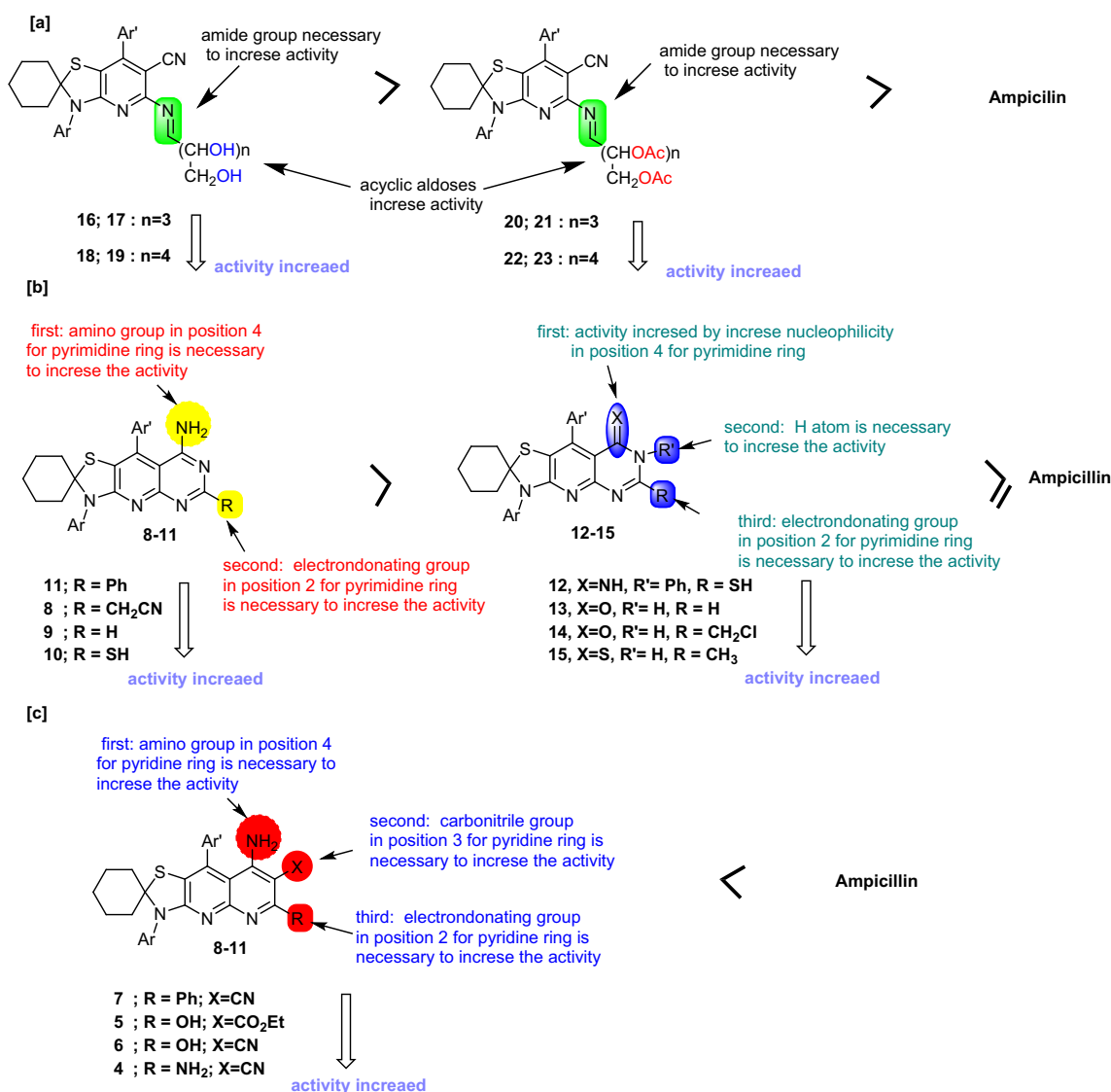
#### General

Melting points were measured using an Electro-Thermal IA 9100 digital melting point apparatus (Büchi, Flawil, Switzerland) and are uncorrected. Infrared spectra were recorded on a PerkinElmer 1600 FTIR (PerkinElmer, Waltham, MA, USA) discs. NMR spectra were determined on a Jeol-Ex-300 NMR spectrometer (JEOL, Tokyo, Japan) and chemical shifts were expressed as part

**Table 2** Minimum Inhibition Concentration (MIC) of new synthesized derivatives 1–29

Compounds No.	% Inhibition of growth ( $\mu\text{g/ml}$ )				
	Gram-positive bacteria		Gram-negative bacteria		Fungi
	St	B.C	E.C	Salm	C. Alb
1	100	99	110	121	92
2	94	80	100	113	79
3	87	95	102	97	67
4	60	69	70	70	55
5	82	66	73	70	61
6	100	93	66	66	74
7	62	62	68	71	66
8	41	43	59	59	80
9	53	64	43	40	49
10	71	69	80	89	77
11	55	59	61	63	47
12	68	67	71	57	60
13	53	40	47	39	59
14	59	53	50	57	71
15	50	60	50	45	50
16	54	55	45	47	67
17	48	41	46	39	51
18	38	43	51	39	44
19	35	35	43	43	37
20	49	43	49	48	33
21	70	68	45	49	34
22	47	67	51	47	53
23	60	41	43	59	41
Ampicillin	4.6	4.7	4	4.2	4.5

per million; ( $\delta$  values, ppm) against TMS as internal reference, National Research Centre, Cairo, Egypt.  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts were referred to the solvent signal (DMSO) at 2.50 and 39.52 ppm, respectively. Data are presented as follows: chemical shift, integration, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) and coupling constant in Hertz (Hz). The mass spectra were run at 70 eV with a Finnigan SSQ 7000 spectrometer (Thermo Electron Corporation, Madison, WI, USA) using EI and the values of m/z are indicated in Dalton. Elemental analyses were performed on a PerkinElmer 2400 analyzer (PerkinElmer) and were found within the accepted range ( $\pm 0.30$ ) of the calculated values. Reaction monitoring and verification of the purity of the compounds was done by *TLC* on silica gel pre-coated aluminum sheets (type 60 F254, Merck, Darmstadt, Germany). All chemical reagents and solvents were purchased from Aldrich (Munich, Germany).



**Fig. 5** Structural Activity relationships for derivatives 1–29

**4-(4-Fluorophenyl)-1-thia-4-azaspiro[4.5]decan-3-one (1) [52]**

A mixture of cyclohexanone (0.01 mol), *p*-fluoroaniline (0.01 mol) and thioglycolic acid (0.01 mol) in dry toluene (50 mL) was refluxed for 10 h. The solution was concentrated and the formed solid was filtered off; dried and crystallized from dioxane/methanol (v; 1:1) to obtain compound **1** as a pale yellow needle. Yield 70%; m.p. 143–145 °C; IR (KBr, v, cm<sup>-1</sup>): 1668 (C=O). <sup>1</sup>H-NMR (DMSO): δ (ppm) 1.45–1.70 (m, 6H, 3CH<sub>2</sub>), 1.79–1.98 (m, 4H, 2CH<sub>2</sub>), 3.52 (s, 2H, SCH<sub>2</sub>CO), 7.01 (d, *J*=8.01, 2H, Ar-H), 7.38 (d, *J*=8.02, 2H, Ar-H). <sup>13</sup>C NMR (DMSO, 75 MHz): δ (ppm) 23.40, 24.39, 25.11, 43.70, 74.13, 115.4, 123.15, 138.2, 162.12, 169.18. MS, m/z (%): 265 (M<sup>+</sup>, 100). Analysis calc. for C<sub>14</sub>H<sub>16</sub>FNOS

(265.35): C, 63.37; H, 6.08; N, 5.28; S, 12.08. Found: C, 63.11; H, 5.82; N, 5.08; S, 11.86.

**5'-Amino-3'-(4-fluorophenyl)-7'-(*p*-tolyl)-3'*H*-spiro[cyclohexane-1,2'-thiazolo [4,5-*b*]pyridine]-6'-carbonitrile (2)[52]**

Compound **1** (0.01 mol); *p*-tolualdehyde (0.01 mol); ammonium acetate (0.02 mol) and malononitrile (0.01 mol) in glacial acetic acid (40 mL) was refluxed for 24 h. The reaction mixture was cooled and poured onto water. The formed solid was filtered off; dried and crystallized from dioxane to give compound **2** as a deep yellow powder. Yield 65%; m.p. 137–139 °C; IR (KBr, v, cm<sup>-1</sup>): 3132 (NH<sub>2</sub>), 2216 (CN). <sup>1</sup>H-NMR (DMSO): δ (ppm) 1.47–1.71 (m, 6H, 3CH<sub>2</sub>), 1.78–1.97 (m, 4H, 2CH<sub>2</sub>), 2.37 (s, 3H, CH<sub>3</sub>), 7.20–7.86 (m, 8H, Ar-H), 8.66 (brs, 2H, NH<sub>2</sub>, D<sub>2</sub>O exchangeable). <sup>13</sup>C



NMR (DMSO, 75 MHz):  $\delta$  (ppm) 21.23, 23.60, 25.12, 38.14, 74.15, 82.21, 114.16, 115.31, 116.25, 121.34, 126.51, 127.25, 131.33, 135.11, 144.12, 151.12, 158.31, 161.60, 163.21. MS,  $m/z$  (%): 430 ( $M^+$ , 70). Analysis calc. for  $C_{25}H_{23}FN_4S$  (430.55): C, 69.74; H, 5.38; N, 13.01; S, 7.45. Found: C, 69.52; H, 5.11; N, 12.79; S, 7.20.

**4'-chloro-8'-(4-fluorophenyl)-5'-(*p*-tolyl)-8'*H*-spiro[cyclohexane-1,7'-thiazolo [5',4':5,6]pyrido[2,3-*d*][1,2,3]triazine] (3)**

A solution of sodium nitrite (0.01 mol) in water (10 mL) was added to a cold solution (0 °C) of compound **2** (0.005 mol) in acetic acid (30 mL) and concentrated hydrochloric acid (15 mL) with stirring in ice bath. After completion of the addition, the ice bath was removed and stirring continued for an additional 2 h at room temperature. The crude product formed was collected by filtration and purification by recrystallized from ethanol to obtain compound **3** as a yellow crystals. Yield 77%; m.p. 148–150 °C.  $^1H$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta$  (ppm) 1.49–2.02 (m, 10H, 5CH<sub>2</sub>), 2.38 (s, 3H, CH<sub>3</sub>), 7.23–7.90 (m, 8H, Ar–H).  $^{13}C$  NMR (DMSO, 75 MHz):  $\delta$  (ppm) 21.09, 23.01, 25.16, 35.02, 76.12, 119.98, 122.31, 124.31, 129.21, 130.55, 131.21, 133.23, 136.88, 141.24, 141.38, 148.31, 154.76, 156.91, 162.21. MS,  $m/z$  (%): 477 ( $M^+$ , 57), 479 ( $M^+$  + 2, 17). Anal. Calcd. for  $C_{25}H_{21}ClFN_5S$  (477.99): C, 62.82; H, 4.43; Cl, 7.42; N, 14.65; S, 6.71. Found: C, 62.57; H, 4.20; Cl, 7.21; N, 14.39; S, 6.44.

**6',8'-diamino-3'-(4-fluorophenyl)-9'-(*p*-tolyl)-3'*H*-spiro[cyclohexane-1,2'-thiazolo[4,5-*b*][1,8]naphthyridine]-7'-carbonitrile (4)**

Compound **2** (0.01 mol) and malononitrile (0.01 mol) in 10 mL DMF containing piperidine (0.1 mL) was heated under reflux for 4 h. The reaction was left to cool at room temperature and then poured onto ice-cold water with stirring. The crude product formed was collected by filtration and purification by recrystallized from acetic acid to give compound **4** as a brown powder, yield 92%, m.p. 160–162 °C; IR (KBr,  $\nu$ ,  $cm^{-1}$ ): 3435 (NH<sub>2</sub>), 3161 (NH<sub>2</sub>), 2219 (CN).  $^1H$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta$  (ppm) 1.48–2.03 (m, 10H, 5CH<sub>2</sub>), 2.39 (s, 3H, CH<sub>3</sub>), 7.24–7.92 (m, 8H, Ar–H), 8.40 (brs, 2H, NH<sub>2</sub>; D<sub>2</sub>O exchangeable), 8.65 (brs, 2H, NH<sub>2</sub>; D<sub>2</sub>O exchangeable).  $^{13}C$  NMR (DMSO, 75 MHz):  $\delta$  (ppm) 21.12, 23.17, 25.35, 35.19, 78.18, 87.95, 119.49, 120.21, 121.52, 123.24, 129.35, 129.71, 132.33, 133.77, 136.89, 137.51, 145.67, 147.09, 148.41, 151.11, 161.53, 163.07. MS,  $m/z$  (%): 496 ( $M^+$ , 80); Anal. Calcd. for  $C_{28}H_{25}FN_6S$  (496.61): C, 67.72; H, 5.07; N, 16.92; S, 6.46. Found: C, 67.49; H, 4.95; N, 16.71; S, 6.23.

**Ethyl 8'-amino-3'-(4-fluorophenyl)-6'-oxo-9'-(*p*-tolyl)-5',6'-dihydro-3'*H*-spiro [cyclohexane-1,2'-thiazolo[4,5-*b*][1,8]naphthyridine]-7'-carboxylate (5).**

Compound **2** (0.01 mol) and diethylmalonate (0.01 mol) was added to 20 mL freshly prepared sodium ethoxide solution [prepared by adding 1 g sodium metal into absolute ethanol (20 mL)] and the mixture was refluxed for 4 h, and left to cool overnight. The solid product formed was collected by filtration, washed with ethanol and recrystallized from ethanol to obtain compound **5** as a white powder. Yield 92%; m.p. 173–175 °C; IR (KBr,  $\nu$ ,  $cm^{-1}$ ): 3272 (NH<sub>2</sub>), 3164 (NH), 1734 (C=O; ester), 1670 (C=O; amide).  $^1H$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta$  (ppm) 1.31 (t,  $J=7.49$ , 3H, CH<sub>2</sub>CH<sub>3</sub>), 1.47–2.02 (m, 10H, 5CH<sub>2</sub>), 2.37 (s, 3H, CH<sub>3</sub>), 4.26 (q,  $J=7.50$ , 2H, CH<sub>2</sub> CH<sub>3</sub>), 7.22–7.97 (m, 9H, 8 Ar–H + NH; D<sub>2</sub>O exchangeable), 8.65 (brs, 2H, NH<sub>2</sub>; D<sub>2</sub>O exchangeable).  $^{13}C$  NMR (DMSO, 75 MHz):  $\delta$  (ppm) 14.82, 21.34, 22.49, 25.73, 34.11, 61.47, 75.31, 92.17, 98.56, 119.91, 122.83, 124.63, 129.72, 130.19, 134.87, 135.79, 136.11, 136.82, 151.90, 158.06, 159.70, 162.27, 167.36, 169.49. MS,  $m/z$  (%): 544 ( $M^+$ , 57); 471 ( $M^+$ -CO<sub>2</sub>Et, 100). Anal. Calcd. for  $C_{30}H_{29}FN_4O_3S$  (544.65): C, 66.16; H, 5.37; N, 10.29; S, 5.89. Found: C, 65.91; H, 5.15; N, 10.02; S, 5.61.

**8'-Amino-3'-(4-fluorophenyl)-6'-oxo-9'-(*p*-tolyl)-5',6'-dihydro-3'*H*-spiro[cyclohexane-1,2'-thiazolo[4,5-*b*][1,8]naphthyridine]-7'-carbonitrile (6).**

Compound **2** (0.01 mol) and ethyl cyanoacetate (0.01 mol) in ethanol (10 mL) containing piperidine (0.1 mL) was heated under reflux for 4 h. The reaction was left to cool at room temperature and the formed was collected by filtration and purification by recrystallized from acetic acid to give compound **6** as a pale yellow crystal, yield 92%, m.p. 158–160 °C; IR (KBr,  $\nu$ ,  $cm^{-1}$ ): 3242 (NH<sub>2</sub>); 3110 (NH); 2217 (CN); 1681 (C=O).  $^1H$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta$  (ppm) 1.48–2.00 (m, 10H, 5CH<sub>2</sub>), 2.39 (s, 3H, CH<sub>3</sub>), 7.19–7.98 (m, 9H, 8Ar-H + NH; D<sub>2</sub>O exchangeable), 8.41 (brs, 2H, NH<sub>2</sub>; D<sub>2</sub>O exchangeable).  $^{13}C$  NMR (DMSO, 75 MHz):  $\delta$  (ppm) 21.11, 22.44, 25.67, 34.45, 69.25, 76.42, 98.71, 114.70, 119.85, 122.34, 124.25, 129.63, 130.25, 134.15, 135.42, 136.34, 137.19, 149.28, 153.91, 161.25, 167.27. MS,  $m/z$  (%): 497 ( $M^+$ , 77). Anal. Calcd. for  $C_{28}H_{24}FN_5OS$  (497.59): C, 67.59; H, 4.86; N, 14.07; S, 6.44. Found: C, 67.28; H, 4.62; N, 13.81; S, 6.21.

**8'-Amino-3'-(4-fluorophenyl)-6'-phenyl-9'-(*p*-tolyl)-3'*H*-spiro[cyclohexane-1,2'-thiazolo[4,5-*b*][1,8]naphthyridine]-7'-carbonitrile (7)**

Equimolar amounts of compound **2** and benzylidenemalononitrile (0.01 mol) was refluxed in dioxane (25 mL)



containing piperidine (0.1 mL) for 5 h. Then allow the reaction mixture to cool and the solid product formed was filtered off and purification by recrystallized from dioxane/dimethylformamide mixture (v; 3:1) to give compound **7** as a brown crystal. Yield 92%; m.p. 170–172 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3290 ( $\text{NH}_2$ ), 2221 (CN).  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta$  (ppm) 1.50–2.03 (m, 10H, 5 $\text{CH}_2$ ), 2.36 (s, 3H,  $\text{CH}_3$ ), 7.17–8.22 (m, 13H, Ar-H), 8.43 (brs, 2H,  $\text{NH}_2$ ;  $\text{D}_2\text{O}$  exchangeable).  $^{13}\text{C}$  NMR (DMSO, 75 MHz):  $\delta$  (ppm) 21.62, 23.21, 25.58, 34.09, 75.96, 87.19, 109.40, 114.58, 119.21, 122.54, 127.26, 128.51, 129.14, 129.92, 130.24, 133.01, 135.57, 136.43, 136.51, 136.88, 137.84, 146.97, 149.40, 151.31, 161.07, 162.68. MS,  $m/z$  (%): 557 ( $\text{M}^+$ , 91), 480 ( $\text{M}^+$ -Ph, 100); Anal. Calcd. for  $\text{C}_{34}\text{H}_{28}\text{FN}_5\text{S}$  (557.69): C, 73.23; H, 5.06; N, 12.56; S, 5.75. Found: C, 73.01; H, 4.82; N, 12.22; S, 5.49.

**2-(8'-Amino-3'-(4-fluorophenyl)-9'-(*p*-tolyl)-3'H-spiro[cyclohexane-1,2'-thiazolo[5,4':5,6]pyrido[2,3-d]pyrimidin]-6'-yl) acetonitrile (8).**

Compound **2** (0.01 mol) and malononitrile (0.01 mol) was added to ethanol (20 mL) and allowed to reflux for 4 h, then left to cool overnight. The solid product formed was collected by filtration, washed with ethanol and purification by recrystallized from ethanol to obtain compound **8** as a white powder. Yield 82%; m.p. 260–262 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3341 ( $\text{NH}_2$ ); 2223 (CN).  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta$  (ppm) 1.47–2.00 (m, 10H, 5 $\text{CH}_2$ ), 2.39 (s, 3H,  $\text{CH}_3$ ), 4.26 (s, 2H,  $\text{CH}_2$ ), 7.20–7.99 (m, 8H, Ar-H), 8.38 (brs, 2H,  $\text{NH}_2$ ;  $\text{D}_2\text{O}$  exchangeable).  $^{13}\text{C}$  NMR (DMSO, 75 MHz):  $\delta$  (ppm) 21.42, 23.36, 24.51, 25.63, 27.96, 34.99, 76.42, 96.84, 114.58, 117.34, 122.52, 129.74, 129.98, 132.89, 136.48, 137.41, 139.31, 143.18, 149.17, 158.41, 161.39, 162.07, 163.05. MS,  $m/z$  (%): 496 ( $\text{M}^+$ , 59); 456 ( $\text{M}^+$ - $\text{CH}_2\text{CN}$ , 100); Anal. Calcd. For  $\text{C}_{28}\text{H}_{25}\text{FN}_6\text{S}$  (496.61): C, 67.72; H, 5.07; N, 16.92; S, 6.46. Found: C, 67.48; H, 4.81; N, 16.67; S, 6.21.

**3'-(4-Fluorophenyl)-9'-(*p*-tolyl)-3'H-spiro[cyclohexane-1,2'-thiazolo[5,4':5,6]pyrido[2,3-d]pyrimidin]-8'-amine (9)**

Compound **2** (0.01 mol) in formamide (10 mL) was refluxed for 5 h, and then left to cool overnight. The formed solid was collected by filtration and purification by recrystallized from dioxane to obtain compound **9** as a gray crystal. Yield 88%; m.p. 150–152 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3244 ( $\text{NH}_2$ ).  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta$  (ppm) 1.48–2.00 (m, 10H, 5 $\text{CH}_2$ ), 2.36 (s, 3H,  $\text{CH}_3$ ), 7.21–7.99 (m, 8H, Ar-H), 8.20 (s, 1H, CH-pyrimidine), 8.55 (brs, 2H,  $\text{NH}_2$ ;  $\text{D}_2\text{O}$  exchangeable).  $^{13}\text{C}$  NMR (DMSO, 75 MHz):  $\delta$  (ppm) 21.12, 22.63, 25.58, 34.14, 75.42, 101.25, 119.88, 122.24, 129.21, 126.74, 132.14,

135.82, 136.11, 140.11, 144.25, 147.02, 153.14, 156.04, 161.46, 163.27. MS,  $m/z$  (%): 457 ( $\text{M}^+$ , 87); Anal. Calcd. For  $\text{C}_{26}\text{H}_{24}\text{FN}_5\text{S}$  (457.57): C, 68.25; H, 5.29; N, 15.31; S, 7.01. Found: C, 68.01; H, 5.05; F, N, 15.09; S, 6.81.

**8'-Amino-3'-(4-fluorophenyl)-9'-(*p*-tolyl)-3'H-spiro [cyclohexane-1,2'-thiazolo[5,4':5,6]pyrido[2,3-d]pyrimidine]-6'(5'H)-thione (10)**

Compound **2** (0.01 mol) and thiourea (0.01 mol) were mixed in a mortar, and then the mixture was allowed to fused at 180 °C in an oil bath for 2 h. The molten product was boiled 10 min with water, cooled and the formed solid was collected by filtration and purification by recrystallized from DMF/ $\text{H}_2\text{O}$  (v; 1:1) to obtain compound **10** as a brown powder. Yield 92%; m.p. 175–177 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3271 ( $\text{NH}_2$ ); 3173 (NH).  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta$  (ppm) 1.48–2.00 (m, 10H, 5 $\text{CH}_2$ ), 2.35 (s, 3H,  $\text{CH}_3$ ), 7.02 (brs, 1H, NH;  $\text{D}_2\text{O}$  exchangeable), 7.21–8.00 (m, 8H, Ar-H), 8.37 (brs, 2H,  $\text{NH}_2$ ;  $\text{D}_2\text{O}$  exchangeable).  $^{13}\text{C}$  NMR (DMSO, 75 MHz):  $\delta$  (ppm) 21.01, 22.12, 25.62, 34.25, 76.15, 99.46, 119.80, 122.24, 129.04, 131.01, 134.00, 135.08, 137.00, 141.24, 146.30, 149.11, 154.10, 161.50, 162.77, 172.19. MS,  $m/z$  (%): 489 ( $\text{M}^+$ , 66); Anal. Calcd. for  $\text{C}_{26}\text{H}_{24}\text{FN}_5\text{S}_2$  (489.63): C, 63.78; H, 4.94; N, 14.30; S, 13.10. Found: C, 63.51; H, 4.69; N, 14.10; S, 12.89.

**3'-(4-Fluorophenyl)-6'-phenyl-9'-(*p*-tolyl)-3'H-spiro [cyclohexane-1,2'-thiazolo [5,4':5,6]pyrido[2,3-d]pyrimidin]-8'-amine (11)**

Compound **2** (0.01 mol) in 20 mL freshly prepared sodium ethoxide solution [prepared by adding 1 g sodium metal into absolute ethanol (20 mL)] and benzonitrile (0.01 mol) in 2-propanol (10 mL) was refluxed for 20 h. On cooling the precipitate formed was collected by filtration and purification by recrystallized from ethanol to give compound **11** as a yellow powder. Yield 92%; m.p. 220–222 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3371 ( $\text{NH}_2$ ).  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta$  (ppm) 1.49–2.01 (m, 10H, 5 $\text{CH}_2$ ), 2.36 (s, 3H,  $\text{CH}_3$ ), 7.19–8.23 (m, 13H, Ar-H), 8.41 (brs, 2H,  $\text{NH}_2$ ;  $\text{D}_2\text{O}$  exchangeable).  $^{13}\text{C}$  NMR (DMSO, 75 MHz):  $\delta$  (ppm) 21.32, 22.17, 25.37, 34.29, 75.14, 96.92, 119.28, 121.35, 125.55, 126.34, 129.74, 130.00, 132.89, 134.88, 136.14, 137.05, 138.02, 141.32, 144.02, 147.00, 151.16, 154.61, 157.07, 162.11. MS,  $m/z$  (%): 533 ( $\text{M}^+$ , 47), 456 ( $\text{M}^+$ -Ph, 100); Anal. Calcd. For  $\text{C}_{32}\text{H}_{28}\text{FN}_5\text{S}$  (533.67): C, 72.02; H, 5.29; N, 13.12; S, 6.01. Found: C, 71.79; H, 5.07; N, 12.85; S, 5.81.

**3'-(4-Fluorophenyl)-8'-imino-7'-phenyl-9'-(*p*-tolyl)-7',8'-dihydro-3'*H*-spiro[cyclohexane-1,2'-thiazolo[5',4':5,6]pyrido[2,3-d]pyrimidine]-6'(5'*H*)-thione (12).**

Compound **2** (0.01 mol) and phenyl isothiocyanate (0.01 mol) in 20 mL freshly prepared sodium ethoxide solution [prepared by adding 1 g sodium metal into absolute ethanol (20 mL)] was refluxed for 20 h. The solvent was distilled off and the obtained solid was collected and purification by recrystallized from tetrahydrofuran (THF) to give compound **12** as a white powder. Yield 90%; m.p. 145–147 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3197 (NH); 3117 (NH).  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta$  (ppm) 1.50–2.04 (m, 10H, 5CH<sub>2</sub>), 2.35 (s, 3H, CH<sub>3</sub>), 7.21–8.41 (m, 14H, 13Ar-H + NH; D<sub>2</sub>O exchangeable), 9.15 (brs, 1H, NH; D<sub>2</sub>O exchangeable).  $^{13}\text{C}$  NMR (DMSO, 75 MHz):  $\delta$  (ppm) 21.19, 23.11, 24.58, 34.09, 75.35, 94.96, 119.09, 123.45, 127.01, 128.02, 129.74, 130.11, 130.79, 132.71, 134.19, 135.88, 137.00, 141.02, 142.11, 143.20, 155.00, 161.14, 162.70, 171.21. MS,  $m/z$  (%): 565 (M<sup>+</sup>, 39); 455 (M<sup>+</sup>-[Ph + SH], 100); Anal. Calcd. For C<sub>32</sub>H<sub>28</sub>FN<sub>5</sub>S<sub>2</sub> (565.73): C, 67.94; H, 4.99; N, 12.38; S, 11.33. Found: C, 67.69; H, 4.71; N, 12.14; S, 11.09.

**3'-(4-Fluorophenyl)-9'-(*p*-tolyl)-3'*H*-spiro[cyclohexane-1,2'-thiazolo[5',4':5,6]pyrido[2,3-d]pyrimidin]-8'(7'*H*)-one (13)**

Compound **2** (0.01 mol) was refluxed in formic acid (20 mL) for 4 h, and left to cool overnight. The solid product formed was collected by filtration and purification by recrystallized from ethanol to give compound **13** as a brown crystal. Yield 92%; m.p. 166–168 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3110 (NH); 1685 (C=O).  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta$  (ppm) 1.49–2.00 (m, 10H, 5CH<sub>2</sub>), 2.36 (s, 3H, CH<sub>3</sub>), 7.21–7.97 (m, 8H, Ar-H), 8.28 (s, 1H, pyrimidine-H), 9.11 (brs, 1H, NH; D<sub>2</sub>O exchangeable).  $^{13}\text{C}$  NMR (DMSO, 75 MHz):  $\delta$  (ppm) 21.17, 22.17, 25.18, 34.21, 74.21, 119.21, 120.25, 121.05, 122.55, 128.74, 131.11, 135.00, 136.88, 137.11, 139.02, 143.21, 146.80, 149.28, 161.76, 162.20. MS,  $m/z$  (%): 458 (M<sup>+</sup>, 69); Anal. Calcd. For C<sub>26</sub>H<sub>23</sub>FN<sub>4</sub>OS (458.56): C, 68.10; H, 5.06; N, 12.22; O, 3.49; S, 6.99. Found: C, 67.85; H, 4.81; N, 12.01; O, 3.21; S, 6.71.

**6'-(Chloromethyl)-3'-(4-fluorophenyl)-9'-(*p*-tolyl)-3'*H*-spiro[cyclohexane-1,2'-thiazolo[5',4':5,6]pyrido[2,3-d]pyrimidin]-8'(7'*H*)-one(14).**

Compound **2** (0.01 mol) and chloroacetyl chloride (0.01 mol) in DMF (20 mL) was stirred at room temperature for 2 h. The reaction mixture was poured onto ice water and the obtained solid was collected by filtration and purification by recrystallized from ethanol to give compound **14** as a yellow crystal.

Yield 85%; m.p. 171–173 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3142 (NH); 1682 (C=O).  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta$  (ppm) 1.47–2.00 (m, 10H, 5CH<sub>2</sub>), 2.36 (s, 3H, CH<sub>3</sub>), 5.17 (s, 2H, CH<sub>2</sub>Cl), 7.21–7.97 (m, 8H, Ar-H); 9.55 (brs, 1H, NH; D<sub>2</sub>O exchangeable).  $^{13}\text{C}$  NMR (DMSO, 75 MHz):  $\delta$  (ppm) 21.71, 22.24, 25.21, 35.09, 47.17, 75.01., 100.22, 119.35, 120.05, 123.15, 128.05, 130.09, 133.89, 135.88, 137.25, 139.15, 143.02, 147.21, 160.16, 161.70, 162.19. MS,  $m/z$  (%): 507 (M<sup>+</sup>, 57), 509 (M<sup>+</sup> + 2, 18), 458 (M<sup>+</sup>-CH<sub>2</sub>Cl, 100); Anal. Calcd. for C<sub>27</sub>H<sub>24</sub>ClFN<sub>4</sub>OS (507.02): C, 63.96; H, 4.77; Cl, 6.99; N, 11.05; S, 6.32. Found: C, 63.71; H, 4.39; Cl, 6.42; N, 11.80; S, 6.05.

**3'-(4-Fluorophenyl)-6'-methyl-9'-(*p*-tolyl)-3'*H*-spiro[cyclohexane-1,2'-thiazolo[5',4':5,6]pyrido[2,3-d]pyrimidin]-8'(7'*H*)-one (15)**

A mixture of compound **2** (0.01 mol) and thioacetamide (0.01 mol) in trifluoroacetic acid (10 mL) was refluxed for 7 h. The reaction mixture was cooled and poured onto ice water. The obtained solid was collected by filtration and purification by recrystallized from ethanol to give compound **15** as a brown crystal. Yield 82%; m.p. 156–158 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3139 (NH).  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta$  (ppm) 1.48–2.02 (m, 10H, 5CH<sub>2</sub>), 2.32 (s, 3H, CH<sub>3</sub>), 2.37 (s, 3H, CH<sub>3</sub>), 7.21–7.98 (m, 8H, Ar-H), 9.39 (brs, 1H, NH; D<sub>2</sub>O exchangeable).  $^{13}\text{C}$  NMR (DMSO, 75 MHz):  $\delta$  (ppm) 20.02, 21.15, 22.37, 25.22, 35.19, 45.29, 119.25, 120.68, 122.45, 129.14, 131.00, 133.82, 135.88, 136.71, 138.21, 142.32, 144.67, 146.61, 161.10, 162.28, 169.32. MS,  $m/z$  (%): 472 (M<sup>+</sup>, 63), 457 (M<sup>+</sup>-Me, 100), Anal. Calcd. for C<sub>27</sub>H<sub>25</sub>FN<sub>4</sub>OS (472.58): C, 68.62; H, 5.33; N, 11.86; S, 6.78. Found: C, 68.35; H, 5.07; N, 11.61; S, 6.54.

**General method for synthesis of amino-sugar derivatives 16–19.**

Aldoses; D-xylose, D-arabinose, D-glucose, and D-galactose (0.05 mol) in water (1 mL) was added to compound **2** (0.01 mol) in ethanol (30 mL) /glacial acetic acid (0.5 mL). The reaction mixture was refluxed for 6 h (TLC; chloroform: methanol; 95:5), then keep the reaction mixture cooled down to room temperature. The excess ethanol was evaporated under reduced pressure, and the residue was treated with diethyl ether (15 mL). The solid formed was filtered off and crystallized from ethanol/DMF (3:1) to give the corresponding amino sugar **16–19**, respectively.

**(D-Xylose) 3'-(4-fluorophenyl)-5'-(methyleneamino)-7'-(*p*-tolyl)-3'*H*-spiro [cyclohexane-1,2'-thiazolo[4,5-b]pyridine]-6'-carbonitrile (16).** Yield 77%, m.p. 242–244 °C. IR spectrum,  $\nu$ ,  $\text{cm}^{-1}$ : 3463 (OH), 2116 (CN).  $^1\text{H}$ -NMR (DMSO):  $\delta$  (ppm) 1.48–2.01 (m, 10H, 5CH<sub>2</sub>), 2.36 (s, 3H,

CH<sub>3</sub>), 3.44–3.52 (m, 2H, H-5' + H-5''), 3.99–4.22 (m, 1H, H-4'), 4.17–4.23 (m, 2H, H-3' + H-4'), 4.30–4.35 (m, 1H, OH, D<sub>2</sub>O exchangeable), 4.44–4.62 (m, 2H, H-2' + OH, D<sub>2</sub>O exchangeable), 5.20–5.24 (m, 1H, OH, D<sub>2</sub>O exchangeable), 7.20–7.86 (m, 9H, 8Ar-H + H-1'). <sup>13</sup>C NMR (DMSO, 75 MHz): δ (ppm) 21.16, 22.36, 25.60, 34.18, 64.43, 72.27, 72.95, 73.10, 85.63, 95.89, 114.88, 115.82, 122.56, 124.66, 129.55, 130.17, 134.11, 136.03, 137.27, 154.97, 155.03, 160.81, 162.92, 164.02. Analysis calc. for C<sub>30</sub>H<sub>31</sub>FN<sub>4</sub>O<sub>4</sub>S: C, 64.04; H, 5.55; N, 9.96; S, 5.70. Found: C, 63.76; H, 5.39; N, 9.70; S, 5.44.

**D-Arabinose 3'-(4-fluorophenyl)-5'-(methyleneamino)-7'-(*p*-tolyl)-3'H-spiro [cyclohexane-1,2'-thiazolo[4,5-b]pyridine]-6'-carbonitrile (17).** Yield 69%, m.p. 161–163 °C. IR spectrum, ν, cm<sup>-1</sup>: 3520 (OH), 2118 (CN). <sup>1</sup>H-NMR (DMSO): δ (ppm) 1.48–2.01 (m, 10H, 5CH<sub>2</sub>), 2.36 (s, 3H, CH<sub>3</sub>), 3.35–3.43 (m, 2H, H-5' + H-5''), 4.11–4.20 (m, 2H, H-3' + H-4'), 4.61–4.64 (m, 1H, H-2'), 4.37–4.41 (m, 1H, OH, D<sub>2</sub>O exchangeable), 4.82 (d, *J* = 6.25 Hz, 1H, OH, D<sub>2</sub>O exchangeable), 4.97–5.01 (m, 1H, OH, D<sub>2</sub>O exchangeable), 5.55–5.58 (m, 1H, OH, D<sub>2</sub>O exchangeable), 7.20–7.86 (m, 9H, 8Ar-H + H-1'). <sup>13</sup>C NMR (DMSO, 75 MHz): δ (ppm) 21.17, 22.35, 25.61, 34.19, 64.44, 72.29, 72.95, 73.35, 85.67, 95.92, 114.91, 115.80, 122.51, 124.71, 129.50, 130.07, 134.11, 136.11, 137.31, 154.97, 155.08, 160.81, 162.75, 163.92. Analysis calc. for C<sub>30</sub>H<sub>31</sub>FN<sub>4</sub>O<sub>4</sub>S: C, 64.04; H, 5.55; N, 9.96; S, 5.70. Found: C, 63.77; H, 5.37; N, 9.71; S, 5.45.

**(D-Glucose) 3'-(4-fluorophenyl)-5'-(methyleneamino)-7'-(*p*-tolyl)-3'H-spiro[cyclohexane-1,2'-thiazolo[4,5-b]pyridine]-6'-carbonitrile (18).** Yield 74%, m.p. 201–203 °C. IR spectrum, ν, cm<sup>-1</sup>: 3450 (OH), 2117 (CN). <sup>1</sup>H-NMR (DMSO): δ (ppm) 1.49–2.01 (m, 10H, 5CH<sub>2</sub>), 2.39 (s, 3H, CH<sub>3</sub>), 3.34–3.41 (m, 2H, H-6' + H-6''), 3.54–3.57 (m, 1H, H-5'), 3.82–3.97 (m, 2H, H-3' + H-4'), 4.28–4.40 (m, 2H, H-2' + OH, D<sub>2</sub>O exchangeable), 4.59 (d, 1H, *J* = 6.19 Hz, OH), 4.61–4.70 (m, 1H, OH, D<sub>2</sub>O exchangeable), 4.78–4.80 (m, 1H, OH, D<sub>2</sub>O exchangeable), 4.92–4.95 (m, 1H, OH, D<sub>2</sub>O exchangeable), 7.20–7.86 (m, 9H, 8Ar-H + H-1'). <sup>13</sup>C NMR (DMSO, 75 MHz): δ (ppm) 21.17, 22.36, 25.62, 34.19, 64.47, 72.05, 74.35, 72.44, 73.75, 85.67, 95.93, 114.97, 115.88, 122.57, 124.71, 129.51, 130.07, 134.04, 136.07, 137.31, 154.97, 155.04, 160.81, 162.75, 163.98. Analysis calc. for C<sub>31</sub>H<sub>33</sub>FN<sub>4</sub>O<sub>5</sub>S: C, 62.82; H, 5.61; N, 9.45; S, 5.41. Found: C, 62.57; H, 5.37; N, 9.19; S, 5.15.

**(D-Galactose) 3'-(4-fluorophenyl)-5'-(methyleneamino)-7'-(*p*-tolyl)-3'H-spiro[cyclohexane-1,2'-thiazolo[4,5-b]pyridine]-6'-carbonitrile (19).** Yield 67%, m.p. 217–219 °C. IR spectrum, ν, cm<sup>-1</sup>: 3461 (OH), 2121 (CN). <sup>1</sup>H-NMR (DMSO): δ (ppm) 1.48–2.00 (m, 10H, 5CH<sub>2</sub>), 2.38 (s, 3H,

CH<sub>3</sub>), 3.29–3.41 (m, 2H, H-6' + H-6''), 3.58–3.59 (m, 1H, H-5'), 3.84–3.99 (m, 2H, H-3' + H-4'), 4.34–4.41 (m, 2H, H-2' + OH, D<sub>2</sub>O exchangeable), 4.61 (d, 1H, *J* = 6.19 Hz, OH), 4.75–4.79 (m, 1H, OH, D<sub>2</sub>O exchangeable), 4.91 (t, *J* = 8.4 Hz, 1H, OH, D<sub>2</sub>O exchangeable), 5.89–4.94 (m, 1H, OH, D<sub>2</sub>O exchangeable), 7.20–7.86 (m, 9H, 8Ar-H + H-1'). <sup>13</sup>C NMR (DMSO, 75 MHz): δ (ppm) 21.14, 22.36, 25.61, 34.17, 62.47, 72.03, 72.18, 72.42, 74.77, 85.64, 95.92, 114.93, 115.86, 122.55, 124.71, 129.49, 130.09, 134.06, 136.09, 137.31, 154.99, 155.06, 160.74, 162.59, 163.91. Analysis calc. for C<sub>31</sub>H<sub>33</sub>FN<sub>4</sub>O<sub>5</sub>S: C, 62.82; H, 5.61; N, 9.45; S, 5.41. Found: C, 62.55; H, 5.39; N, 9.21; S, 5.16.

#### General method for synthesis of acetylated amino sugar derivatives 20–23.

A solution of the amino sugars **16–19** (1 mmol) in acetic anhydride/acetic acid (10 mL; 1:1) was heated at 100 °C until *TLC* (chloroform: methanol; 96:4) showed completion of the reaction. The resulting solution was poured onto ice-cold water, and then extracted with chloroform (40 mL). Sodium hydrogen carbonate was added to the organic layer and mixture was stirred for 1 h and filtered. The chloroform layer was washed with water, dried with sodium sulfate anhydrous and evaporated till dryness to afford the corresponding acetyl sugar derivatives **20–23**, respectively.

**(1,2,3,4-Tetra-O-acetyl-D-xylose) 3'-(4-fluorophenyl)-5'-(methyleneamino)-7'-(*p*-tolyl)-3'H-spiro[cyclohexane-1,2'-thiazolo[4,5-b]pyridine]-6'-carbonitrile (20).** Yield 62%, m.p. 169–171 °C. IR spectrum, ν, cm<sup>-1</sup>: 2116 (CN), 1748 (C=O). <sup>1</sup>H-NMR (DMSO): δ (ppm) 1.48–2.01 (m, 10H, 5CH<sub>2</sub>), 2.10 (s, 3H, COCH<sub>3</sub>), 2.13 (s, 3H, COCH<sub>3</sub>), 2.17 (s, 3H, COCH<sub>3</sub>), 2.20 (s, 3H, COCH<sub>3</sub>), 2.37 (s, 3H, CH<sub>3</sub>), 3.84–3.92 (m, 2H, H-5' + H-5''), 5.11–5.17 (m, 2H, H-3' + H-4'), 5.61–5.72 (m, 1H, H-2'), 7.29–7.89 (m, 9H, 8Ar-H + H-1'). <sup>13</sup>C NMR (DMSO, 75 MHz): δ (ppm) 20.72, 21.14, 21.29, 22.37, 25.57, 34.18, 36.67, 62.44, 68.51, 68.92, 71.45, 85.60, 95.00, 114.75, 115.84, 122.55, 125.19, 129.51, 130.19, 134.33, 136.30, 137.49, 156.56, 157.11, 160.73, 165.44, 165.99, 170.88, 171.22, 171.85. Analysis calc. for C<sub>38</sub>H<sub>39</sub>FN<sub>4</sub>O<sub>8</sub>S: C, 62.45; H, 5.38; N, 7.67; S, 4.39. Found: C, 62.21; H, 5.11; N, 7.41; S, 4.10.

**(1,2,3,4-Tetra-O-acetyl-D-arabinose) 3'-(4-fluorophenyl)-5'-(methyleneamino)-7'-(*p*-tolyl)-3'H-spiro[cyclohexane-1,2'-thiazolo[4,5-b]pyridine]-6'-carbonitrile (21).** Yield 73%, m.p. 157–159 °C. IR spectrum, ν, cm<sup>-1</sup>: 2117 (CN), 1755 (C=O). <sup>1</sup>H-NMR (DMSO): δ (ppm) 1.48–2.01 (m, 10H, 5CH<sub>2</sub>), 2.08 (s, 3H, COCH<sub>3</sub>), 2.011 (s, 3H, COCH<sub>3</sub>), 2.13 (s, 3H, COCH<sub>3</sub>), 2.18 (s, 3H, COCH<sub>3</sub>), 2.38 (s, 3H, CH<sub>3</sub>), 3.97 (dd, 1H, *J* = 11.19, 2.81 Hz, H-5'), 4.16 (dd, 1H, *J* = 11.20, 3.20 Hz, H-5''), 4.29–4.33 (m, 1H, H-4'), 5.21 (dd, 0.1H,

$J=7.80, 7.2$  Hz, H-3'), 5.32 (dd, 0.1H,  $J=7.80, 7.2$  Hz, H-2') 7.20–7.86 (m, 9H, 8Ar-H + H-1').  $^{13}\text{C}$  NMR (DMSO, 75 MHz):  $\delta$  (ppm) 20.70, 21.12, 21.27, 22.36, 25.56, 34.18, 62.44, 67.51, 67.92, 68.45, 85.60, 95.03, 114.75, 115.84, 122.55, 124.35, 129.51, 130.19, 134.33, 136.30, 137.49, 156.56, 157.11, 160.73, 164.98, 165.44, 165.99, 170.88, 171.22, 171.85. Analysis calc. for  $\text{C}_{38}\text{H}_{39}\text{FN}_4\text{O}_8\text{S}$ : C, 62.45; H, 5.38; N, 7.67; S, 4.39. Found: C, 62.23; H, 5.15; N, 7.40; S, 4.15.

**(1,2,3,4,5-Penta-O-acetyl-D-glucose) 3'-(4-fluorophenyl)-5'-(methyleneamino)-7'-(p-tolyl)-3'H-spiro[cyclohexane-1,2'-thiazolo[4,5-b]pyridine]-6'-carbonitrile (22).** Yield 67%, m.p. 211–213 °C. IR spectrum,  $\nu$ ,  $\text{cm}^{-1}$ : 2118 (CN), 1741 (C=O).  $^1\text{H}$ -NMR (DMSO):  $\delta$  (ppm) 1.47–1.99 (m, 10H, 5CH<sub>2</sub>), 2.09 (s, 3H, COCH<sub>3</sub>), 2.011 (s, 3H, COCH<sub>3</sub>), 2.14 (s, 3H, COCH<sub>3</sub>), 2.20 (s, 3H, COCH<sub>3</sub>), 2.22 (s, 3H, COCH<sub>3</sub>), 2.389 (s, 3H, CH<sub>3</sub>), 3.81–3.88 (m, 2H, H-6' + H-6''), 4.04–4.06 (m, 1H, H-5'), 4.33–4.42 (m, 2H, H-3' + H-4'), 4.80–4.88 (m, 1H, H-2'), 7.28–7.88 (m, 9H, 8Ar-H + H-1').  $^{13}\text{C}$  NMR (DMSO, 75 MHz):  $\delta$  (ppm) 20.66, 21.14, 21.21, 22.31, 25.54, 34.18, 62.39, 67.15, 67.70, 67.88, 69.55, 85.40, 95.33, 114.77, 115.85, 122.15, 124.35, 129.50, 130.09, 134.13, 136.10, 137.39, 154.56, 156.11, 156.88, 160.63, 163.98, 170.22, 170.88, 171.22, 171.92. Analysis calc. for  $\text{C}_{41}\text{H}_{43}\text{FN}_4\text{O}_{10}\text{S}$ : C, 61.34; H, 5.40; N, 6.98; S, 3.89. Found: C, 61.09; H, 5.14; N, 6.76; S, 3.74.

**(1,2,3,4,5-penta-O-acetyl-D-galactose) 3'-(4-fluorophenyl)-5'-(methyleneamino)-7'-(p-tolyl)-3'H-spiro[cyclohexane-1,2'-thiazolo[4,5-b]pyridine]-6'-carbonitrile (23).** Yield 59%, m.p. 254–256 °C. IR spectrum,  $\nu$ ,  $\text{cm}^{-1}$ : 2121 (CN), 1738 (C=O).  $^1\text{H}$ -NMR (DMSO):  $\delta$  (ppm) 1.48–2.00 (m, 10H, 5CH<sub>2</sub>), 2.09 (s, 3H, COCH<sub>3</sub>), 2.11 (s, 3H, COCH<sub>3</sub>), 2.14 (s, 3H, COCH<sub>3</sub>), 2.19 (s, 3H, COCH<sub>3</sub>), 2.22 (s, 3H, COCH<sub>3</sub>), 2.37 (s, 3H, CH<sub>3</sub>), 3.79–3.83 (m, 2H, H-6 + H-6''), 4.11–4.16 (m, 1H, H-5'), 4.34–4.66 (m, 2H, H-3' + H-4'), 4.84–4.61 (m, 1H, H-2'), 7.27–7.87 (m, 9H, 8Ar-H + H-1').  $^{13}\text{C}$  NMR (DMSO, 75 MHz):  $\delta$  (ppm) 20.68, 21.15, 21.24, 22.37, 25.55, 34.17, 62.40, 67.18, 67.82, 67.88, 69.51, 85.42, 95.35, 114.73, 115.84, 122.16, 124.37, 129.51, 130.08, 134.15, 136.11, 137.38, 154.58, 156.14, 156.86, 160.61, 163.95, 170.25, 170.85, 171.20, 171.95. Analysis calc. for  $\text{C}_{41}\text{H}_{43}\text{FN}_4\text{O}_{10}\text{S}$ : C, 61.34; H, 5.40; N, 6.98; S, 3.89. Found: C, 61.11; H, 5.17; N, 6.74; S, 3.72.

### Anti-microbial activity

The antimicrobial potency of the tested samples was examined against some targeted pathogenic microorganisms obtained from the American type culture collection (ATCC; Rockville, MD, USA). The tested organisms were *Staphylococcus aureus* ATCC-47,077 (St.), *Bacillus cereus*

ATCC-12228 (B.C.), *Escherichia coli* ATCC-25922 (E.C.), *Salmonella typhi* ATCC-15566 (Salm.) and *Candida albicans* ATCC-10231 (C. Alb.). The stock cultures of pathogens utilized in this study were kept on nutrient agar slants at 4 °C. The Agar well diffusion method was employed to study the antimicrobial activities of the samples according to the method described. Reference antibacterial drug ampicillin was evaluated for their antibacterial and antifungal action and compared with the tested samples. Seventy microliters of bacterial and yeast cells ( $10^6$  CFU/mL) of each pathogen were spread on the nutrient agar plates. The wells (6 mm diameter) were dug on the inoculated agar plates and 100  $\mu\text{L}$  of the samples and its derivatives suspended in DMSO, were added to the wells. The reference antibiotics disks (10  $\mu\text{g}$ /disk of ampicillin) were potted onto surface of agar inoculated plates. The plates were allowed to stand at 4 °C for 2 h before incubation to allow for diffusion. The plates were incubated at 37 °C for 24 h except yeast strain that were incubated at 28 °C for 24 h then followed by the measurement of the diameter of the inhibition zone (mm), and three replicates were averaged [53–55].

### Minimum inhibition concentration (MIC):

The MIC calculation of the prepared materials was performed according to a slightly modified previous method. In brief, serial dilutions were prepared of the tested materials dissolved in DMSO. 150  $\mu\text{L}$  of double strength Mueller Hinton broth medium were loaded in each well of the 96 well micro liter plate followed by 150  $\mu\text{L}$  of the twofold appropriate concentration and mixed well to obtain the final concentration. Overnight broth cultures of the tested bacterial and yeast strains prepared as an inoculum of 5% (V/V) (OD = 0.5 McFarland standard) was inoculated into the respective wells. For the positive growth control, the same inoculum size of each test strain was inoculated in wells that did not containing any of the tested materials. DMSO solution was tested as negative control. The plates were statically incubated at 37 °C for 24 h. 30  $\mu\text{L}$  of prepared solution (0.18%) was added to each well to act as an electron acceptor and reduce to a pink, red or purple resourcing colored product by active micro-organisms (i.e., inhibition of bacterial growth was visible as a dark blue well and the presence of growth was detected by the presence of pink, red or purple color). The MIC was defined as the concentration at which the bacteria and yeast do not show visible growth with respect to the positive control [53–55].

### Statistical analysis

Statistical analyses were carried out using GraphPad Prism 5.0 (Graph Pad Software, LaJolla, CA). In One-way model ANOVA, the observed variance is partitioned into

components due to different explanatory variables. A level of  $p < 0.05$  was considered to be statistically significant.

**Funding** Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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