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# Diastereoselective Synthesis, Characterization, Investigation of Anticancer, Antibacterial Activities, *In Silico* Approaches and DNA/BSA Binding Affinities of Novel Pyrimidine-Sugar Derivatives

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ABSTRACT

Eighteen novel carbohydrate conjugates (**1-9**) and their tetra-*O*-acetyl derivatives (**10-18**) were synthesized in total and evaluated for their biological properties, including antimicrobial and anticancer functions, and DNA/protein binding affinities. The compounds were prepared by firstly glycosylation and secondly acetylation methods. The structures of all compounds were elucidated by spectral analysis and the results showed that the glycoconjugates were obtained by diastereoselectivity as pure $\beta$- anomer. To observe cell proliferation, cytotoxicity and microdilution, different cancer cell lines (Hep3B, A549, HeLa, C6, HT29, MCF7) were treated with pyrimidine *N*-$\beta$-D-glycosides (**1-9**) and their tetra-*O*-acetyl derivatives (**10-18**). These new carbohydrate conjugates and the controls showed the same non-toxic property to the cells, while **10-18** displayed lower cytotoxic potency than **1-9**. And to support the experimental results of some compounds (**8, 9, 17**, and **18**) whose pharmacological properties were determined, molecular docking studies were performed, which belonged to the *in silico* methods. The values of the binding parameters of these compounds with different receptors were determined by molecular docking. Studies on pathogenic bacteria revealed that both groups of new compounds exhibited significant antimicrobial activity with low concentrations (31.25-125 μg/mL). Significant data have been obtained indicating that they can bind to DNA via groove binding, with binding constants ranging from $1.1 \times 10^3$ to $4.0 \times 10^4$. In summary, preliminary information indicates that acetylated derivatives (**10-18**) have effective pharmacological properties.

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## 1. Introduction

Multidrug-resistant bacteria have recently caused and exacerbated life-threatening infestations worldwide. The increase in antibiotic resistance is of great importance for the treatment of infections and poses a risk. Therefore, the research of a new and influential antibacterial agent is essential to combat drug resistance [1]. Moreover, some important experimental results have shown the close relationship between pathogenic microbial flora and certain diseases such as gastric, oropharyngeal, hepatic, genitourinary, cervical cancer, and lymph node disorder [2]. It can well be proven that such pathogenic microbial flora often causes some chronic inflammations due to its toxic microbial metabolites, which may lead to an increased risk of cancer or development of cancer-related diseases [3,4].

Cancer, defined as the uncontrolled growth and spread of an abnormal cell, has long been recognised as one of the most common fatal diseases of our time. Cancer and cancer-related diseases have recently become one of the leading causes of death, right after traffic accidents and common injuries [5,6]. Chemotherapy has a critical role in cancer treatment, but agents used in chemotherapy cause many side effects due to their cytotoxic and mutagenic effects on healthy cells [5,6]. Therefore, the development of alternative drugs with or without negligible side effects on the human body and without multi-drug resistance is essential.

In the treatment of both cancer and inflammation, dual- acting compounds with antibacterial and anticancer properties could be a good alternative. In practice, this may let physicians to effectively cure cancer and inflammation, provided that intelligent dose adjustment is made [7].

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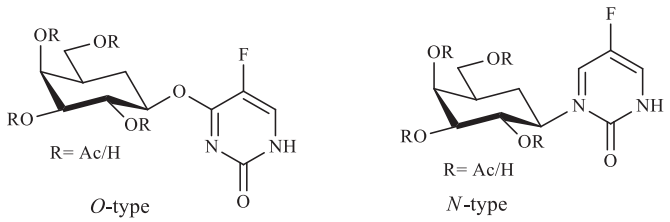



**Figure 1.** *O*- and *N*-galactopyranosides of 5-Fluorouracil [10]

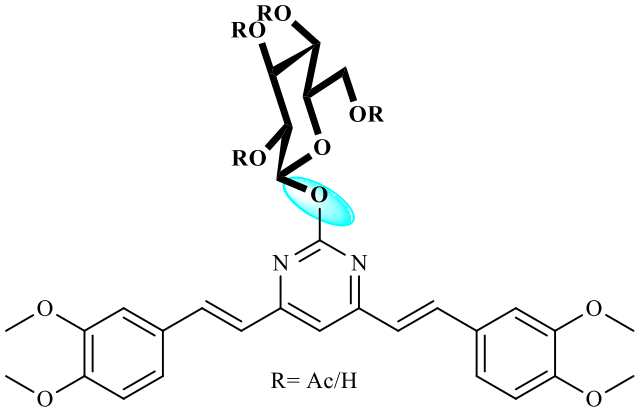
Carbohydrates allow extremely limitless structural variations among major classes of biomolecules, and a wide variety of combined compounds can be formed by combining different monosaccharides with different stereochemistry [8]. The structural differences of carbohydrates allow them to be considered as "special" molecules that can be transformed into a variety of biological properties [8]. Moreover, due to their rigid structure with a high degree of functionality and the presence of several adjacent stereogenic centers, they have a wide usage as chiral templates to introduce chirality in synthetic asymmetric methods [9].

The chemistry and glycobiology of glycoconjugates have attracted considerable great attention in recent years with increasing importance due to the recognition of the role of carbohydrates in biological processes such as immune responses, adhesion, inflammation, and cell growth [10,11]. Among these structures, heterocyclic linked glycosides are essential and reliable sites for the development of many anticancer and antitumor drugs [10] and are considered good glycosyl donors in addition to their biological properties, such as enzyme inhibitory activity [10]. The toxic effect of 5-fluorouracil, which is used as an excellent drug in the treatment of antimetabolic cancer, on mammalian cells can be reduced by the synthesis of *N*- or *O*-galactopyranoside derivatives, and the inhibitory effect of this drug on cancer cells can be improved (Figure 1) [10].

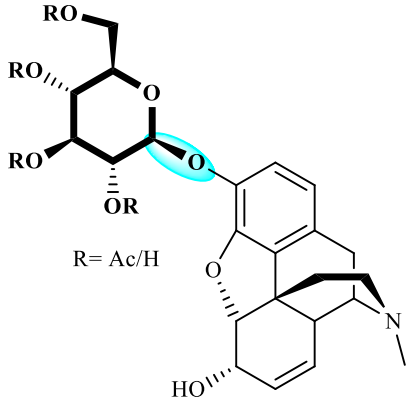
In addition, the pharmacological properties and bioavailability of the compounds can be improved by glycosylation, which contributes to the water solubility and stability of the organic molecules. Furthermore, glycoside derivatives of physiologically active compounds such as vitamin glycosides have been reported to be useful antiallergic agents [12].

There are many synthetic glycosides in the literature with striking properties. Curcuminoids, for example, are bioactive compounds of turmeric plant used as a medicinal herb. Literature shows that these compounds have hypoglycemic, anti-rheumatic, anti-inflammatory, anticancer, and antioxidant activities [12]. But low water solubility and bioavailability, poor stability and rapid systematic excretion limit the usefulness of curcumin compounds. Therefore, it is necessary to improve the bioavailibility and biological properties of curcumin derivatives. For this reason, pyrimidine-*O*-glycosides resembling curcuminoids were prepared by Gao et al. [12]. In addition, morphine-*O*-glycosides [13] and pyrazole-linked glycosides are some of the other glycoside compounds that have been reported by researchers in the literature [10]. Terfestatin A, reported as a novel auxin signaling inhibitor, is another compound known in the literature as a glycoside derivative (Figure 2) [14].

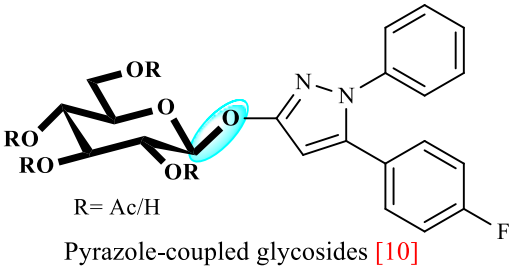
Despite the difficulties in syntheses, the biological activities of glycosides and glycoconjugates have always inspired glycochemists to new syntheses [15,16]. The synthesis of *N*-glycosides has also never been thoroughly explored because the stereoselective induction of nitrogen scaffolds at the anomeric position is a particularly difficult task [15]. The main reason for the increasing interest in the synthesis of *N*-glycosides is that they are integral components of glycoproteins, glycolipids, peptidoglycans, and proteoglycans in biological systems. In addition, the literature review has shown that *N*-glycosyl amines are potential therapeu-



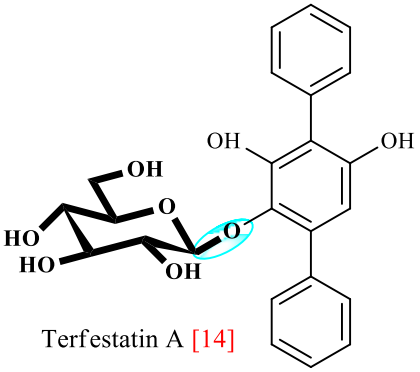

Pyrimidine-*O*- glycosides of curcuminoids[12]




Morphine-*O*- glycosides [13]




Pyrazole-coupled glycosides [10]




Terfestatin A [14]

**Figure 2.** Structures of some synthetic and bioactive *O*-glycosides

tic candidates for the treatment of inflammation and infection [17,18].

In recent years, the concept of hybrid drugs, in which two or more pharmacophore groups are combined to produce a synergistic effect, has attracted much attention [19]. Using this approach, several researchers have recently reported numerous hybrid compounds combining medically privileged heterocycles with biologically important pharmacophores [10,19]. Therefore, the efficient synthesis of heterocycle-linked glycosides/arylglycosides is invaluable and plays an important role at the forefront of organic chemistry. Aryl glycosides can be obtained by linking of carbohydrate to an aromatic aglycone via *C*-, *O*-, and *N*-glycosidic bonds to yield *C*-, *O*-, and *N*-aryl glycosides.

Due to their biological significance and the limited synthetic methodologies available, there is an urgent need for a simple and efficient synthesis protocol for *N*-glycosides. Since pyrimidine motifs are common heterocyclic moieties found in natural products, functional materials, biological systems, and agrochemicals with pharmaceutical and chemical properties [20,21], in this study the pyrimidine ring was combined with glucose to obtain sugar-modified heterocyclic derivatives.

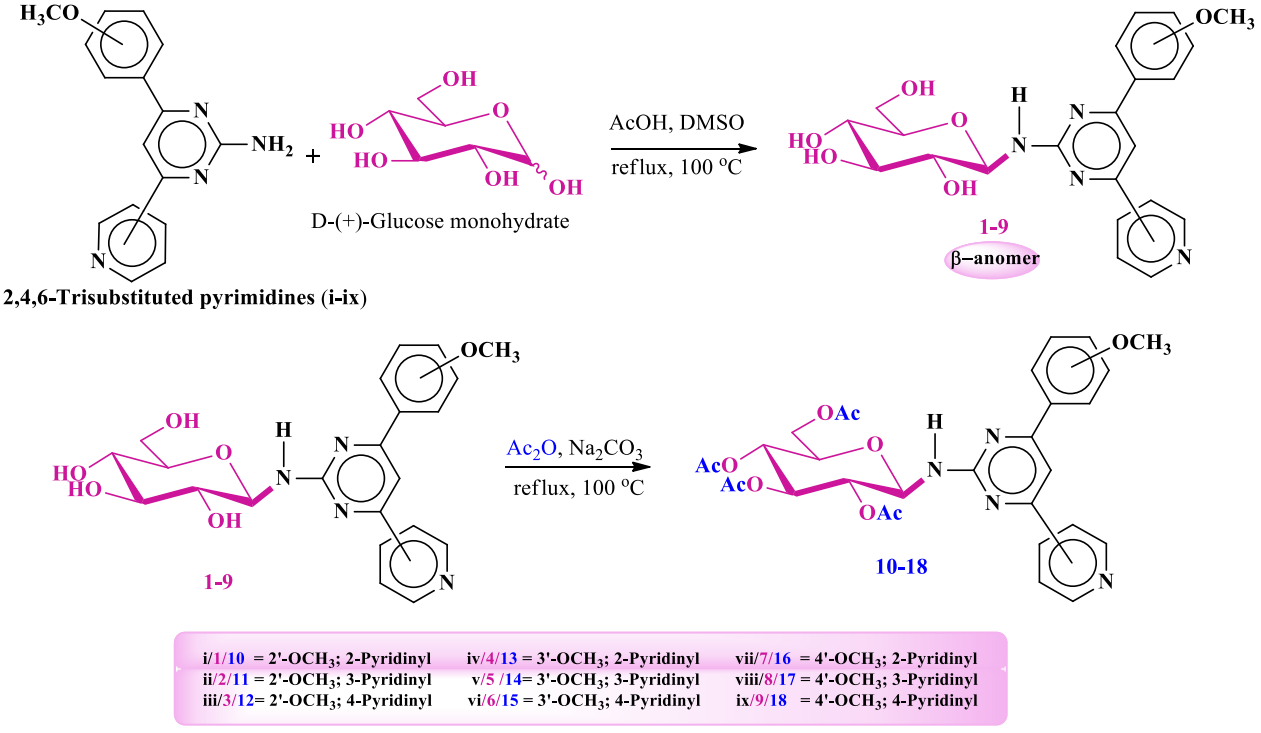
Herein, the operationally simple synthesis of pyrimidine-*N*-glycosides was reported, and in view of the expected biological activities, their anticancer and antibacterial activities, and DNA/BSA binding affinities were evaluated.

## 2. Results and discussion

### 2.1. Chemistry

The detailed synthetic routes for the preparation of the novel methoxy-substituted pyrimidine-sugar derivatives (**1-9**) and their tetra-*O*-acetyl derivatives (**10-18**) have shown in Scheme 1 [22]. The purity of all compounds was checked by TLC, and if necessary, they were purified by column chromatography and their structures were determined spectroscopically. The characterization of all the newly synthesized compounds were performed by $^{1}$H, $^{13}$C, APT, COSY, ACD-NMR, FT-IR, LC-MS/MS spectral and elemental analyzes.

In the first part, new methoxy substituted pyrimidine coupled *N*-$\beta$-glycosides (**1-9**) were synthesized by the condensation between 2,4,6-trisubstituted pyrimidines and D-(+)-glucose monohydrate in acidic medium [8,22]. New signals were observed between $\delta$ 3.4-5.5 ppm, originating from seven protons indicating binding of the sugar moiety, in addition to the signals of 13 protons corresponding to the 2,4,6-trisubstituted pyrimidine compound. The stereochemistry of the *N*-glycosides was determined and subtracted from the NMR spectra. The $^{1}$H-NMR spectra of **1-9** showed that the signal of the anomeric hydrogen (H-1′″) appeared as a doublet with $J_{1'''\text{-}2'''}$= 8.0-9.0 Hz at $\delta$=5.5 ppm. This coupling constant confirmed the $\beta$-anomeric form [10,11,15,23]. All resulting compounds adopted the $\beta$-configuration. For pyranoses, the usual energetically preferred form is the equatorial orientation of the substituents [24]. The other H-atoms of the glucopyranosyl (H-2′″-H-6′″) formed a coupling system and the multiplet peak arised at 3.4-3.6 ppm was defined as the H-2′″-H-5′″ protons. The -$CH_2$ protons (H-6a′″/6b′″) exhibited different chemical shifts due to the different chemical environment. H-6a′″ gave a peak as a singlet or doublet (d, $J$=12.0 Hz) or doublet of doublet (dd, $J$=12.0/2.0 Hz) at $\delta$ 3.9 ppm. Also, H-6b′″ appeared as a dd at 3.7 ppm with coupling constant $J$=12.0-11.2/4.0-5.2 Hz. On the other hand, due to the dissolution of almost all *N*-$\beta$-D-glucopyranoside derivatives in methanol and the preparation of NMR samples in deuteromethanol ($CD_3OD$), the –NH and –OH protons of the glycopyranosyl ring were subjected to deuterium exchange. Preliminary assignment of $^{13}$C-NMR data was done with the help of Attached Proton Test (APT) experiments. In addition to pyrimidine carbons between 166.4-103.1 ppm, the analysis of the $^{13}$C-NMR spectra of compounds 1-9 indicated that the peaks around ~82.7 ppm and peaks between 78.1-61.7 ppm confirmed the presence of




**Scheme 1.** Consecutive synthesis of novel pyrimidine-sugar derivatives (**1-18**)

anomeric carbon (C-1′′) bonded to pyrimidine and other glycopyranosyl carbons (C-2′′′-6′′′), respectively. The FT-IR spectrum of compounds **1-9** showed characteristic broad absorption bands for both the –OH and –NH groups between 3332-3301 $cm^{-1}$. These bands were appeared due to the attachment of the glycopyranosyl group to the pyrimidine core. When the LC-MS/MS spectra were evaluated, besides the ion peaks consisting of different fragmentation as the base peak, $[M+1]^+$ and $[M+Na]^+$ ion peaks were also seen at m/z 441 and 463, respectively.

In the next step, tetra-*O*-acetyl derivatives (**10-18**) were obtained in high yields (75-91%) as a result of the nucleophilic acyl substitution reaction between pyrimidine-linked *N*-$\beta$-glycosides and acetic anhydride in basic medium [25]. As a result of the deshielding effect of the C=O or O-acetylated glucopyranosyl group by the electron-withdrawing property, the anomeric H-1′′′ appeared in the low field ($\delta$ 6.1-5.9 ppm) as a doublet with a coupling constant of between 8.0-9.6 Hz. The $\beta$-anomeric form was also confirmed by the coupling constant. The AcO H-atoms of **10-18** resonated in the region of 2.1-1.9 ppm in $^1$H-NMR spectra, and the $^{13}$C-NMR signals were observed around 20.8.6-20.6 ppm. In addition, four new quaternary peaks coming in view around 170.7-169.6 ppm originated from the carbonyl groups confirmed the structure. In general, $[M+Na]^+$ ions were seen as the base peak at m/z 632 in the LC-MS/MS spectra for acetylated compounds (**10-18**). Compared to the starting *N*-$\beta$-glycosides, the –OH stretches were disappeared in the acetylated compounds and only the –NH absorption band was seen as a small sharp peak at about 3420 $cm^{-1}$ in the FT-IR spectra. In addition, the sharp carbonyl peaks arisen around 1742 $cm^{-1}$ also supported the structures. Some spectra of compounds **8** and **13** were given in supplementary information.

### 2.2. Biological evaluation

#### 2.2.1. Evaluation of anticancer properties of the compounds

Although many anticancer drugs have been developed in modern chemotherapy, it has not been possible to develop a therapeutic product at the desired level. Moreover, the therapeutic effect of these drugs decreasing day by day due to the resistance mechanisms to chemotherapeutic agents for various reasons and their undesirable side effects. Therefore, even today, intensive research is being carried out to develop anticancer drugs that can be used in the treatment of cancer. In this regard, the effect 18 novel pyrimidine-sugar derivatives, designated as *N*-pyrimidinyl-$\beta$-D-glucopyranosylamines (**1-9**) and 2,3,4,6-tetra-O-acetyl-*N*-pyrimidinyl-$\beta$-D-glucopyranosylamines (**10-18**), on cell proliferation was demonstrated by MTT assay. $GI_{50}$, TGI, and $LC_{50}$ parameters, as well as $IC_{50}$ inhibition values, were determined using absorbance data of these compounds from the MTT assay. Compounds **1-9** had no measurable parameters against C6, HeLa, A549, and Hep3B cancer cell lines even at high concentrations such as 500 $\mu$g/mL, so they were ineffective on cell proliferation (Tables 1-2).

However, the lack of anticancer activities of these compounds was not sufficient evidence to consider the compounds as insufficient interms of their pharmacological activities. This is because they had very little toxic effects on cells. Genomic, transcriptomic and proteomic analyses are planned in future studies to investigate the various pharmacological activities of these molecules. Interestingly, compared with the anticancer drugs used as controls (Table 3), caompound **9** was found to exhibit significant anticancer activity on the HT29 colon cancer cell line ($IC_{50}$=58.01 $\mu$g/mL, TGI value 187.59 $\mu$g/mL).

This result also tells us that it is a nontoxic molecule that could be used in cancer therapy, considering the high $LC_{50}$ value ($IC_{50}$ and TGI values, >500 $\mu$g/mL) and low $GI_{50}$ value (1.90 $\mu$g/mL) of compound **9** on HT29 cancer cell lines (Tables 1 and 3). When the cytotoxicity measurement results were examined given in Table 4, it was found that compound **9** had very low toxicity to HT29 cell.

The $IC_{50}$ data of the test results showed that 2,3,4,6-tetra-*O*-acetyl-*N*-$\beta$-D-glucopyranosylamines exhibited stronger or similar anticancer properties than cisplatin and 5-fluorouracil (5FU) against C6 cells,with the exception of compounds **10** and **17** ($IC_{50}$ values ranging from 23.08 to 69.26 $\mu$g/mL and TGI values ranging from 15.92 to 48.71 $\mu$g/mL) (Tables 1 and 3). Examination of the $GI_{50}$ values shown in Table 1 revealed that compound **16** caused greater growth inhibition than cisplatin and compounds **12** and **14** caused greater inhibition than 5FU in the C6 cell line. In the A549 and HT29 cancer cell lines, none of the 2,3,4,6-tetra-*O*-acetyl-*N*-$\beta$-D-glucopyranosylamines showed sufficient anticancer activity ($IC_{50}$ and TGI values >500 $\mu$g/mL) (Tables 1 and 2). For total growth inhibition (TGI) and inhibitor concentration ($IC_{50}$) values, only compound **11** showed anticancer activity against MCF7 cell line (TGI value 60.99, $IC_{50}$ value 52.27 µg/mL), while compounds **11, 16** and **17** exhibited anticancer activity against Hep3B cell line (TGI values 14.34, 44.35 and 36.53 $\mu$g/mL, $IC_{50}$ values 14.12, 42.78 and 35.00 $\mu$g/mL) (Table 2). Evaluation of the high $LC_{50}$ (>500 $\mu$g/mL) and low $GI_{50}$ values (1.77-3.92 $\mu$g/mL) shown in both cell lines led to the conclusion that these compounds have the potential for use in advanced pharmacological studies for liver and breast cancer. The lower growth inhibition ($GI_{50}$) and total inhibition (TGI) values of the test compounds indicated a higher anticancer effects, while the higher lethal concentration ($LC_{50}$) values indicated that the test compounds had a lower cytocidal effects. For this reason, among the tested molecules, the researchers selected those with high $LC_{50}$ and low $GI_{50}$ and TGI values for the pharmacological studies. When the TGI and $IC_{50}$ data of the test results were examined, only compounds **11, 13**, and **16** were found to be effective in the HeLa cell line (TGI values 19.25-23.71 $\mu$g/mL and $IC_{50}$ values 29.82-41.63 $\mu$g/mL). When the $GI_{50}$ and $LC_{50}$ results of these compounds were evaluated, it was found that all related compounds were more effective than the control agents 5FU and cisplatin (Tables 1 and 3). In this case, they can be used for further studies because they have the proper molecular structure that can be used in cervical cancer research. Although compounds **11, 16**, and **18** possessed potent anticancer properties (TGI values 23.00-42.52 $\mu$g/mL and $IC_{50}$ values 20.97-40.86 $\mu$g/mL), unfortunately, they could not meet NCI criteria due to their partial toxicity to normal cells (FL cells) (Table 2). For this reason, the molecular design of the test compounds in question can be redesigned with Lipinski's rules in mind and the toxic effects can be obtained at a more reasonable level without lowering the anticancer efficacy. In general, compounds **12-15** for C6 cells, **13** and **16** for HeLa cells, **11** and **17** for Hep3B cells, **9** for HT29 cells, and **11** for MCF7 cells showed high anticancer activity and largely met the NCI criteria for the corresponding anticancer therapies without having toxic effects on the normal cell (FL) line (Table 2). The LDH assay, which measured cell membrane damage caused by the test molecules, showed that the above- mentioned anticancer agents (**11-17**) caused approximately 10-15% membrane damage at effective concentrations ($IC_{50}$ values ranging from 25 to 50 $\mu$g/mL) (Table 4). This value is very close to thepercentage cytotoxicity values caused by our positive controls (5FU and Cisplatin) (Table 5). Despite the low cytotoxicity values caused by the remaining test molecules, they did not prove to be suitable for the further pharmacological studies because they were not able to cause anticancer activity at the desired level. In conclusion, acetylation of the pyrimidine-*N*-$\beta$-D-glycosides affected the pharmacological properties and significantly enhanced the antiproliferative effects of the compounds without increasing the toxic properties on normal cells, thus increasing their suitability as anticancer agents.

**Table 1**
$GI_{50}$. TGI. $LC_{50}$ and $IC_{50}$ values for **1-9** and **10-18** against C6, HeLa, and HT29.

| Compound (*μg/mL*) | C6 | | | | HeLa | | | | HT29 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $GI_{50}$ | TGI | $LC_{50}$ | $IC_{50}$ | $GI_{50}$ | TGI | $LC_{50}$ | $IC_{50}$ | $GI_{50}$ | TGI | $LC_{50}$ | $IC_{50}$ |
| **1** | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | 2.11 | >500 | >500 | 201.65 |
| **2** | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | 2.35 | >500 | >500 | >500 |
| **3** | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | 1.77 | >500 | >500 | >500 |
| **4** | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | 2.62 | >500 | >500 | >500 |
| **5** | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | 1.82 | >500 | >500 | >500 |
| **6** | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | 1.79 | >500 | >500 | >500 |
| **7** | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | 1.43 | >500 | >500 | >500 |
| **8** | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | 1.71 | >500 | >500 | 102.07 |
| **9** | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | 1.90 | 187.59 | >500 | 58.01 |
| **10** | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | 1.21 | >500 | >500 | >500 |
| **11** | 13.14 | 33.10 | 116.19 | 42.59 | 6.09 | 19.25 | 126.53 | 30.32 | 4.46 | >500 | >500 | 378.59 |
| **12** | 7.93 | 26.64 | 181.71 | 41.69 | >500 | >500 | >500 | >500 | 2.64 | >500 | >500 | >500 |
| **13** | 16.75 | 48.71 | 212.41 | 69.26 | 6.45 | 19.93 | 121.64 | 29.82 | 3.87 | >500 | >500 | >500 |
| **14** | 8.19 | 27.45 | 184.14 | 43.44 | >500 | >500 | >500 | >500 | 4.52 | >500 | >500 | >500 |
| **15** | 12.38 | 43.00 | 276.55 | 67.68 | >500 | >500 | >500 | >500 | 1.42 | >500 | >500 | 129.68 |
| **16** | 6.06 | 15.92 | 69.96 | 23.08 | 6.20 | 23.71 | 242.62 | 41.63 | 1.38 | >500 | >500 | >500 |
| **17** | 22.17 | 68.85 | 323.45 | 105.05 | >500 | >500 | >500 | >500 | 3.64 | >500 | >500 | >500 |
| **18** | 11.79 | 33.81 | 151.88 | 47.00 | >500 | >500 | >500 | >500 | 10.10 | >500 | >500 | >500 |

**Table 2**
$GI_{50}$. TGI. $LC_{50}$ and $IC_{50}$ values for **1-18** against MCF7, A549, Hep3B, and FL.

| Compound (*μg/mL*) | MCF7 | | | | A549 | | | | Hep3B | | | | FL | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $GI_{50}$ | TGI | $LC_{50}$ | $IC_{50}$ | $GI_{50}$ | TGI | $LC_{50}$ | $IC_{50}$ | $GI_{50}$ | TGI | $LC_{50}$ | $IC_{50}$ | $GI_{50}$ | TGI | $LC_{50}$ | $IC_{50}$ |
| **1** | 1.99 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | 15.38 | >500 | >500 | >500 | 66.34 | >500 | >500 | >500 |
| **2** | 1.54 | 406.42 | >500 | 291.94 | >500 | >500 | >500 | >500 | 5.07 | >500 | >500 | >500 | 13.64 | >500 | >500 | >500 |
| **3** | 1.25 | >500 | >500 | >500 | 204.27 | >500 | >500 | >500 | 5.80 | >500 | >500 | >500 | 4.02 | >500 | >500 | >500 |
| **4** | 1.63 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | 3.93 | >500 | >500 | >500 | 4.56 | >500 | >500 | >500 |
| **5** | 2.19 | >500 | >500 | >500 | 24.02 | >500 | >500 | >500 | 4.67 | >500 | >500 | >500 | 3.15 | >500 | >500 | >500 |
| **6** | 1.25 | >500 | >500 | >500 | 86.94 | >500 | >500 | >500 | 3.37 | >500 | >500 | >500 | 2.77 | >500 | >500 | >500 |
| **7** | 1.04 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | 4.05 | >500 | >500 | >500 | 7.12 | >500 | >500 | >500 |
| **8** | 1.19 | >500 | >500 | >500 | 78.96 | >500 | >500 | >500 | 3.83 | >500 | >500 | >500 | 5.58 | >500 | >500 | >500 |
| **9** | 1.43 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | 3.50 | >500 | >500 | >500 | 5.24 | >500 | >500 | >500 |
| **10** | 3.77 | >500 | >500 | >500 | 85.49 | >500 | >500 | >500 | 5.16 | >500 | >500 | >500 | 15.35 | >500 | >500 | >500 |
| **11** | 1.77 | 60.99 | >500 | 52.27 | 4.47 | >500 | >500 | 261.27 | 3.28 | 14.34 | 393.65 | 14.12 | 3.25 | 24.33 | >500 | 20.97 |
| **12** | 2.18 | 221.01 | >500 | 181.38 | 4.54 | >500 | >500 | >500 | 3.78 | >500 | >500 | >500 | 3.28 | >500 | >500 | >500 |
| **13** | 1.58 | >500 | >500 | >500 | 6.81 | >500 | >500 | >500 | 3.77 | >500 | >500 | >500 | 2.83 | >500 | >500 | >500 |
| **14** | 2.12 | >500 | >500 | >500 | 3.78 | >500 | >500 | 372.77 | 3.10 | 469.17 | >500 | 408.49 | 3.92 | >500 | >500 | >500 |
| **15** | 3.64 | >500 | >500 | >500 | 2.39 | >500 | >500 | >500 | 3.75 | >500 | >500 | >500 | 16.42 | >500 | >500 | >500 |
| **16** | 4.40 | >500 | >500 | >500 | 11.26 | >500 | >500 | >500 | 3.92 | 44.35 | >500 | 42.78 | 3.14 | 23.00 | >500 | 22.39 |
| **17** | 2.18 | >500 | >500 | >500 | 7.79 | >500 | >500 | >500 | 3.19 | 36.53 | >500 | 35.00 | 4.60 | >500 | >500 | >500 |
| **18** | 2.03 | >500 | >500 | >500 | 255.09 | >500 | >500 | >500 | 4.81 | >500 | >500 | >500 | 3.44 | 42.52 | >500 | 40.86 |

**Table 3**
$IC_{50}$ (*μg/mL*) of positive controls in cell lines.

| | HeLa | HT29 | A549 | MCF7 | C6 | Hep3B | FL |
|---|---|---|---|---|---|---|---|
| ***Cisplatin*** | 50.29 | 40.39 | 60.49 | 63.79 | 33.08 | 48.69 | 52.79 |
| ***5FU*** | 61.59 | 65.19 | 69.79 | 74.19 | 54.30 | 62.89 | 59.09 |

*2.2.2. Changes in cell morphology after the treatments with novel compounds*

To evaluate the effect of potent compounds determined by MTT assay on cell morphology, exponentially growing cells were incubated in log phase and these compounds at different concentrations for 24 hours. As a result of the phase contrast microscopy performed, some morphological changes were observed in the cells. Accordingly, these morphological changes on the cells were the transformation of the epithelial shape into a globular shape, very weak binding to the flask surface, formation of spikes and bubbles, shrinkage of the cells or cytoplasm, formation of pockets on apoptotic cells, and disrupted cellular structures in places (Figure 3). These morphological changes of the cells and the decrease in the number of adherent cells are directly proportional to the concentration of the test substance used.

*2.2.3. Evaluation of antibacterial effects of the compounds*

The increasing number of resistant bacterial species to current first-, second-, and even fourth- generation antibacterial agents and the extraordinary increase in the number of hospital-acquired infections are pushing scientists to develop new, effective antibacterial agents. On this occasion, the antibacterial activity of newly synthesized compounds on some Gram-positive and Gram-negative bacteria causing diseases in the human body was investigated by the minimum inhibitory concentration (MIC) method. Discussing the obtained MIC values of the new compounds on Gram (+) bacteria, it was found that the antibacterial activity of compounds **10-15** against *E. faecalis* ATCC 29212 (31.25 – 125 μg/mL), compounds **5** and **8** against *S. aureus* ATCC 25923 (125 μg/mL, each), compounds **9** against *S. aureus* MSSA ATCC 29213 (62.5 μg/mL), compounds **8** and **9** against *S. aureus* MRSA ATCC 46300 (62.5 and 31.25 μg/mL, respectively), compounds **8-9, 11-12**, and **18** against *S. mutans* ATCC 35668 (125 μg/mL, each), and compound 8 against *S. gordonii* NCTC 7870 (125 μg/mL) were more or similar to the SCF antibiotic used as positive control (Tables 6-7).

On the other hand, when the effects of the compounds on Gram-negative bacteria were considered, it was found that only compound **9** showed higher antibacterial activity against *A. actinomycetemcomitans* ATCC 33384 (31.25 μg/mL) than the antibiotic

**Table 4**
% Cytotoxicity of **1-18** at various concentrations against C6, HeLa and HT29.

| Compound (μg/mL) | C6 | | | | HeLa | | | | HT29 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 25 | 50 | 75 | 100 | 25 | 50 | 75 | 100 | 25 | 50 | 75 | 100 |
| ***1*** | 8.75 | 19.27 | 37.14 | 48.42 | 14.06 | 32.49 | 36.48 | 45.66 | 9.55 | 25.47 | 32.70 | 41.70 |
| **2** | 11.41 | 14.08 | 35.24 | 43.29 | 19.06 | 26.66 | 37.62 | 50.92 | 9.77 | 21.80 | 30.80 | 45.43 |
| **3** | 9.11 | 11.11 | 30.17 | 54.24 | 25.40 | 30.91 | 33.38 | 53.89 | 8.45 | 22.31 | 30.80 | 44.93 |
| **4** | 9.05 | 18.39 | 37.14 | 41.15 | 18.81 | 26.98 | 37.56 | 46.93 | 6.47 | 22.94 | 38.90 | 58.66 |
| **5** | 8.53 | 20.53 | 29.54 | 38.98 | 14.25 | 26.28 | 31.48 | 39.27 | 8.79 | 24.34 | 36.95 | 43.34 |
| **6** | 10.96 | 23.63 | 21.44 | 32.40 | 17.99 | 31.03 | 37.68 | 46.36 | 9.46 | 15.28 | 25.93 | 41.89 |
| **7** | 11.03 | 17.37 | 22.07 | 49.49 | 12.54 | 22.61 | 34.77 | 41.80 | 9.91 | 15.35 | 36.69 | 55.05 |
| **8** | 7.86 | 14.52 | 23.20 | 47.52 | 8.74 | 21.72 | 32.36 | 50.03 | 9.56 | 20.92 | 35.17 | 40.18 |
| **9** | 8.20 | 15.53 | 30.17 | 41.20 | 9.37 | 25.71 | 32.74 | 39.39 | 9.20 | 21.67 | 35.93 | 54.61 |
| **10** | 7.29 | 21.36 | 32.58 | 57.78 | 8.11 | 27.68 | 36.16 | 40.79 | 10.15 | 18.70 | 39.97 | 47.39 |
| **11** | 15.14 | 31.42 | 55.17 | 76.76 | 10.64 | 30.21 | 37.94 | 42.75 | 16.40 | 27.36 | 47.44 | 76.76 |
| **12** | 14.44 | 28.38 | 49.16 | 70.43 | 11.91 | 23.50 | 37.37 | 42.94 | 10.46 | 19.27 | 37.19 | 59.16 |
| **13** | 11.09 | 27.37 | 57.14 | 74.62 | 8.74 | 31.29 | 39.01 | 43.70 | 11.41 | 15.66 | 30.17 | 55.18 |
| **14** | 13.12 | 21.29 | 52.57 | 74.10 | 10.01 | 22.36 | 37.37 | 44.02 | 9.39 | 14.64 | 39.79 | 50.43 |
| **15** | 11.34 | 27.87 | 43.70 | 67.00 | 17.99 | 45.35 | 47.18 | 56.17 | 27.11 | 41.04 | 75.24 | 83.47 |
| **16** | 14.13 | 28.64 | 53.34 | 74.23 | 17.10 | 27.36 | 40.34 | 50.47 | 10.27 | 29.27 | 48.90 | 67.46 |
| **17** | 9.38 | 22.31 | 51.44 | 77.21 | 25.65 | 37.18 | 47.63 | 55.73 | 9.54 | 21.80 | 40.80 | 62.71 |
| **18** | 16.21 | 23.57 | 46.06 | 68.08 | 17.54 | 30.21 | 35.21 | 43.00 | 14.20 | 29.91 | 48.14 | 56.63 |

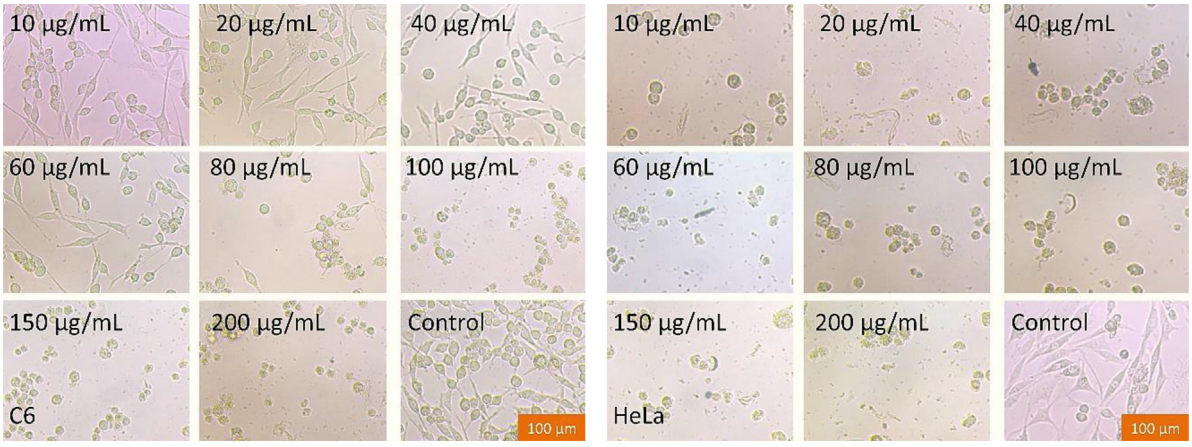



**Figure 3.** Effect of **15** on the morphologies of C6 and HeLa cell lines. Exponentially growing cells were incubated overnight with various concentrations of **15** at 37°C. Control cells were treated with only DMSO. All measurements were 100 $\mu$m.

**Table 5**
% Cytotoxicity of positive controls at $IC_{50}$ concentrations.

| | HeLa | HT29 | A549 | MCF7 | C6 | Hep3B | FL |
|---|---|---|---|---|---|---|---|
| ***Cisplatin*** | 9.85 | 11.23 | 8.63 | 10.71 | 9.04 | 8.46 | 8.33 |
| ***5FU*** | 8.83 | 7.91 | 9.19 | 7.69 | 10.01 | 9.67 | 8.44 |

SCF used as positive control (Tables 6-7). The results of the *in vitro* antibacterial assays of the compounds showed that all the pyrimidine-sugar compounds and their tetra-*O*-acetyl derivatives had similar antibacterial properties. Overall, both functional groups were it was found to be effective against some bacterial pathogens in the human body and inhibited bacterial growth even at very low

**Table 6**
Minimum-inhibitory concentrations (MIC, in μg/mL) of **1-9**.

| Microorganisms | MIC (μg/mL) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **KCN** | **SCF** |
| *E. faecalis* **VRE** ATCC 19433 | 500 | >1000 | 1000 | 1000 | >1000 | >1000 | >1000 | >1000 | 1000 | NE | 250 |
| *E. faecalis* ATCC 29212 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | 250 | NE | 62.5 |
| *S. aureus* ATCC 25923 | >1000 | >1000 | >1000 | >1000 | 125 | >1000 | 250 | 125 | 500 | NE | 250 |
| *S. aureus* **MSSA** ATCC 29213 | 1000 | >1000 | 250 | 500 | 1000 | 500 | 500 | 250 | 62.5 | NE | NA |
| *S. aureus* **MRSA** ATCC 46300 | >1000 | >1000 | 1000 | 500 | 250 | 1000 | 500 | 62.5 | 31.25 | NE | 250 |
| *E. coli* ATCC 25922 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | NE | 15.62 |
| *E. coli* **ESBL** ATCC 35218 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | NE | 31.25 |
| *P. aeruginosa* **AGME** ATCC 27853 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | NE | 250 |
| *S. mutans* ATCC 35668 | 250 | 1000 | 500 | 500 | 500 | 1000 | 250 | 125 | 125 | NE | 125 |
| *S. gordonii* NCTC 7870 | 1000 | 1000 | >1000 | 1000 | 1000 | >1000 | 1000 | 125 | 250 | NE | 125 |
| *A. actinomycetemcomitans* ATCC 33384 | 1000 | 1000 | 1000 | 500 | 1000 | >1000 | 500 | 250 | 31.25 | NE | 62.5 |

SCF. sulbactam (30 μg) + cefoperazone (75 μg), as a positive control
KCN. potassium cyanide, as a negative control, NE: No effect, NA: Not available

**Table 7**
Minimum-inhibitory concentrations (MIC. in µg/mL) of **10-18**.

| Microorganisms | **MIC** (µg/mL) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **KCN** | **SCF** |
| *E. faecalis* **VRE** ATCC 19433 | >1000 | 1000 | 1000 | >1000 | >1000 | >1000 | >1000 | >1000 | 250 | NE | 250 |
| *E. faecalis* ATCC 29212 | 125 | 62.5 | 62.5 | 31.25 | 31.25 | 31.25 | 250 | 1000 | 250 | NE | 62.5 |
| *S. aureus* ATCC 25923 | >1000 | >1000 | >1000 | >1000 | 500 | 1000 | >1000 | >1000 | 500 | NE | 250 |
| *S. aureus* **MSSA** ATCC 29213 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | 1000 | NE | NA |
| *S. aureus* **MRSA** ATCC 46300 | 500 | >1000 | 1000 | >1000 | >1000 | 1000 | >1000 | >1000 | 500 | NE | 250 |
| *E. coli* ATCC 25922 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | NE | 15.62 |
| *E. coli* **ESBL** ATCC 35218 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | NE | 31.25 |
| *P. aeruginosa* **AGME** ATCC 27853 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | NE | 250 |
| *S. mutans* ATCC 35668 | 1000 | 125 | 125 | 500 | 500 | 500 | 500 | 250 | 125 | NE | 125 |
| *S. gordonii* NCTC 7870 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | NE | 125 |
| *A. actinomycetemcomitans* ATCC 33384 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | NE | 62.5 |

SCF. sulbactam (30 µg) + cefoperazone (75 µg), as a positive control
KCN. potassium cyanide, as a negative control, NE: No effect, NA: Not available

concentrations, making their potential for use as antibiotics possible.

### 2.2.4. Examination of DNA/BSA binding properties of the compounds

The vast majority of current chemotherapeutic chemistry targets biomacromolecules such as DNA, RNA and proteins. Therefore, when developing a pharmacologically active agent, the relationship of the agentin question to biomacromolecules is necessarily examined. For many laboratories, a simple way to verify this relationship is to use spectrophotometric methods. The DNA/BSA binding properties of the new chemicals synthesized by our group were determined using a UV-Vis spectrophotometer. The interactions of these compounds with DNA were as follows. Compounds **10, 12**, and **13** caused a hyperchromic effect and their maximum peaks showed a slight red shift (2 nm). Compounds **11, 6**, and **7** also caused a slight hyperchromic effect without causing astrong shift in the absorption band. While compounds **14, 15**, and **16** exhibited a strong hyperchromic feature, only compound **15** caused a slight red shift (2 nm) among them. Compound **17** caused a strong hypochromic effect and did not show a clear shift. In addition, compounds **1-5, 8, 9**, and **18** displayed slight hypochromic change but no shift in their maximum peak. The interactions of the new compounds with BSA gave the following results. Compounds **10** and **11** showed a hyperchromic spectrum without a significant shift of themaximum peak. And compounds **12-17** also showed strong hyperchromic spectrum properties and caused no net shift of the maximum peak. Compound **2** demonstrated hypochromic spectrum with a slight red shift at maximum peak. Compounds **1, 4-7**, and **18** caused a slight hypochromic effect but did not show a significant shift. A slightly hyperchromic spectrum was given by compounds **3, 8**, and **9** without a net shift in the maximum peak. The hypochromic effect of the molecules is generally considered to indicate that the complex is electrostatically or mainly intercalatively bound to DNA. In the hyperchromic effect, the molecules bind covalently to DNA and cause some twisting of the helical structure. When looking at the spectra, the observed decrease or increase in absorbance likely indicates that the complexes are physically interacting directly with the DNA. In addition to this change in the spectra, the red shift in the absorption bands of the complexes may indicate the stability of the complex-DNA structure [26].

In titration studies based on absorption, binding constants (Kb) can be calculated from data obtained after interaction of the complexes with DNA using the following Wolfe–Shimmer equation [27]; [DNA]/($\varepsilon$a - $\varepsilon$f) = [DNA]/($\varepsilon$b - $\varepsilon$f) + 1 / [Kb($\varepsilon$b - $\varepsilon$f)]. In this equation, [DNA] is the DNA concentration in the base pairs. $\varepsilon$a, $\varepsilon$f and $\varepsilon$b correspond to the molar absorption coefficients of the $A_{observed}$/[complex], free complex and complex-DNA solutions, respectively. Kb represents the bindingstrength of the complex to DNA and is calculated from the slope of the line between [DNA] / ($\varepsilon$a - $\varepsilon$f) and [DNA]. Table 8 shows the values of the binding constants for the complexes. The binding constants (Kb) of the complexes varied from 1.1 × $10^3$ to 4.0 × $10^4$. These results indicate that the compounds can bind tightly enough to exert their effect on DNA when compared with the binding constants of cisplatin (5.73 × $10^4$ $M^{-1}$) and 5FU (9.7 × $10^4$ $M^{-1}$), two clinically used anticancer drugs that can bind to DNA [28,29].

**Table 8**
The binding constants ($K_b$) of these compounds.

| **No** | $K_b$ ($M^{-1}$) | **No** | $K_b$ ($M^{-1}$) |
|---|---|---|---|
| **1** | 1.9 × $10^4$ | **10** | 3.1 × $10^4$ |
| **2** | 6.2 × $10^3$ | **11** | 1.5 × $10^3$ |
| **3** | 3.2 × $10^4$ | **12** | 3.9 × $10^4$ |
| **4** | 4.0 × $10^4$ | **13** | 1.1 × $10^4$ |
| **5** | 8.9 × $10^3$ | **14** | 2.5 × $10^4$ |
| **6** | 6.7 × $10^3$ | **15** | 2.5 × $10^4$ |
| **7** | 3.1 × $10^4$ | **16** | 6.4 × $10^3$ |
| **8** | 4.3 × $10^3$ | **17** | 2.2 × $10^4$ |
| **9** | 1.1 × $10^3$ | **18** | 3.3 × $10^3$ |

### 2.3. In silico studies

Molecular docking studies were applied to determine the binding parameters of the compounds. In molecular docking studies, compounds (**8, 9, 17** and **18**) were interacted *in silico* with PBD IDS: 4QL3 [30], 6MPP [31], 4E26 [32], 6QGG [33], 5MU8 [34], 4QUG [35], 2DBF, 6SL6 [36], 1CGL [37], 5Z62 [38], 6EB6 [39], 5ITD [40], 4EKK [41], 6GU7 [42], 7BG9 [43], 3GUS [44] receptors, respectively.

As a result of these interactions, binding parameter values and active amino acids in the binding site were determined. XP GScore, $\Delta G_{Bind}$ energy, docking score, Glide gscore, and complex energy values for each compound of active receptors were presented in Table 9. When Table 9 is analyzed, it can be said that the best binding parameter values among these receptors belong to BRAF (PDB ID: 4E26), Cytochrome c oxidase (PDB ID: 5Z62) and PI3K alpha (PDB ID: 5ITD) receptors.

When the parameter values of the PI3K alpha protein in Table 9 were examined, the XP GScore, docking score, Glide gscore, $\Delta G_{Bind}$, complex energy values of the results for compound **8** were calculated as -10.712 kcal/mol, -10.712 kcal/mol, -10.712 kcal/mol, -57.07 kcal/mol, -43474.927, respectively. The XP GScore, docking score, Glide gscore, $\Delta G_{Bind}$, complex energy values of BRAF protein, which has one of the best binding parameter values, for compound **9** are shown in Table 9 as -9.175 kcal/mol, -9.175 kcal/mol, -9.175 kcal/mol, -65.47 kcal/mol and -11696.121, respectively.

**Table 9**
MM-GBSA $\Delta G_{Bind}$, XP GScore, docking score, Glide gscore and complex energy values of compounds **8, 9, 17 and 18** interacting with different identified proteins.

| Protein | PDB ID | Compound 8 | | | | | Compound 9 | | | | | Compound 17 | | | | | Compound 18 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | XP GScore | Docking Score | Glide GScore | MM-GBSA $\Delta G_{Bind}$ | Complex Energy | XP GScore | Docking Score | Glide GScore | MM-GBSA $\Delta G_{Bind}$ | Complex Energy | XP GScore | Docking Score | Glide GScore | MM-GBSA $\Delta G_{Bind}$ | Complex Energy | XP GScore | Docking Score | Glide GScore | MM-GBSA $\Delta G_{Bind}$ | Complex Energy |
| **KRAS** | ***4QL3*** | -4.139 | -4.139 | -4.139 | -36.99 | -7969.438 | -4.097 | -4.097 | -4.097 | -36.24 | -8034.531 | -1.987 | -1.987 | -1.987 | -71.33 | -8091.559 | -1.904 | -1.904 | -1.904 | -58.34 | -8143.341 |
| **NRAS** | ***6MPP*** | -7.442 | -7.442 | -7.442 | -76.35 | -12751.391 | -7.359 | -7.359 | -7.359 | -80.19 | -12818.116 | -6.559 | -6.559 | -6.559 | -80.24 | -12838.307 | -6.594 | -6.594 | -6.594 | -67.27 | -12894.672 |
| **BRAF** | ***4E26*** | **-8.090** | **-8.090** | **-8.090** | **-58.43** | **-11621.887** | **-9.175** | **-9.175** | **-9.175** | **-65.47** | **-11696.121** | **-4.027** | **-4.027** | **-4.027** | **-64.50** | **-11714.183** | **-1.256** | **-1.256** | **-1.256** | **-67.04** | **-11777.962** |
| **Bcl-2** | ***6QGG*** | -7.015 | -7.015 | -7.015 | 60.05 | -6638.522 | -5.076 | -5.076 | -5.076 | -55.58 | -6697.401 | 0.729 | 0.729 | 0.729 | -57.78 | -6712.707 | -4.023 | -4.023 | -4.023 | -61.35 | -6791.860 |
| **TNF-alfa** | ***5MU8*** | -3.957 | -3.957 | -3.957 | -46.13 | -5893.861 | -4.725 | -4.725 | -4.725 | -46.53 | -5959.573 | -3.232 | -3.232 | -3.232 | -59.74 | -5992.130 | -1.586 | -1.586 | -1.586 | -45.14 | -6042.289 |
| **Caspase 3** | ***4QUG*** | -5.327 | -5.327 | -5.327 | -47.93 | -10597.672 | -5.277 | -5.277 | -5.277 | -47.22 | -10659.769 | 3.635 | 3.635 | 3.635 | -58.98 | -10685.987 | 0.901 | 0.901 | 0.901 | -58.36 | -10761.151 |
| **NF-kB p105 subunit** | ***2DBF*** | -3.260 | -3.260 | -3.260 | -35.74 | -4133.662 | -2.678 | -2.678 | -2.678 | -49.91 | -4203.080 | 1.410 | 1.410 | 1.410 | -33.83 | -4217.961 | 4.239 | 4.239 | 4.239 | −33.80 | -4274.175 |
| **p53** | ***6SL6*** | -1.878 | -1.878 | -1.878 | -47.76 | -8779.310 | -2.466 | -2.466 | -2.466 | -44.43 | -8837.720 | -1.037 | -1.037 | -1.037 | -42.87 | -8859.992 | -1.779 | -1.779 | -1.779 | -29.26 | -8912.467 |
| **Fibroblast collagenase** | ***1CGL*** | -5.782 | -5.782 | -5.782 | -57.97 | -7330.479 | -5.476 | -5.476 | -5.476 | -36.30 | -7374.606 | -3.571 | -3.571 | -3.571 | -55.25 | -7413.712 | 0.171 | 0.171 | 0.171 | -56.59 | -7475.436 |
| **Cytochrome c oxidase** | ***5Z62*** | **-10.969** | **-10.969** | **-10.969** | **-91.62** | **-19671.585** | **-10.412** | **-10.412** | **-10.412** | **-79.25** | **-19728.931** | **-4.085** | **-4.085** | **-4.085** | **-50.45** | **-19721.489** | **-3.439** | **-3.439** | **-3.439** | **-66.97** | **-19799.634** |
| **BAX W139A** | ***6EB6*** | -2.642 | -2.642 | -2.642 | -40.30 | -7722.599 | -1.780 | -1.780 | -1.780 | -41.45 | -7790.156 | 1.806 | 1.806 | 1.806 | -52.07 | -7818.123 | -1.754 | -1.754 | -1.754 | -55.94 | -7896.072 |
| **PI3K alpha** | ***5ITD*** | **-10.712** | **-10.712** | **-10.712** | **-57.07** | **-43474.927** | **-10.339** | **-10.339** | **-10.339** | **-58.14** | **-43544.832** | **-5.264** | **-5.264** | **-5.264** | **-54.20** | **-43558.852** | **-7.012** | **-7.012** | **-7.012** | **-82.19** | **-43656.639** |
| **Akt1** | ***4EKK*** | -6.826 | -6.826 | -6.826 | -63.43 | -14195.328 | -7.513 | -7.513 | -7.513 | -68.99 | -14262.150 | 0.121 | 0.121 | 0.121 | -64.31 | -14277.075 | 3.892 | 3.892 | 3.892 | -74.34 | -14345.457 |
| **Cdk1** | ***6GU7*** | -6.437 | -6.437 | -6.437 | -52.70 | -12016.156 | -5.037 | -5.037 | -5.037 | -43.08 | -12078.371 | 0.526 | 0.526 | 0.526 | -68.02 | -12126.254 | 3.919 | 3.919 | 3.919 | -47.66 | -12171.131 |
| **Tert** | ***7BG9*** | -2.880 | -2.880 | -2.880 | -39.04 | -34166.994 | -4.729 | -4.729 | -4.729 | -51.76 | -34254.092 | -2.604 | -2.604 | -2.604 | -42.84 | -34268.486 | -0.089 | -0.089 | -0.089 | -38.10 | -34328.465 |
| **Gstp1** | ***3GUS*** | -6.051 | -6.051 | -6.051 | -58.40 | -9391.116 | -6.807 | -6.807 | -6.807 | -44.60 | -9443.611 | -6.426 | -6.426 | -6.426 | -75.53 | -9495.300 | -4.656 | -4.656 | -4.656 | -69.87 | -9556.772 |

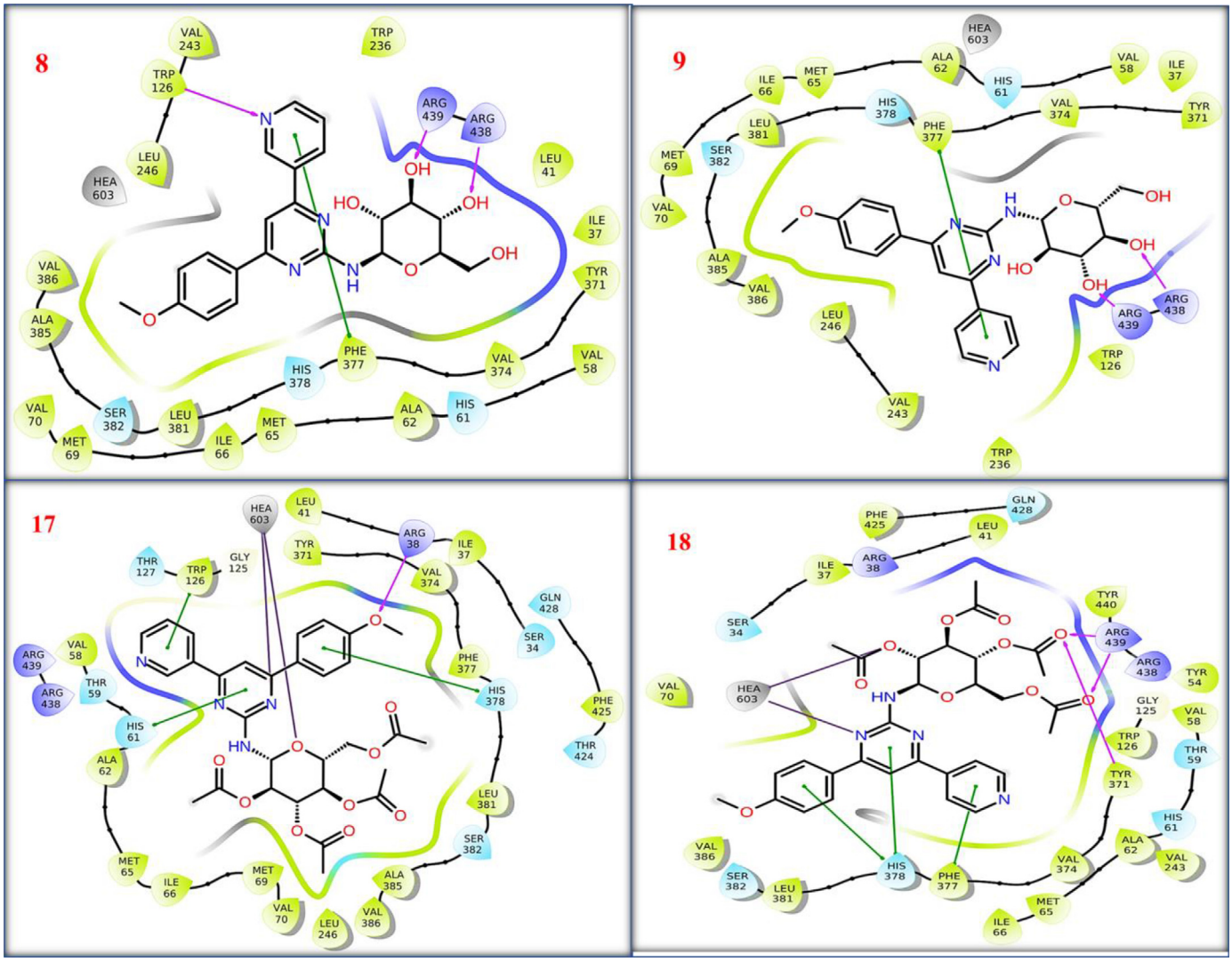



**Figure 4.** 2D interaction diagrams of compounds **(8, 9, 17,** and **18)** with the Cytochrome c oxidase protein (PDB ID: 5Z62).

According to the theoretically calculated molecular docking analysis of *in silico* approaches, the protein that gives the best results in **Table 9** is Cytochrome c oxidase. When the binding parameter values of Cytochrome c oxidase (PDB ID: 5Z62) were examined, XP GScore, docking score, Glide gsore, $\Delta G_{Bind}$, complex energy values for each compound respectively were calculated -10.969 kcal/mol, -10.969 kcal/mol, -10.969 kcal/mol, -91.62, -19671.585 for compound **8**, -10.412 kcal/mol, -10.412 kcal/mol, -10.412 kcal/mol -79.25, -19728.931 for compound **9,** -4.085 kcal/mol, -4.085 kcal/mol, -4.085kcall/mol, -50.45, -19721.489 for compound **17**, -3.439 kcal/mol, -3.439 kcal/mol, -3.439 kcal/mol, -66.97,-19799.634 kcal/mol for compound **18**. While analyzing the results of molecular docking studies, not only the binding parameter values, but also the amino acid residues in the binding site are analyzed in detail.

For Cytochrome c oxidase, which was calculated according to *in silico* approaches and has the best binding parameter values, the 2D interaction diagrams in the binding site are presented in Figure 4, and the 3D interaction is presented in Figure 5.

When the compounds were examined separately in Figure 4, it was determined that all the compounds were docked in the active binding site of Cytochrome c oxidase. It was determined that compound **8** had hydrogen bond interaction with aminoacids Arg438, Arg439, Trp126 and $\pi$-$\pi$ interaction with Phe377 in the binding site. In the 2D interaction diagram of compound **9**, Phe377 aminoacid $\pi$-$\pi$ interaction, Arg438, Arg439 aminoacid residues hydrogen bond interaction were determined.

When the 2D interaction diagram of compound **17** in Figure 4 is examined, there is $\pi$-$\pi$ interaction with His61 and His378, metal coordination with Hea603, and hydrogen bond interaction with Arg38. In the 2D interaction diagram in Figure 4 for compound **18**, there is metal coordination with Hea603, hydrogen bond interaction with amino acids Arg438, Arg439 and Tyr371, and $\pi$-$\pi$ interaction with amino acids His378 and Phe377.

The 3D interactions of amino acid residues in the active binding site of the Cytochrome c oxidase protein of compounds **8, 9, 17** and **18** are presented in detail in Figure 5.

## 3. Conclusion

In summary, a new series of methoxy substituted pyrimidine-*N*-glycosides (**1-9**) and their acetylated derivatives (**10-18**) were effectively synthesized in good yields, and characterized using spectral techniques. This synthesis allowed us to obtain one diastereomer ($\beta$-anomer) of enantiomerically pure pyrimidine-*N*-$\beta$-D-glycosides [9]. The $\beta$-anomeric forms for the sugar derivatives were assigned using NMR studies [11]. In total, 18 compounds were synthesized,

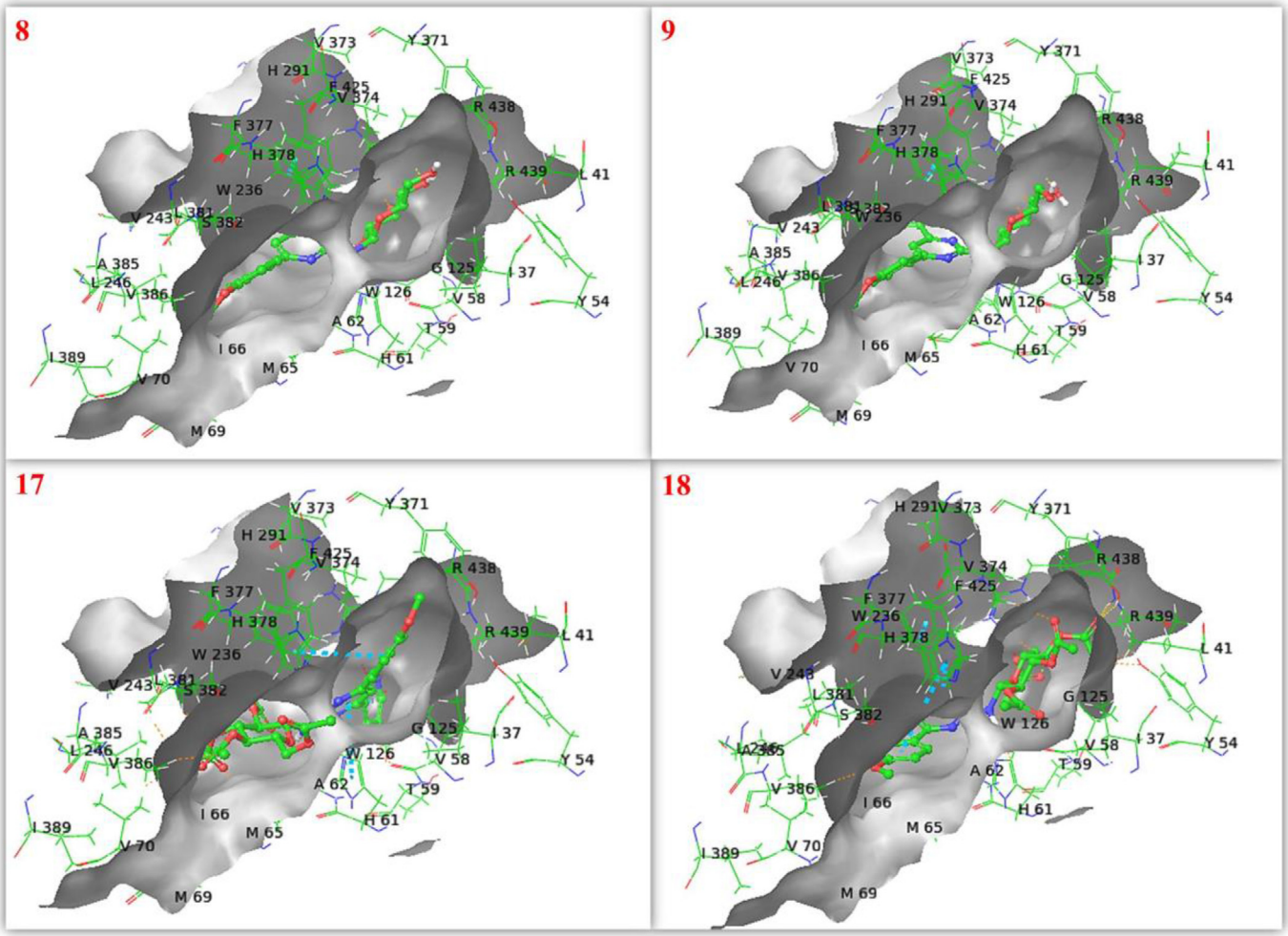



**Figure 5.** 3D interaction of compounds **(8, 9, 17 and 18)** with the Cytochrome c oxidase protein (PDB ID: 5Z62).

and it is determined by the literature review that all of them are new.

In this work, the biological activities -antiproliferative, cytotoxic properties and DNA/protein binding affinities- of the synthesized compounds (**1-18)** were explored. According to the proliferation measurements, compounds **12-15** for C6 cells, **13** and **16** for HeLa cells, **11** and **17** for Hep3B cells, **9** for HT29 cells, and **11** for MCF7 cells exhibited high anticancer activity by largely meeting the NCI criteria for the relevant cancer therapies without causing any toxic effects. On the other hand, when the *in vitro* antibacterial test results of these compounds were evaluated as a whole and it was found that there were similar antibacterial properties between pyrimidine-*N*-$\beta$-D-glycosides (**1-9**) and their tetra-*O*-acetyl derivatives (**10-18**). In addition, the new molecules were able to bind strongly enough to DNA. In particular, tetra-*O*-acetyl derivatives of pyrimidine-*N*-$\beta$-glycosides were found to show more affinity in DNA than others. For this reason, they could be candidates for ADME/Tox and advanced phase studies due to the significant *in vitro* biological activity shown by the respective test compounds.

In our study, the binding parameter values of the compounds examined experimentally were determined *via* applying molecular docking calculations with *in silico* approaches. According to the results of molecular dockig analysis of compounds **8, 9, 17** and **18**, it was determined that they had the best interaction with BRAF, Cytochrome c oxidase and PI3K alpha. According to the results of these determinations, it can be said that the compounds can be drug candidates and lead to the next studies.

## 4. Experimental

The materials and equipment used in this study were presented in supplementary information.

### *4.1. Methods*

#### *4.1.1. General procedure for synthesis of compounds 1-9*

A mixture of pyrimidine derivative (15 mmol, 4.2 g), D-glucose monohydrate (15 mmol, 2.97 g) and glacial acetic acid (15 mmol, 0.85 mL) in dimethyl sulfoxide (6 mL) was heated and refluxed at 100 °C for 24 h under progress control by TLC examination [22]. The control of UV inactive glucose was done by spraying 10% alcoholic sulfuric acid solution onto TLC plate using an atomizer and the TLC plate was then burned on a hot plate. After the reaction completion, the mixture was allowed to cool to room temperature and placed in the separating funnel, shaken by adding chloroform, and then distilled water. When the shaken mixture came to rest, phase separation and the formation of precipitate between the two phases were observed. The precipitate was recovered after separation of the solvents and was washed again with chloroform and water in a the separatory funnel to try to remove impurities

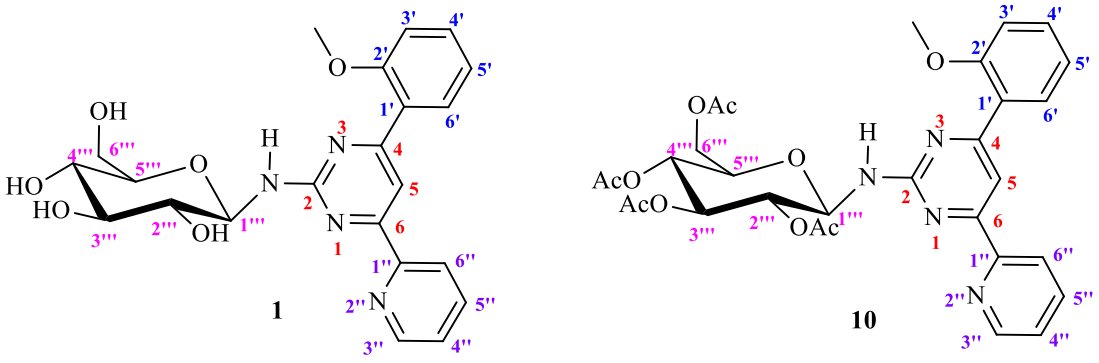



**Figure 6.** Numbering of the atoms in compounds **1** and **10.**

that may arise from unreacted pyrimidine and glucose. The purity of the precipitate was checked again with TLC and dried with a freeze dryer. The structure was confirmed by spectroscopic methods ($^1$H, $^1$H-$^1$H COSY and $^{13}$C-APT NMR, LC-MS/MS and FT-IR) and elemental analysis. The numbering of atoms in compounds **8** and **13** was given in Figure 6 as an example for spectroscopic analysis of pyrimidine-*N*-glycosides and acetylated derivatives.

*4.1.1.1. N-[4-(2-methoxyphenyl)-6-pyridin-2-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (1).* Yield: 56%. White solid, M.p.: 132-134°C. Rf: 0.73 (Ethyl acetate-methanol: 1:1).

FT-IR (cm$^{-1}$): 3332, 2923, 1541, 1360, 1246, 1014, 754.

$^1$H-NMR (400 MHz, $CD_3OD$, ppm): $\delta$=8.2 (s, 1H, H-5); $\delta$= 7.2 (d, $J$= 8.0 Hz, 1H, H-3′); $\delta$= 7.5 (t, $J$= 8.0 Hz, 1H, H-4′); $\delta$= 7.1 (t, $J$= 8.0 Hz, 1H, H-5′); $\delta$= 7.9 (d, $J$= 8.0 Hz, 1H, H-6′); $\delta$= 8.7 (d, $J$= 4.0 Hz, 1H, H-3′′); $\delta$= 7.4 (t, $J$= 4.0 Hz,1H, H-4′′); $\delta$= 8.0 (dd, $J$= 8.0/4.0 Hz, 1H, H-5′′); $\delta$= 8.5 (8.0, 1H, H-6′′); $\delta$=5.5 (d, $J$= 8.0 Hz, 1H, H-1′′′); $\delta$= 3.4-3.6 (m, 4H, H-2′′′/H-3′′′/H-4′′′/ H-5′′′); $\delta$= 3.9 (d, $J$= 12.0 Hz, 1H, H-6a′′′); $\delta$= 3.7 (dd, $J$= 12.0/4.0 Hz,1H, H-6b′′′); $\delta$=3.9 (s, 3H, -$OCH_3$).

$^{13}$C-NMR (100 MHz, $CD_3OD$, ppm): 162.4 (C-2), 152.1 (C-4), 109.1 (C-5), 158.0 (C-6), 126.9 (C-1′), 163.1 (C-2′), 111.4 (C-3′), 130.5 (C-4′), 120.4 (C-5′), 131.2 (C-6′), 154.8 (C-1′′), 148.9 (C-3′′), 125.0 (C-4′′), 137.3 (C-5′′), 122.0 (C-6′′), 82.6 (C-1′′′), 70.5 (C-2′′′), 78.0 (C-3′′′), 73.0 (C-4′′′), 77.9 (C-5′′′), 61.5 (C-6′′′), 54.8 (-$OCH_3$).

Poz. LC-MS/MS m/z (%): 441 (25) $[M+1]^+$, 463 (100) $[M+Na]^+$.

Anal. cal. for $C_{22}H_{24}N_4O_6$ (440.45 g/mol): C 59.99, H 5.49, N 12.72, found: C 59.86, H 5.52, N 12.70.

*4.1.1.2. N-[4-(2-methoxyphenyl)-6-pyridin-3-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (2).* Yield:61%. White solid, M.p.:60-62°C. Rf: 0.77 (Ethyl acetate-methanol: 1:1).

FT-IR (cm$^{-1}$): 3319, 2919, 1584, 1542, 1356, 1245, 1076, 1016, 757.

$^1$H-NMR (400 MHz, $CD_3OD$, ppm): $\delta$=7.6 (s, 1H, H-5); $\delta$= 7.0 (bs, 1H, H-3′); $\delta$= 7.4 (m, 1H, H-4′); $\delta$= 7.0 (d, $J$= 8.3 Hz, 1H, H-5′); $\delta$= 7.8 (bs, 1H, H-6′); $\delta$= 9.1 (s, 1H, H-2′′); $\delta$= 8.5 (bs, 1H, H-4′′); $\delta$= 7.4 (t, $J$= 7.5 Hz, 1H, H-5′′); $\delta$= 8.3 (bs, 1H, H-6′′); $\delta$=5.5 (d, $J$= 8.8 Hz, 1H, H-1′′′); $\delta$= 3.4-3.6 (m, 4H, H-2′′′/H-3′′′/H-4′′′/ H-5′′′); $\delta$= 3.9 (d, $J$= 12.3 Hz, 1H, H-6a′′′); $\delta$=3.7 (dd, $J$= 11.8/5.2 Hz, 1H, H-6b′′′); $\delta$=3.9 (s, 3H, -$OCH_3$).

$^{13}$C-NMR (100 MHz, $CD_3OD$, ppm): 162.4 (C-2), 157.9 (C-4), 108.6 (C-5), 161.4 (C-6), 126.3 (C-1′), 162.3 (C-2′), 111.3 (C-3′), 130.5 (C-4′), 120.4 (C-5′), 131.3 (C-6′), 133.6 (C-1′′), 149.9 (C-2′′), 147.4 (C-4′′), 120.4 (C-5′′), 135.2 (C-6′′), 82.5 (C-1′′′), 70.4 (C-2′′′), 78.0 (C-3′′′), 73.0 (C-4′′′), 77.8 (C-5′′′), 61.5 (C-6′′′), 54.8 (-$OCH_3$).

Poz. LC-MS/MS m/z (%):463 (100) $[M+Na]^+$, 464 (97) $[M+1+Na]^+$.

Anal. cal. for $C_{22}H_{24}N_4O_6$ (440.45 g/mol): C 59.99, H 5.49, N 12.72, found: C 59.96, H 5.53, N 12.69.

*4.1.1.3. N-[4-(2-methoxyphenyl)-6-pyridin-4-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (3).* Yield: 71%. White solid, M.p.: 154-158°C. Rf: 0.54 (Ethyl acetate-methanol: 1:1).

FT-IR (cm$^{-1}$): 3304, 2905, 1582, 1538, 1358, 1245, 1015, 826, 757.

$^1$H-NMR (400 MHz, $CD_3OD$, ppm): $\delta$=7.9 (s, 1H, H-5); $\delta$= 7.2 (d, $J$= 8.5 Hz, 1H, H-3′); $\delta$= 7.5 (t, $J$= 8.0 Hz, 1H, H-4′); $\delta$= 7.1 (t, $J$= 7.5 Hz, 1H, H-5′); $\delta$= 8.0 (d, $J$= 7.8 Hz, 1H, H-6′); $\delta$= 8.1 (d, $J$= 5.8 Hz, 1H, H-2′′); $\delta$= 8.7 (d, $J$= 5.0 Hz, 1H, H-3′′); $\delta$= 8.7 (d, $J$= 5.0 Hz, 1H, H-5′′); $\delta$= 8.1 (d, $J$= 5.8 Hz, 1H, H-6′′); $\delta$=5.5 (d, $J$= 9.0 Hz, 1H, H-1′′′); $\delta$= 3.4-3.6 (m, 4H, H-2′′′/H-3′′′/H-4′′′/ H-5′′′); $\delta$= 3.9 (dd, $J$= 12.0/2.0 Hz, 1H, H-6a′′′); $\delta$=3.7 (dd, $J$= 11.9/5.2 Hz, 1H, H-6b′′′); $\delta$=4.0 (s, 3H, -$OCH_3$).

$^{13}$C-NMR (100 MHz, $CD_3OD$, ppm): 165.9 (C-2), 161.7 (C-4), 109.1 (C-5), 158.2 (C-6), 126.7 (C-1′), 162.7 (C-2′), 111.8 (C-3′), 130.5 (C-4′), 120.6 (C-5′), 131.4 (C-6′), 146.1 (C-1′′), 121.4 (C-2′′), 149.5 (C-3′′), 149.5 (C-5′′), 121.4 (C-6′′), 82.6 (C-1′′′), 70.7 (C-2′′′), 78.0 (C-3′′′), 73.1 (C-4′′′), 78.0 (C-5′′′), 61.7 (C-6′′′), 55.0 (-$OCH_3$).

Poz. LC-MS/MS m/z (%): 263 (100) $[M-(NH\text{-}Glucopyranosyl)]^+$, 441 (65) $[M+1]^+$, 463 (32) $[M+Na]^+$.

Anal. cal. for $C_{22}H_{24}N_4O_6$ (440.45 g/mol): C 59.99, H 5.49, N 12.72, found: C 59.94, H 5.53, N 12.70.

*4.1.1.4. N-[4-(3-methoxyphenyl)-6-pyridin-2-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (4).* Yield: 72%. White solid, M.p.: 94-96°C. Rf: 0.58 (Ethyl acetate-methanol: 1:1).

FT-IR (cm$^{-1}$): 3321, 2928, 1572, 1546, 1360, 1077, 1010, 779.

$^1$H-NMR (400 MHz, $CD_3OD$, ppm): $\delta$=8.1 (s, 1H, H-5); $\delta$= 7.5 (bs, 1H, H-2′); $\delta$= 7.1 (t, $J$= 8.0 Hz, 1H, H-4′); $\delta$= 7.8 (m, 1H, H-5′); $\delta$= 7.8 (bs, 1H, H-6′); $\delta$= 8.7 (bs, 1H, H-3′′); $\delta$= 7.4 (t, $J$= 7.3 Hz, 1H, H-4′′); $\delta$= 8.0 (t, $J$= 7.0 Hz, 1H, H-5′′); $\delta$= 8.5 (d, $J$= 7.0 Hz, 1H, H-6′′); $\delta$=5.5 (d, $J$= 8.6 Hz, 1H, H-1′′′); $\delta$= 3.4-3.6 (m, 4H, H-2′′′/H-3′′′/H-4′′′/ H-5′′′); $\delta$= 3.9 (s, 1H, H-6a′′′); $\delta$= 3.7 (dd, $J$= 11.2/4.2 Hz, 1H, H-6b′′′); $\delta$=4.0 (s, 3H, -$OCH_3$).

$^{13}$C-NMR (100 MHz, $CD_3OD$, ppm): 162.7 (C-2), 164.3 (C-4), 104.1 (C-5), 148.4 (C-6), 138.9 (C-1′), 119.3 (C-2′), 166.1 (C-3′), 112.1 (C-4′), 129.4 (C-5′), 116.5 (C-6′), 160.3 (C-1′′), 148.9 (C-3′′), 121.8 (C-4′′), 137.3 (C-5′′), 125.1 (C-6′′), 82.8 (C-1′′′), 70.7 (C-2′′′), 78.1 (C-3′′′), 73.1 (C-4′′′), 78.0 (C-5′′′), 61.7 (C-6′′′), 54.6 (-$OCH_3$).

Poz. LC-MS/MS m/z (%): 263 (45) $[M-(NH\text{-}Glycopyranosyl)]^+$, 441 (25) $[M+1]^+$, 463 (100) $[M+Na]^+$.

Anal. cal. for $C_{22}H_{24}N_4O_6$ (440.45 g/mol): C 59.99, H 5.49, N 12.72, found: C 59.91, H 5.54, N 12.71.

*4.1.1.5. N-[4-(3-methoxyphenyl)-6-pyridin-3-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (5).* Yield: 77%. White solid, M.p.: 130-132°C. Rf: 0.67 (Ethyl acetate-methanol: 1:1).

FT-IR (cm$^{-1}$): 3312, 2900, 1579, 1545, 1355, 1079, 1030, 786, 701.

$^1$H-NMR (400 MHz, $CD_3OD$, ppm): $\delta$=7.7 (s, 1H, H-5); $\delta$= 7.4 (t, $J$= 7.8 Hz, 1H, H-2′); $\delta$= 7.1 (d, $J$= 7.0 Hz, 1H, H-4′); $\delta$= 7.6 (m, 1H, H-5′); $\delta$= 7.8 (m, 1H, H-6′); $\delta$= 9.3 (bs, 1H, H-2′′); $\delta$= 8.7 (bs, 1H, H-4′′); $\delta$= 7.7 (m, 1H, H-5′′); $\delta$= 8.6 (d, $J$= 6.8 Hz, 1H, H-6′′); $\delta$=5.5 (d, $J$= 8.8 Hz, 1H, H-1′′′); $\delta$= 3.4-3.6 (m, 4H, H-2′′′/H-3′′′/H-4′′′/ H-5′′′); $\delta$=3.9 (s, 1H, H-6a′′′); $\delta$=3.7 (dd, $J$= 11.6/5.2 Hz, 1H, H-6b′′′); $\delta$=3.9 (s, 3H, -$OCH_3$).

$^{13}$C-NMR (100 MHz, $CD_3OD$, ppm): 166.2 (C-2), 162.0 (C-4), 104.0 (C-5), 150.6 (C-6), 138.5 (C-1′), 123.8 (C-2′), 112.3 (C-4′), 129.4 (C-5′), 116.4 (C-6′), 147.6 (C-2′′), 147.8 (C-4′′), 119.4 (C-5′′), 135.2 (C-6′′), 82.7 (C-1′′′), 70.7 (C-2′′′), 78.0 (C-3′′′), 73.1 (C-4′′′), 78.1 (C-5′′′), 61.7 (C-6′′′), 55.0 (-$OCH_3$).

Poz. LC-MS/MS m/z (%):441 (72) $[M+1]^+$, 463 (100) $[M+Na]^+$.

Anal. cal. for $C_{22}H_{24}N_4O_6$ (440.45 g/mol): C 59.99, H 5.49, N 12.72, found: C 59.97, H 5.52, N 12.70.

*4.1.1.6. N-[4-(3-methoxyphenyl)-6-pyridin-4-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (6).* Yield: 73 %. White solid, M.p.: 145-147°C. Rf: 0.58 (Ethyl acetate-methanol: 1:1).

FT-IR ($cm^{-1}$): 3303, 2910, 1540, 1360, 1078, 1023, 776.

$^1$H-NMR (400 MHz, $CD_3OD$, ppm): $\delta$=7.7 (s, 1H, H-5); $\delta$= 7.8 (m, 1H, H-2′); $\delta$= 7.1 (d, $J$= 7.4 Hz, 1H, H-4′); $\delta$= 7.4 (t, $J$= 7.9 Hz, 1H, H-5′); $\delta$= 7.8 (m, 1H, H-6′); $\delta$= 8.2 (bs, 1H, H-2′′); $\delta$= 8.7 (d, $J$= 4.4 Hz, 1H, H-3′′); $\delta$= 8.7 (d, $J$= 4.4 Hz, 1H, H-5′′); $\delta$= 8.2 (bs, 1H, H-6′′); $\delta$=5.5 (d, $J$= 8.8 Hz, 1H, H-1′′′); $\delta$= 3.4-3.6 (m, 4H, H-2′′′/H-3′′′/H-4′′′/ H-5′′′); $\delta$=3.9 (s, 1H, H-6a′′′); $\delta$= 3.7 (dd, $J$= 11.6/5.2 Hz, 1H, H-6b′′′); $\delta$=3.9 (s, 3H, -$OCH_3$).

$^{13}$C-NMR (100 MHz, $CD_3OD$, ppm): 166.4 (C-2), 161.4 (C-4), 104.2 (C-5), 160.1 (C-6), 138.4 (C-1′), 129.4 (C-2′), 162.8 (C-3′), 119.3 (C-4′), 121.5 (C-5′), 116.4 (C-6′), 145.9 (C-1′′), 112.0 (C-2′′), 149.4 (C-3′′), 149.4 (C-5′′), 112.0 (C-6′′), 82.6 (C-1′′′), 70.4 (C-2′′′), 77.8 (C-3′′′), 72.9 (C-4′′′), 78.1 (C-5′′′), 61.5 (C-6′′′), 54.5 (-$OCH_3$).

Poz. LC-MS/MS m/z (%):441 (100) $[M+1]^+$.

Anal. cal. for $C_{22}H_{24}N_4O_6$ (440.45 g/mol): C 59.99, H 5.49, N 12.72, found: C 59.97, H 5.53, N 12.71.

*4.1.1.7. N-[4-(4-methoxyphenyl)-6-pyridin-2-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (7).* Yield: 69 %. White solid, M.p.: 232-234°C. Rf: 0.54 (Ethyl acetate-methanol: 1:1).

FT-IR ($cm^{-1}$): 3315, 2928, 1579, 1542, 1366, 1235, 1173, 1079, 1012, 833, 783.

$^1$H-NMR (400 MHz, $CD_3OD$, ppm): $\delta$=8.1 (s, 1H, H-5); $\delta$= 7.8 (m, 1H, H-2′); $\delta$= 7.1 (d, $J$= 7.4 Hz, 1H, H-4′); $\delta$= 7.4 (t, $J$= 7.9 Hz, 1H, H-5′); $\delta$= 7.8 (m, 1H, H-6′); $\delta$= 8.2 (bs, 1H, H-2′′); $\delta$= 8.7 (d, $J$= 4.4 Hz, 1H, H-3′′); $\delta$= 8.7 (d, $J$= 4.4 Hz, 1H, H-5′′); $\delta$= 8.2 (bs, 1H, H-6′′); $\delta$=5.5 (d, $J$= 8.8 Hz, 1H, H-1′′′); $\delta$= 3.4-3.6 (m, 4H, H-2′′′/H-3′′′/H-4′′′/ H-5′′′); $\delta$=3.9 (s, 1H, H-6a′′′); $\delta$= 3.7 (dd, $J$= 11.6/5.2 Hz, 1H, H-6b′′′); $\delta$=3.9 (s, 3H, -$OCH_3$).

$^{13}$C-NMR (100 MHz, $CD_3OD$, ppm): 159.4 (C-2), 162.2 (C-4), 103.0 (C-5), 162.7 (C-6), 129.7 (C-1′), 128.5 (C-2′), 113.7 (C-3′), 165.1 (C-4′), 113.7 (C-5′), 128.5 (C-6′), 154.8 (C-1′′), 148.9 (C-3′′), 121.8 (C-4′′), 137.4 (C-5′′), 125.1 (C-6′′), 82.7 (C-1′′′), 70.4 (C-2′′′), 78.1 (C-3′′′), 73.0 (C-4′′′), 77.9 (C-5′′′), 61.5 (C-6′′′), 54.5 (-$OCH_3$).

Poz. LC-MS/MS m/z (%):106 (100) [methoxyphenyl+1]$^+$, 441 (40) $[M+1]^+$.

Anal. cal. for $C_{22}H_{24}N_4O_6$ (440.45 g/mol): C 59.99, H 5.49, N 12.72, found: C 59.93, H 5.52, N 12.71.

*4.1.1.8. N-[4-(4-methoxyphenyl)-6-pyridin-3-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (8).* Yield: 63 %. White solid, M.p.: 147-149°C. Rf: 0.63 (Ethyl acetate-methanol: 1:1).

FT-IR ($cm^{-1}$): 3331, 2910, 1586, 1544, 1514, 1367, 1242, 1173, 1072, 1019, 817.

$^1$H-NMR (400 MHz, $CD_3OD$, ppm): $\delta$=7.6 (s, 1H, H-5); $\delta$= 8.1 (d, $J$= 8.8 Hz, 1H, H-2′); $\delta$= 7.0 (d, $J$= 8.8 Hz, 1H, H-3′); $\delta$= 7.0 (d, $J$= 8.8 Hz, 1H, H-5′); $\delta$= 8.1 (d, $J$= 8.8 Hz, 1H, H-6′); $\delta$= 9.3 (bs, 1H, H-2′′); $\delta$= 8.6 (d, $J$= 3.8 Hz, 1H, H-4′′); $\delta$= 7.5 (m, 1H, H-5′′); $\delta$= 8.5 (d, $J$= 8.0 Hz, 1H, H-6′′); $\delta$=5.5 (d, $J$= 8.8 Hz, 1H, H-1′′′); $\delta$= 3.4-3.6 (m, 4H, H-2′′′/H-3′′′/H-4′′′/ H-5′′′); $\delta$= 3.9 (dd, $J$= 12.0/2.0 Hz, 1H, H-6a′′′); $\delta$= 3.7 (dd, $J$= 11.2/5.2 Hz, 1H, H-6b′′′); $\delta$=3.9 (s, 3H, -$OCH_3$).

$^{13}$C-NMR (100 MHz, DMSO-$d_6$, ppm): 162.0 (C-2), 164.7 (C-4), 102.8 (C-5), 162.8 (C-6), 133.3 (C-1′), 129.3 (C-2′), 114.5 (C-3′), 165.3 (C-4′), 114.5 (C-5′), 129.3 (C-6′), 129.7 (C-1′′), 151.6 (C-2′′), 148.8 (C-4′′), 124.2 (C-5′′), 134.9 (C-6′′), 82.9 (C-1′′′), 70.8 (C-2′′′), 78.9 (C-3′′′), 72.8 (C-4′′′), 78.4 (C-5′′′), 61.7 (C-6′′′), 55.8 (-$OCH_3$).

Poz. LC-MS/MS m/z (%):441 (100) $[M+1]^+$, 442 (80) $[M+2]^+$.

Anal. cal. for $C_{22}H_{24}N_4O_6$ (440.45 g/mol): C 59.99, H 5.49, N 12.72, found: C 59.96, H 5.51, N 12.70.

*4.1.1.9. N-[4-(4-methoxyphenyl)-6-pyridin-4-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (9).* Yield: 55 %. White solid, M.p.: 163-165°C. Rf: 0.58 (Ethyl acetate-methanol: 1:1).

FT-IR ($cm^{-1}$): 3301, 2900, 1581, 1536, 1512, 1363, 1239, 1079, 1022, 806.

$^1$H-NMR (400 MHz, $CD_3OD$, ppm): $\delta$=7.7 (s, 1H, H-5); $\delta$= 8.2 (m, 1H, H-2′); $\delta$= 7.1 (d, $J$= 8.3 Hz, 1H, H-3′); $\delta$= 7.1 (d, $J$= 8.3 Hz, 1H, H-5′); $\delta$= 8.2 (m, 1H, H-6′); $\delta$= 8.1 (m, 1H, H-2′′); $\delta$= 8.7 (bs, 1H, H-3′′); $\delta$= 8.7 (bs, 1H, H-5′′); $\delta$= 8.1 (m, 1H, H-6′′); $\delta$=5.5 (d, $J$= 8.6 Hz, 1H, H-1′′′); $\delta$= 3.4-3.6 (m, 4H, H-2′′′/H-3′′′/H-4′′′/ H-5′′′); $\delta$= 3.9 (s, 1H, H-6a′′′);$\delta$= 3.7 (dd, $J$= 11.3/5.0 Hz, 1H, H-6b′′′); $\delta$=3.9 (s, 3H, -$OCH_3$).

$^{13}$C-NMR (100 MHz, $CD_3OD$, ppm): 162.9 (C-2), 165.1 (C-4), 103.5 (C-5), 160.1 (C-6), 131.8 (C-1′), 126.6 (C-2′), 113.9 (C-3′), 166.3 (C-4′), 113.9 (C-5′), 126.6 (C-6′), 146.2 (C-1′′), 121.5 (C-2′′), 149.4 (C-3′′), 149.4 (C-5′′), 121.5 (C-6′′), 82.7 (C-1′′′), 70.7 (C-2′′′), 78.1 (C-3′′′), 73.1 (C-4′′′), 78.0 (C-5′′′), 61.7 (C-6′′′), 54.6 (-$OCH_3$).

Poz. LC-MS/MS m/z (%):441 (100) $[M+1]^+$, 442 (60) $[M+2]^+$.

Anal. cal. for $C_{22}H_{24}N_4O_6$ (440.45 g/mol): C 59.99, H 5.49, N 12.72, found: C 59.98, H 5.52, N 12.70.

*4.1.2. General procedure for synthesis of compounds 10-18*

Methoxy substituted pyrimidine-*N*-glycoside (**1-9**) (3 mmol, 1.3 g each), acetic anhydride (12 mmol, 1.2 g) and $Na_2CO_3$ (12 mmol, 1.1 g) were mixed and this mixture was stirred at 100 °C under reflux conditions for 10-20 min, with progress monitored by TLC examination [25]. Water and then chloroform were added to the completed reaction and the mixture was placed in the separatory funnel, shaken andallowed to rest. After phase separation, the organic phase was taken and the solvent was removed in vacuo. The product was washed with diethyl ether and dried at room temperature. This class of compounds was found to dissolve in chloroform. Purity control of the compounds was performed by TLC and their structures were elucidated by spectroscopic methods ($^1$H, $^1$H-$^1$H COSY and $^{13}$C-APT NMR, LC-MS/MS and FT-IR) and elemental analysis.

*4.1.2.1. 2,3,4,6-tetra-O-acetyl-N-[4-(2-methoxyphenyl)-6-pyridin-2-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (10).* Yield: 81 %. White solid, M.p.: 12-129°C. Rf: 0.70 (Diethyl ether-ethyl acetate: 1:1).

FT-IR ($cm^{-1}$): 3419, 2944, 1741, 1580, 1543, 1362, 1219, 1031, 755.

$^1$H-NMR (400 MHz, $CDCl_3$, ppm): $\delta$=8.4 (s, 1H, H-5); $\delta$= 7.0 (d, $J$=8.0 Hz, 1H, H-3′); $\delta$= 7.4 (t, $J$=8.0 Hz, 1H, H-4′); $\delta$= 7.1 (t, $J$=8.0 Hz, 1H, H-5′); $\delta$= 7.9 (d, $J$=8.0 Hz,1H, H-6′); $\delta$= 8.7 (d, $J$=4.0 Hz, 1H, H-3′′); $\delta$= 7.4 (t, $J$=4.0 Hz, 1H, H-4′′); $\delta$= 8.0 (t, $J$=8.0 Hz, 1H, H-5′′); $\delta$= 8.4 (d, $J$=8.0 Hz, 1H, H-6′′); $\delta$=5.9 (d, $J$=8.0 Hz, 1H, H-1′′′); $\delta$= 5.8 (t, $J$=8.0 Hz, 1H, H-2′′′); $\delta$= 5.2 (t, $J$=8.0 Hz, 1H, H-3′′′); $\delta$= 5.5 (t, $J$=8.0 Hz, 1H, H-4′′′); $\delta$= 5.2 (t, $J$=8.0 Hz, 1H, H-5′′′); $\delta$= 4.3 (dd, $J$=12.0/8.0 Hz 1H, H-6a′′′); $\delta$=4.2 (d, $J$=12.0 Hz, 1H, H-6b′′′); $\delta$=3.9 (s, 3H, -$OCH_3$); $\delta$=4.0 (bs, 1H, NH); $\delta$= 2.0-2.1 (m, 12H, acetyl $CH_3$).

$^{13}$C-NMR (100 MHz, $CDCl_3$, ppm): 161.0 (C-2), 154.9 (C-4), 110.6 (C-5), 158.0 (C-6), 127.0 (C-1′), 161.0 (C-2′), 111.5 (C-3′), 130.9 (C-4′), 120.8 (C-5′), 131.4 (C-6′), 154.9 (C-1′′), 149.4 (C-3′′), 124.9 (C-4′′), 136.8 (C-5′′), 121.7 (C-6′′), 81.3 (C-1′′′), 68.9 (C-2′′′), 73.4 (C-3′′′), 73.2 (C-4′′′), 70.6 (C-5′′′), 62.2 (C-6′′′), 20.6-20.8 (acetyl $CH_3$), 170.7/170.6/170.2/169.6 (C=O), 55.7 (-$OCH_3$).

Poz. LC-MS/MS m/z (%):632 (100) $[M+Na+1]^+$.

Anal. cal. for $C_{30}H_{32}N_4O_{10}$ (608.21 g/mol): C 59.21, H 5.30, N 9.21, found: C 59.20, H 5.34, N 9.20.

*4.1.2.2. 2,3,4,6-tetra-O-acetyl-N-[4-(2-methoxyphenyl)-6-pyridin-3-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (11).* Yield: 75 %. White solid, M.p.: 77-79°C. Rf: 0.45 (Diethyl ether-ethyl acetate: 1:1).

FT-IR ($cm^{-1}$): 3427, 2945, 1740, 1586, 1544, 1362, 1221, 1031, 753.

$^1$H-NMR (400 MHz, $CDCl_3$, ppm): δ=7.9 (s, 1H, H-5); δ= 7.1 (bs, 1H, H-3′); δ= 7.5 (bs, 1H, H-4′); δ= 7.5 (bs, 1H, H-5′); δ= 8.0 (bs, 1H, H-6′); δ= 9.3 (bs, 1H, H-2′′); δ= 8.8 (bs, 1H, H-4′′); δ= 7.2 (bs, 1H, H-5′′); δ= 8.4 (bs, 1H, H-6′′); δ=6.0 (bs, 1H, H-1′′′); δ= 5.8 (d, J=8.0 Hz, 1H, H-2′′′); δ= 5.2 (m, 2H, H-3′′′/ H-5′′′); δ= 5.5 (d, J=8.0 Hz, 1H, H-4′′′); δ= 4.3 (bs, 1H, H-6a′′′); δ=4.2 (bs, 1H, H-6b′′′); δ=4.0 (s, 3H, -$OCH_3$); δ=4.0 (bs, 1H, NH); δ= 2.0-2.1 (bs, 12H, acetyl $CH_3$).

$^{13}$C-NMR (100 MHz, $CDCl_3$, ppm): 161.2 (C-2), 158.0 (C-4), 110.3 (C-5), 155.9 (C-6), 126.3 (C-1′), 165.9 (C-2′), 111.5 (C-3′), 131.0 (C-4′), 121.2.8 (C-5′), 131.7 (C-6′), 133.3 (C-1′′), 151.2 (C-2′′), 148.6 (C-4′′), 123.6 (C-5′′), 134.6 (C-6′′), 81.2 (C-1′′′), 68.8 (C-2′′′), 73.3 (C-3′′′), 73.2 (C-4′′′), 70.5 (C-5′′′), 62.2 (C-6′′′), 20.8-20.7 (acetyl $CH_3$), 170.8/170.7/170.1/169.7 (C=O), 55.7 (-$OCH_3$).

Poz. LC-MS/MS m/z (%):609 (75) $[M]^+$, 610 (78) $[M+1]^+$, 631 (100) $[M+Na]^+$.

Anal. cal. for $C_{30}H_{32}N_4O_{10}$ (608.21 g/mol): C 59.21, H 5.30, N 9.21, found: C 59.19, H 5.33, N 9.19.

*4.1.2.3. 2,3,4,6-tetra-O-acetyl-N-[4-(2-methoxyphenyl)-6-pyridin-4-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (12).* Yield: 81 %. White solid, M.p.: 157-159°C. Rf: 0.63 (Diethyl ether-ethyl acetate: 1:1).

FT-IR ($cm^{-1}$): 3362, 2946, 1743, 1582, 1535, 1362, 1215, 1028, 759.

$^1$H-NMR (400 MHz, $CDCl_3$, ppm): δ=7.9 (s, 1H, H-5); δ= 7.0 (d, J= 8.3 Hz, 1H, H-3′); δ= 7.5 (t, J=7.0 Hz, 1H, H-4′); δ= 7.1 (t, J=7.0 Hz, 1H, H-5′); δ= 7.9 (bs, 1H, H-6′); δ= 7.9 (d, J=3.5 Hz, 1H, H-2′′); δ= 8.8 (d, J=3.5 Hz, 1H, H-3′′); δ= 8.8 (d, J= 3.5 Hz, 1H, H-5′′); δ= 7.9 (d, J=3.5 Hz, 1H, H-6′′); δ=6.1 (bs, 1H, H-1′′′); δ= 5.8 (t, J= 9.2 Hz, 1H, H-2′′′); δ= 5.2 (m, 1H, H-3′′′); δ= 5.5 (t, J=9.2 Hz, 1H, H-4′′′); δ= 5.1 (m, 1H, H-5′′′); δ= 4.3 (dd, J= 11.8/4.8 Hz, 1H, H-6a′′′); δ=4.2 (d, J= 12.2 Hz, 1H, H-6b′′′); δ=3.9 (s, 3H, -$OCH_3$); δ=4.0 (bs, 1H, NH); δ= 1.9-2.1 (m, 12H, acetyl $CH_3$).

$^{13}$C-NMR (100 MHz, $CDCl_3$, ppm): 161.4 (C-2), 158.1 (C-4), 110.5 (C-5), 158.0 (C-6), 126.2 (C-1′), 161.4 (C-2′), 111.6 (C-3′), 130.9 (C-4′), 121.1 (C-5′), 131.8 (C-6′), 144.3 (C-1′′), 121.2 (C-2′′), 150.3 (C-3′′), 150.3 (C-5′′), 121.2 (C-6′′), 81.1 (C-1′′′), 69.8 (C-2′′′), 73.3 (C-3′′′), 73.2 (C-4′′′), 70.5 (C-5′′′), 62.2 (C-6′′′), 20.7-20.6 (acetyl $CH_3$), 170.6/170.1/169.6 (C=O), 55.7 (-$OCH_3$).

Poz. LC-MS/MS m/z (%): 609 (44) $[M+1]^+$, 631 (100) $[M+Na]^+$.

Anal. cal. for $C_{30}H_{32}N_4O_{10}$ (608.21 g/mol): C 59.21, H 5.30, N 9.21, found: C 59.20, H 5.31, N 9.20.

*4.1.2.4. 2,3,4,6-tetra-O-acetyl-N-[4-(3-methoxyphenyl)-6-pyridin-2-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (13).* Yield: 78 %. White solid, M.p.: 175-177°C. Rf: 0.67 (Diethyl ether-ethyl acetate: 1:1).

FT-IR ($cm^{-1}$): 3417, 2944, 1740, 1570, 1548, 1364, 1220, 1033, 779.

$^1$H-NMR (400 MHz, $CDCl_3$, ppm): δ=8.3 (s, 1H, H-5); δ= 7.3 (bs, 1H, H-2′); δ= 7.1 (d, J=7.4 Hz, 1H, H-4′); δ= 7.4 (m, 1H, H-5′); δ= 7.7 (bs, 1H, H-6′); δ= 8.7 (bs, 1H, H-3′′); δ= 7.4 (m, 1H, H-4′′); δ= 7.9 (t, J= 7.0 Hz, 1H, H-5′′); δ= 8.4 (d, J=7.4 Hz, 1H, H-6′′); δ=6.0 (d, J=8.8 Hz, 1H, H-1′′′); δ= 5.8 (t, J= 9.2 Hz, 1H, H-2′′′); δ= 5.2 (m, 1H, H-3′′′); δ= 5.5 (t, J=8.8 Hz, 1H, H-4′′′); δ= 5.2 (m, 1H, H-5′′′); δ= 4.3 (dd, J= 12.1/4.4 Hz, 1H, H-6a′′′);δ=4.2 (d, J= 12.2 Hz, 1H, H-6b′′′); δ=3.9 (s, 3H, -$OCH_3$); δ=4.0 (bs, 1H, NH); δ= 1.9-2.1 (m, 12H, acetyl $CH_3$).

$^{13}$C-NMR (100 MHz, DMSO-$d_6$, ppm): 159.9 (C-2), 105.7 (C-5), 154.6 (C-6), 125.2 (C-1′), 119.8 (C-2′), 161.2 (C-3′), 112.1 (C-4′), 129.7 (C-5′), 117.8 (C-6′), 154.5 (C-1′′), 137.0 (C-3′′), 121.6 (C-4′′), 134.1 (C-5′′), 125.2 (C-6′′), 81.4 (C-1′′′), 68.9 (C-2′′′), 73.3 (C-3′′′), 73.3 (C-4′′′), 70.6 (C-5′′′), 62.2 (C-6′′′), 20.8-20.6 (acetyl $CH_3$), 170.7/170.1/169.6 (C=O), 55.5 (-$OCH_3$).

Poz. LC-MS/MS m/z (%):631 (100) $[M+Na]^+$.

Anal. cal. for $C_{30}H_{32}N_4O_{10}$ (608.21 g/mol): C 59.21, H 5.30, N 9.21, found: C 59.19, H 5.33, N 9.21.

*4.1.2.5. 2,3,4,6-tetra-O-acetyl-N-[4-(3-methoxyphenyl)-6-pyridin-3-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (14).* Yield: 88%. White solid, M.p.: 203-205°C. Rf: 0.50 (Diethyl ether-ethyl acetate: 1:1).

FT-IR ($cm^{-1}$): 3412, 2941, 1741, 1584, 1572, 1546, 1216, 1034, 785.

$^1$H-NMR (400 MHz, $CDCl_3$, ppm): δ=7.6 (s, 1H, H-5); δ= 7.7 (bs, 1H, H-2′); δ= 7.1 (d, J=6.2 Hz, 1H, H-4′); δ= 7.4 (m, 1H, H-5′); δ= 7.7 (bs, 1H, H-6′); δ= 9.3 (bs, 1H, H-2′′); δ= 8.7 (d, J=3.1 Hz, 1H, H-4′′); δ= 7.4 (m, 1H, H-5′′); δ= 8.4 (d, J=7.5 Hz, 1H, H-6′′); δ=6.1 (d, J=9.7 Hz, 1H, H-1′′′); δ= 5.7 (t, J= 9.2 Hz, 1H, H-2′′′); δ= 5.2 (m, 1H, H-3′′′); δ= 5.5 (t, J=9.2 Hz, 1H, H-4′′′); δ= 5.2 (m, 1H, H-5′′′); δ= 4.3 (dd, J= 12.3/4.8 Hz, 1H, H-6a′′′); δ=4.1 (d, J= 11.8 Hz, 1H, H-6b′′′); δ=3.9 (s, 3H, -$OCH_3$); δ=4.0 (bs, 1H, NH); δ= 1.9-2.1 (m, 12H, acetyl $CH_3$).

$^{13}$C-NMR (100 MHz, $CDCl_3$, ppm): 161.9 (C-2), 152.9 (C-4), 104.7 (C-5), 154.6 (C-6), 129.6 (C-1′), 161.1 (C-2′), 114.0 (C-3′), 121.6 (C-4′), 114.0 (C-5′), 125.0 (C-6′), 129.6 (C-1′′), 149.2 (C-2′′), 136.9 (C-4′′), 129.1 (C-5′′), 128.8 (C-6′′), 81.4 (C-1′′′), 68.9 (C-2′′′), 73.3 (C-3′′′), 73.2 (C-4′′′), 70.6 (C-5′′′), 62.2 (C-6′′′), 20.8-20.7 (acetyl $CH_3$), 170.7/170.7/170.1/169.6 (C=O), 55.4 (-$OCH_3$).

Poz. LC-MS/MS m/z (%): 631 (100) $[M+Na]^+$.

Anal. cal. for $C_{30}H_{32}N_4O_{10}$ (608.21 g/mol): C 59.21, H 5.30, N 9.21, found: C 59.21, H 5.32, N 9.20.

*4.1.2.6. 2,3,4,6-tetra-O-acetyl-N-[4-(3-methoxyphenyl)-6-pyridin-4-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (15).* Yield: 79 %. White solid, M.p.: 163-165°C. Rf: 0.65 (Diethyl ether-ethyl acetate: 1:1).

FT-IR ($cm^{-1}$): 3414, 2944, 1736, 1538, 1562, 1223, 1033, 779.

$^1$H-NMR (400 MHz, $CDCl_3$, ppm): δ=7.6 (s, 1H, H-5); δ= 7.3 (bs, 1H, H-2′); δ= 7.1 (d, J=7.0 Hz, 1H, H-4′); δ= 7.4 (m, 1H, H-5′); δ= 7.7 (bs, 1H, H-6′); δ= 8.0 (bs, 1H, H-2′′); δ= 8.8 (bs, 1H, H-3′′); δ= 8.8 (bs, 1H, H-5′′); δ= 8.0 (bs, 1H, H-6′′); δ=6.0 (d, J=8.3 Hz, 1H, H-1′′′); δ= 5.7 (t, J= 8.4 Hz, 1H, H-2′′′); δ= 5.2 (m, 1H, H-3′′′); δ= 5.5 (t, J=8.4 Hz, 1H, H-4′′′); δ= 5.2 (m, 1H, H-5′′′); δ= 4.3 (dd, J= 12.0/4.0 Hz, 1H, H-6a′′′); δ=4.1 (d, J= 12.0 Hz, 1H, H-6b′′′); δ=3.9 (s, 3H, -$OCH_3$); δ=4.0 (bs, 1H, NH); δ= 1.9-2.1 (m, 12H, acetyl $CH_3$).

$^{13}$C-NMR (100 MHz, $CDCl_3$, ppm): 161.5 (C-4), 105.6 (C-5), 160.1 (C-6), 119.5 (C-2′), 133.0 (C-5′), 115.9 (C-6′), 129.9 (C-2′′), 150.6 (C-3′′), 150.6 (C-5′′), 129.9 (C-6′′), 81.2 (C-1′′′), 68.8 (C-2′′′), 73.3 (C-3′′′), 73.2 (C-4′′′), 70.5 (C-5′′′), 61.1 (C-6′′′), 20.8-20.6 (acetyl $CH_3$), 170.6/170.5/170.1/169.6 (C=O), 55.5 (-$OCH_3$).

Poz. LC-MS/MS m/z (%): 609 (83) $[M+1]^+$, 610 (100) $[M+2]^+$.

Anal. cal. for $C_{30}H_{32}N_4O_{10}$ (608.21 g/mol): C 59.21, H 5.30, N 9.21, found: C 59.22, H 5.32, N 9.21.

*4.1.2.7. 2,3,4,6-tetra-O-acetyl-N-[4-(4-methoxyphenyl)-6-pyridin-2-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (16).* Yield: 86 %. White solid, M.p.: 192-194°C. Rf: 0.68 (Diethyl ether-ethyl acetate: 1:1).

FT-IR ($cm^{-1}$): 3411, 2944, 1736, 1544, 1365, 1223, 1032.

$^1$H-NMR (400 MHz, $CDCl_3$, ppm): $\delta$=8.2 (s, 1H, H-5); $\delta$= 8.2 (d, $J$=7.9 Hz, 1H, 1H, H-2′); $\delta$= 7.0 (d, $J$=7.9 Hz, 1H, H-3′); $\delta$= 7.0 (d, $J$=7.9 Hz, 1H, H-5′); $\delta$= 8.2 (d, $J$=7.9 Hz, 1H, H-6′); $\delta$= 8.7 (bs, 1H, H-3′′); $\delta$= 7.4 (m, 1H, H-4′′); $\delta$= 7.9 (t, $J$=7.0 Hz, 1H, H-5′′); $\delta$= 8.4 (d, $J$=7.0 Hz, 1H, H-6′′); $\delta$=5.9 (d, $J$=9.7 Hz, 1H, H-1′′′); $\delta$= 5.8 (t, $J$= 9.2 Hz, 1H, H-2′′′); $\delta$= 5.2 (m, 1H, H-3′′′); $\delta$= 5.5 (t, $J$=9.7 Hz, 1H, H-4′′′);$\delta$= 5.2 (m, 1H, H-5′′′); $\delta$= 4.3 (dd, $J$= 12.1/4.8 Hz, 1H, H-6a′′′); $\delta$=4.1 (d, $J$= 12.0 Hz, 1H, H-6b′′′); $\delta$=3.9 (s, 3H, -$OCH_3$); $\delta$=4.0 (bs, 1H, NH); $\delta$= 1.9-2.1 (m, 12H, acetyl $CH_3$).

$^{13}$C-NMR (100 MHz, $CDCl_3$, ppm): 160.0 (C-2), 154.9 (C-4), 105.3 (C-5), 154.9 (C-6), 132.9 (C-1′), 134.5 (C-2′), 119.5 (C-3′), 161.5 (C-4′), 119.5 (C-5′), 134.5 (C-6′), 138.4 (C-1′′), 151.4 (C-3′′), 129.9 (C-4′′), 148.5 (C-5′′), 123.6 (C-6′′), 81.2 (C-1′′′), 68.8 (C-2′′′), 73.3 (C-3′′′), 73.3 (C-4′′′), 70.5 (C-5′′′), 62.2 (C-6′′′), 20.8-20.6 (acetyl $CH_3$), 170.7/170.0/169.6/169.6 (C=O), 55.5 (-$OCH_3$).

Poz. LC-MS/MS m/z (%): 631 (100) $[M+Na]^+$.

Anal. cal. for $C_{30}H_{32}N_4O_{10}$ (608.21 g/mol): C 59.21, H 5.30, N 9.21, found: C 59.21, H 5.33, N 9.21.

*4.1.2.8. 2,3,4,6-tetra-O-acetyl-N-[4-(4-methoxyphenyl)-6-pyridin-3-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (17).* Yield: 91 %. White solid, M.p.: 201-203°C. Rf: 0.50 (Diethyl ether-ethyl acetate: 1:1).

FT-IR ($cm^{-1}$): 3409, 2942, 1742, 1585, 1545, 1364, 1219, 1026.

$^1$H-NMR (400 MHz, $CDCl_3$, ppm): $\delta$=7.5 (s, 1H, H-5); $\delta$= 8.1 (d, $J$=7.9 Hz, 1H, 1H, H-2′); $\delta$= 7.0 (d, $J$=6.9 Hz, 1H, H-3′); $\delta$= 7.0 (d, $J$=6.9 Hz, 1H, H-5′); $\delta$= 8.0 (d, $J$=7.9 Hz, 1H, H-6′); $\delta$= 9.3 (bs, 1H, H-2′′); $\delta$= 8.7 (bs, 1H, H-4′′); $\delta$= 7.4 (m, 1H, H-5′′); $\delta$= 8.4 (d, $J$=7.0 Hz, 1H, H-6′′); $\delta$=6.0 (d, $J$=9.6 Hz, 1H, H-1′′′); $\delta$= 5.8 (t, $J$= 9.7 Hz, 1H, H-2′′′); $\delta$= 5.2 (m, 1H, H-3′′′); $\delta$= 5.5 (t, $J$=9.2 Hz, 1H, H-4′′′); $\delta$= 5.2 (m, 1H, H-5′′′); $\delta$= 4.3 (dd, $J$= 11.8/4.8 Hz, 1H, H-6a′′′); $\delta$=4.1 (d, $J$= 11.8 Hz, 1H, H-6b′′′); $\delta$=3.9 (s, 3H, -$OCH_3$); $\delta$=4.0 (bs, 1H, NH); $\delta$= 1.9-2.0 (m, 12H, acetyl $CH_3$).

$^{13}$C-NMR (100 MHz, $CDCl_3$, ppm): 161.4 (C-2), 146.8 (C-4), 104.3 (C-5), 151.8 (C-6), 133.2 (C-1′), 128.7 (C-2′), 114.2 (C-3′), 162.0 (C-4′), 114.2 (C-5′), 128.7 (C-6′), 129.3 (C-1′′), 151.9 (C-2′′), 151.9 (C-4′′), 123.6 (C-5′′), 148.5 (C-6′′), 81.3 (C-1′′′), 68.8 (C-2′′′), 73.3 (C-3′′′), 73.3 (C-4′′′), 70.5 (C-5′′′), 62.2 (C-6′′′), 20.8-20.6 (acetyl $CH_3$), 170.7/170.7/170.1/169.6 (C=O), 55.4 (-$OCH_3$).

Poz. LC-MS/MS m/z (%):631 (100) $[M+Na]^+$.

Anal. cal. for $C_{30}H_{32}N_4O_{10}$ (608.21 g/mol): C 59.21, H 5.30, N 9.21, found: C 59.20, H 5.32, N 9.20.

*4.1.2.9. 2,3,4,6-tetra-O-acetyl-N-[4-(4-methoxyphenyl)-6-pyridin-4-ylpyrimidin-2-yl]-β-D-glucopyranosylamine (18).* Yield: 88 %. White solid, M.p.: 166-169°C. Rf: 0.46 (Diethyl ether-ethyl acetate: 1:1).

FT-IR ($cm^{-1}$): 3418, 2971, 1742, 1580, 1535, 1364, 1221, 1030, 817.

$^1$H-NMR (400 MHz, $CDCl_3$, ppm): $\delta$=7.6 (s, 1H, H-5); $\delta$= 8.1 (d, $J$=7.5 Hz, 1H, 1H, H-2′); $\delta$= 7.0 (d, $J$=7.9 Hz, 1H, H-3′); $\delta$= 7.0 (d, $J$=7.9 Hz, 1H, H-5′); $\delta$= 8.1 (d, $J$=7.5 Hz, 1H, H-6′); $\delta$= 7.9 (bs, 1H, H-2′′); $\delta$= 8.8 (bs, 1H, H-3′′); $\delta$= 8.8 (bs, 1H, H-5′′); $\delta$= 7.9 (bs, 1H, H-6′′); $\delta$=6.0 (d, $J$=9.2 Hz, 1H, H-1′′′); $\delta$= 5.7 (t, $J$= 9.2 Hz, 1H, H-2′′′); $\delta$= 5.2 (m, 1H, H-3′′′); $\delta$= 5.5 (t, $J$=9.6 Hz, 1H, H-4′′′); $\delta$= 5.2 (m, 1H, H-5′′′); $\delta$= 4.3 (dd, $J$= 12.0/4.8 Hz, 1H, H-6a′′′); $\delta$=4.1 (d, $J$= 12.2 Hz, 1H, H-6b′′′); $\delta$=3.9 (s, 3H, -$OCH_3$); $\delta$=4.0 (bs, 1H, NH); $\delta$= 1.9-2.0 (m, 12H, acetyl $CH_3$).

$^{13}$C-NMR (100 MHz, $CDCl_3$, ppm): 162.1 (C-2), 151.8 (C-4), 104.6 (C-5), 144.8 (C-6), 129.2 (C-1′), 128.7 (C-2′), 114.2 (C-3′), 161.5 (C-4′), 114.2 (C-5′), 128.7 (C-6′), 144.8 (C-1′′), 121.0 (C-2′′), 150.5 (C-3′′), 150.5 (C-5′′), 121.0 (C-6′′), 81.2 (C-1′′′), 68.8 (C-2′′′), 73.3 (C-3′′′), 73.2 (C-4′′′), 70.5 (C-5′′′), 62.2 (C-6′′′), 20.8-20.6 (acetyl $CH_3$), 170.7/170.7/170.1/169.6 (C=O), 55.5 (-$OCH_3$).

Poz. LC-MS/MS m/z (%): 610 (100) $[M+2]^+$.

Anal. cal. for $C_{30}H_{32}N_4O_{10}$ (608.21 g/mol): C 59.21, H 5.30, N 9.21, found: C 59.21, H 5.32, N 9.21.

*4.1.3. Pharmacology*

Pharmacological experiments that include the preparation of cell culture, cell proliferation assay (MTT assay), cytotoxic activity assay, microdilution assay, and DNA binding studies were provided in supplementary information. The calculation of $IC_{50}$ and % inhibition were also explained in the supplementary information.

*4.1.4. In silico Studies*

Molecular docking studies were applied to determine the amino acid residues in the active site of compounds **8, 9, 17 and 18**, which interact with all the crystal structures on **Table A** *in silico* approaches, and to calculate the binding parameters. Schrödinger 2021-2 software (Schrödinger Release 2021-2: Glide, LLC New York, USA) [45] was used in all molecular docking studies.

*4.1.4.1. Preparation of ligands.* Compounds **8, 9, 17 and 18** were optimized using the LigPrep wizard (Schrödinger Release 2021-2: LigPrep) [17] utility of the software Schrödinger 2021-2 (Schrödinger, LLC New York, USA) [46]. With this method, a net negative change in substituents was produced in each case using possible tautomeric states Epic at pH 7.0 ± 2.0 [47,48].

*4.1.4.2. Determination and preparation of proteins.* The crystal structures of the proteins with which compounds **8, 9, 17 and 18** interact were obtained from the Protein Data Bank (https://www.rcsb.org/). Based on the pathways in the research study, crystal structures with PDB access codes 4QL3 [30], 6MPP [31], 4E26 [32], 6QGG [33], 5MU8 [34], 4QUG [35], 2DBF, 6SL6 [36], 1CGL [37], 5Z62 [38], 6EB6 [39], 5ITD [40], 4EKK [41], 6GU7 [42], 7BG9 [43], 3GUS [44] respectively, were used. All crystal structures have different resolution values and binding sites. Crystal structures obtained from the protein database, respectively, were prepared separately with the "Protein Preparation Wizard" [49] module of Schrödinger 2021-2 software. Proteins were prepared by sequential processes such as deletion of water molecules, addition of missing side chains and hydrogen atoms, protonation states, assignment of partial charges, optimization and minimization using the OPLS-2005 force field [48].

*4.1.4.3. Molecular docking and MM-GBSA.* Molecular docking studies were applied for compounds **8, 9, 17 and 18**, which are compounds with the best cytotoxicity effect. After ligand and proteins were prepared separately, the docking score was calculated by interacting with the ligand docking wizard. Ligands were docked using Schrödinger 2021-2 software (Schrödinger Release 2021-2: Glide, LLC New York, USA) [45] to investigate the binding modes of their compounds. The protocol used in molecular docking was applied as in previous studies [47,48,50].

Prime MM/GBSA (Schrödinger Release 2021-2: Prime) [51] analysis was used to calculate ligand binding energies using the OPLS_2005 force field and the VSGB solvent model. MM-GBSA analysis was applied to calculate [52] the free binding energies and complex energies of compounds **8, 9, 17 and 18** with all proteins mentioned in the pathway, respectively.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

## CRediT authorship contribution statement

**Nuran Kahriman:** Conceptualization, Writing – review & editing, Methodology, Supervision. **Vildan Serdaroğlu:** Writing – original draft, Methodology, Investigation. **Ali Aydın:** Methodology, Investigation. **Burçin Türkmenoğlu:** Methodology, Software. **Asu Usta:** Methodology, Investigation.

## Data Availability

No data was used for the research described in the article.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2022.134821.

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