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Synthesis and biological evaluation of novel urea, thiourea and squaramide diastereomers possessing sugar backbone

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ABSTRACT

A series of novel chiral 14 urea, thiourea and squaramide stereoisomers possessing carbohydrate backbones as well as amide functional groups was synthesized and characterized by their, ^{1}H NMR, ^{13}C NMR, FT-IR, HRMS, optical rotation, and melting points. Their antiproliferative activities were investigated against HeLa and PC3 cell lines. The compounds **9**, **11** and **12** showed better activities at 25 μM against PC3 cell line with respect to the standard 5-fluorouracil (5-FU). Especially, the compounds **9** and **11** showed higher activities than the standard 5-FU even at low concentration (5 μM) against HeLa cell line. IC_{50} results also confirm these activities. The compounds **9**, **10** and **11** have the IC_{50} values of 1.10 μM, 1.51 μM and 1.02 μM, respectively while 5-FU has 2.51 μM. Moreover, their cytotoxicity tests have proven that their viabilities were in between 50% and 100%.

1. Introduction

Cancer is a major global public health problem. In addition, the incidence and mortality rates of cancer continue to increase. It has been reported that there will be an estimated 18.1 million new cancer cases and 9.6 million cancer deaths in 2018 [1]. Cervix cancer is the most common cancer worldwide among women. Among men, prostate cancer was the second most common cancer type diagnosed with 15.0% [2].

Over the past decades, sugar derivatives have been much attraction for the cytotoxic studies [3–8]. Their unique chiral structures are giving valuable contributions to the drugs. Thus, new derivatives of sugars as drugs are important stereoisomers. Apart from their role as drugs, new sugar synthesis can be considered as “chiral pool” in asymmetric reaction methods since the starting compound can be used in large scale [9]. Another advantage, particularly for drug industry, is that reactions can be achieved without the use of a chiral catalyst.

It was reported that 53% of the drugs which used in market have at least one chiral centre whereas in clinical use, 27% of the total are in single enantiomer form [10]. It is conceivable that chiral drug compounds can display different biological and pharmacological activities from their enantiomers or racemate forms. For instance, the use of D,L-dopa can cause granulocytopenia, yet this adverse effect has not been observed in the use of the pure enantiomer L-dopa [11]. The timolol can be given as another classical example for different pharmaceutical behaviours of enantiomers as drugs. While (S) isomer becomes effective for hypertension by decreasing intraocular pressure in the treatment of glaucoma, (R) isomer is considerably less potent [12,13]. With all the explanations given above, it is clear that pharmaceutical consequences of chirality might not be interpreted correctly without taking stereochemistry into consideration.

All the compounds **9–22** that we have in the present study include Brønsted acidic hydrogens which are bound to nitrogen atoms. It is well known that the inhibitory effect of nitrogen-bound acidic hydrogen (i.e. amide hydrogen) [14]. Hence, new molecules containing amide are important to drug industry due to their biological activities (Fig. 1). It has been reported that there are many applications of functional amides in biological activities such as fungicidal [15], herbicidal [16], insecticidal [17], anticancer [18], and antibacterial [19]. Some cholic acid-aryl amide derivatives were tested against three human cancer cell lines (HT29, MDAMB231, U87MG) and a human normal cell line (HEK293T). Good promising results were obtained as follows: 1.35 μM, 1.41 μM and 4.52 μM against the breast cancer cell line compared to Cisplatin (7.21 μM) [20]. Furthermore, a number of several bile acid derivatives were synthesized and screened towards MCF-7, MDA-MB-231, PC3, HeLa and HT-29 with high activities (IC_{50} < 5 μM) [21].

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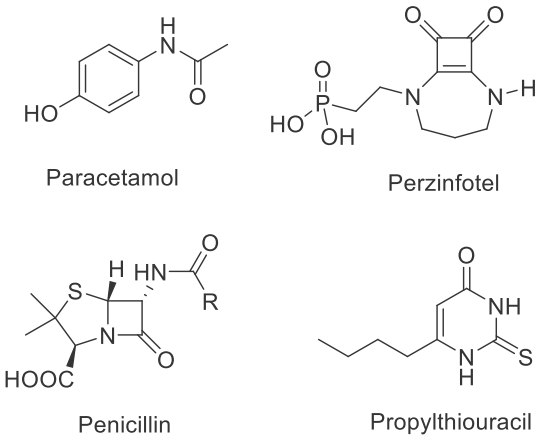


Fig. 1. The structures of some amide drugs.

2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds **9–22** can be divided into two parts as follows: First, synthesis of amino sugars, secondly; synthesis of urea, thiourea and squaramide derivatives. In the first part, the tosylated sugar **2** was prepared in the usual manner in which *p*-toluenesulfonyl chloride and the sugars **1** having free hydroxyl group is reacted in the presence of 4-dimethylaminopyridine (DMAP) with catalytic amount (Scheme 1). Next, the azido derivative **3** was obtained by nucleophilic displacement azide ion with the tosyl group of **2**. It is noteworthy that the displacement reaction between the azide ion and tosyl group of **2** changed the stereochemistry at C-3 position of **2**. Thus, *allo*-tosyl derivative **2** gave the *gluco*-azido derivative **3** (Scheme 1). However, the synthesis of 3-azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*allofuranose* failed when the same protocol was followed. The hindrance of the replacement reaction on C3-OTs arises from the repulsions between the *endo*oxygens of the isopropylidene group to the bicyclic system and the nucleophilic azide ion (Fig. 2) [22].

In the last step of this part, the amino sugar **4** was obtained by reduction of the azido sugar **3** with $LiAlH_4$ as pointed out in the literature [22]. Similar synthetic methodology was followed for the synthesis of the amino sugar **8** as given in Scheme 1.

In the second part, the amino sugars **4** and **8** were readily converted into the urea and thiourea derivatives **9**, **10**, **11**, and **12** respectively in high yields (Scheme 2) [23].

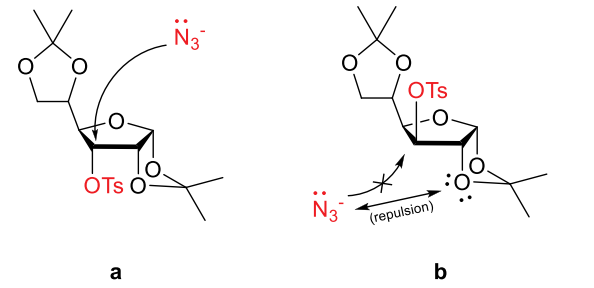
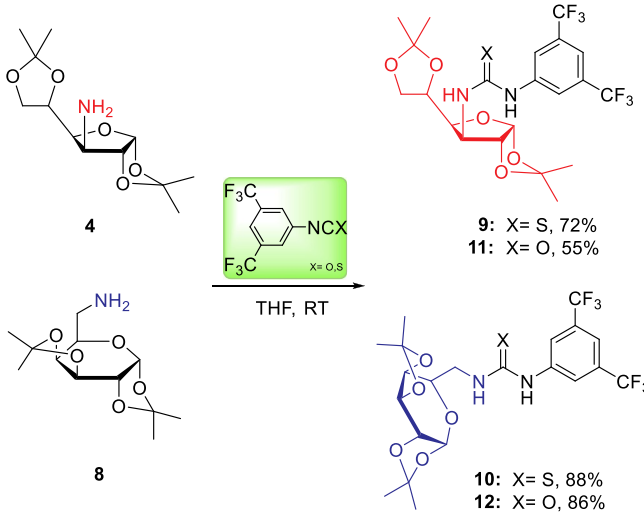


Fig. 2. a) Free nucleophilic attack in *allo*-sugar. **b)** Forbidden nucleophilic attack in *gluco*-sugar.



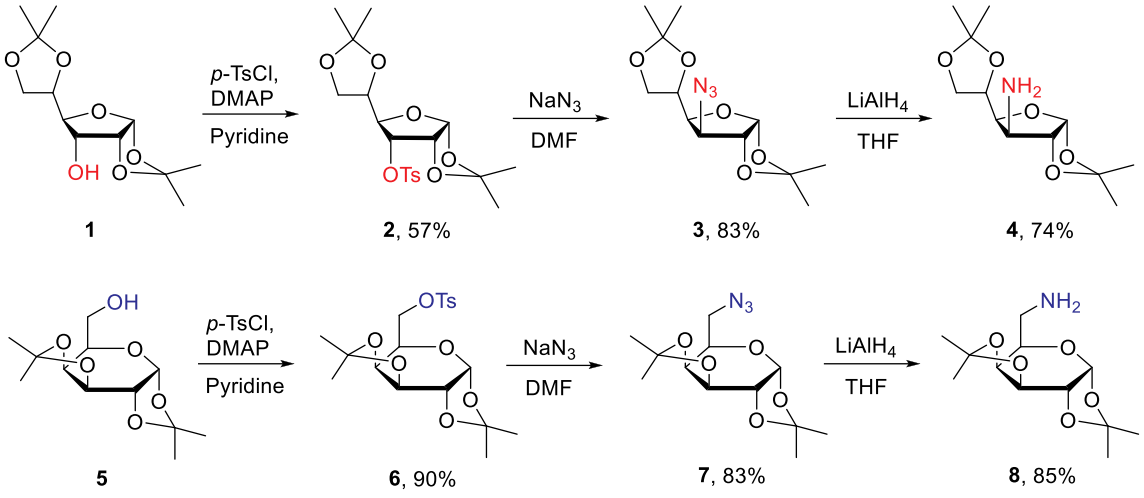
Scheme 2. Synthesis of urea and thiourea based sugars **9–12**.

Moreover, the squaramide derivatives **14–22** were obtained by the reaction of 3,4-dimethoxy-3-cyclobutene-1,2-dione and the corresponding amines (sugar amines or aromatic amines) [24]. (Scheme 3).

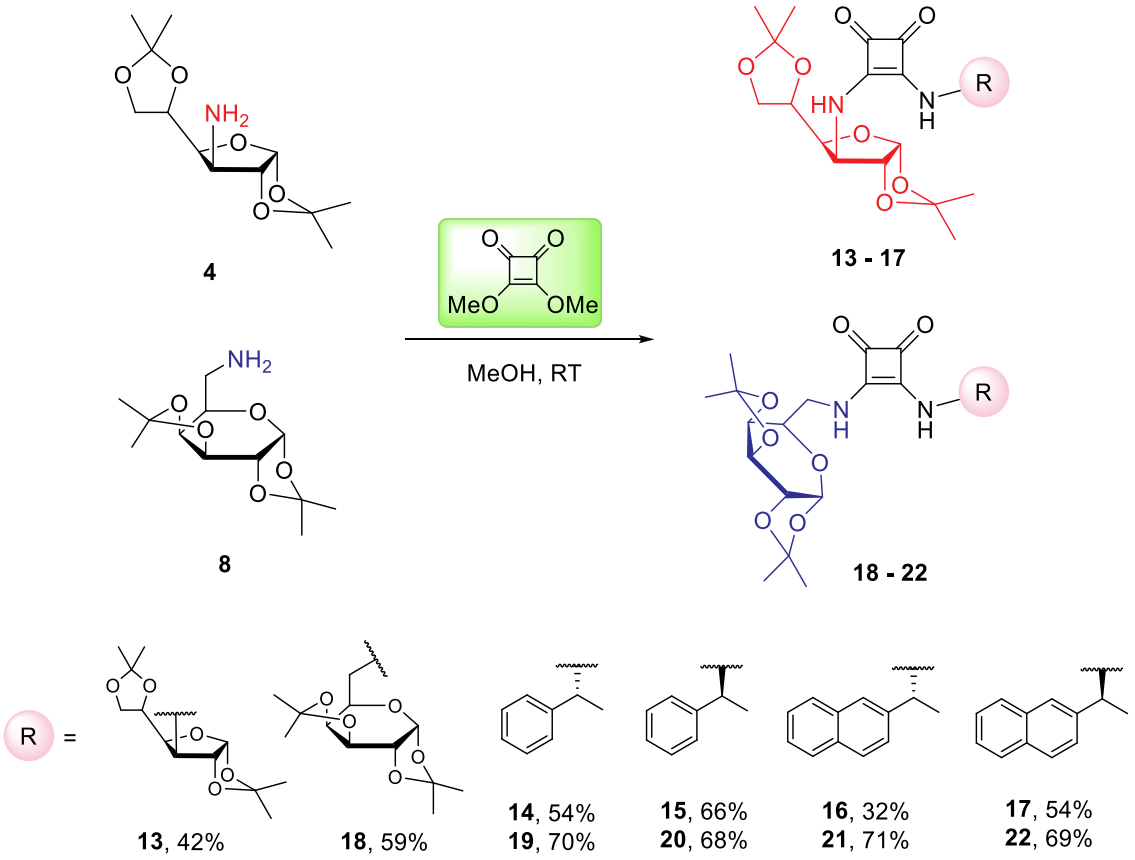
All the analyses (presented in experimental and supplementary data sections) for the characterization of the newly synthesized novel urea, thiourea, and squaramides are in good agreement with the expected structures.

2.2. Antiproliferative activity results against HeLa and PC3 cells

Antiproliferative activities of the compounds **9–22** and 5-



Scheme 1. Synthesis of sugar amines **4–8**.



Scheme 3. Synthesis of squaramide based sugars **13–22**.

Table 1
IC_{50} values of compounds **9–22** and 5-FU.

Entry	Compound	HeLa (μM)	PC3 (μM)
1	**9**	1.10	11.84
2	**10**	1.51	45.42
3	**11**	1.02	14.47
4	**12**	13.22	17.14
5	**13**	58.39	53.68
6	**14**	50.74	nd[a]
7	**15**	51.36	nd[a]
8	**16**	41.26	59.01
9	**17**	nd[a]	67.95
10	**18**	nd[a]	64.84
11	**19**	24.04	27.13
12	**20**	19.37	49.05
13	**21**	4.02	nd[a]
14	**22**	16.59	16.20
15	**5-FU**	2.51	3.10

[a] Not detected.

Florouracil (5-FU) against HeLa and PC3 cells were investigated at four different concentrations (100, 50, 25 and 5 μM). IC_{50} values of compounds **9–22** against HeLa and PC3 cells are given in Table 1.

As can be seen in Table 1, IC_{50} inhibition activities proved that the compounds **9** (IC_{50} = 1.10 μM), **10** (IC_{50} = 1.51 μM) and **11** (IC_{50} = 1.02 μM) gave more promising results than the standard 5-FU (IC_{50} = 2.51 μM) against HeLa cell line. Compound **21** (IC_{50} = 4.02 μM) showed moderate activity compare with against HeLa cell line. On the other hand, compound **9** (IC_{50} = 11.84 μM), **11** (IC_{50} = 14.47 μM), **22** (IC_{50} = 16.20 μM) and **12** (IC_{50} = 17.14 μM) have moderate activity in comparison with the 5-FU against PC3 cancer cell line. Another result that we have seen in Table 1 is that 10 squaramide derivatives **13–22** except **21** did not show satisfactory results compared to the urea and thiourea derivatives **9–12**.

The antiproliferative activities of the compounds **9–22** and 5-FU were determined to have increased depending on concentration against the HeLa cell line (Fig. 3). At all doses, **9** and **11** were determined to have higher activities than 5-FU against HeLa cell. The compound **10** was observed to have higher activity than 5-FU at 100, 50 and 25 μM concentrations but not at 5 μM. In other words, the compound **10** showed a cell selective activity against HeLa cells at high concentrations, although it had a satisfactory IC_{50} value (IC_{50} = 1.51 μM). Interestingly the only active squaramide derivative against HeLa cell was **21** which showed moderate activity compared with 5-FU at all doses (Fig. 3). It should not be neglected that the compound **21** also had a satisfactory IC_{50} value (Table 1).

In the case of PC3 cell line, similar antiproliferative activities were detected for the compounds **9–22** as in the HeLa cell line (Fig. 4). In other words, while the urea and thiourea derivatives **9–12** except **10** gave good results compared to the standard, the squaramides **13–22** showed moderate activities. As can be seen in Fig. 4, urea or thiourea derivatives **9–12** gave high activities at 100 μM, 50 μM and 25 μM concentrations compared to the 5-FU standard. Unfortunately, they are almost inactive at 5 μM in PC3 cell line.

More specifically, the urea and thiourea derivatives **9–12** containing 3,5-bis(trifluorophenyl) and sugar groups showed fairly good activity against HeLa and PC3 cell lines compared to squaramide derivatives **13–20** which bear the same sugars (**4** or **8**) but different aromatics (phenyl or naphthyl).

2.3. Cytotoxicity results against L929 fibroblast cell

The aforementioned results prompted us to test the compounds **9–12** in the cytotoxicity test namely MTT in L929 cell line. The cell viability was found to above 50% when different concentrations of

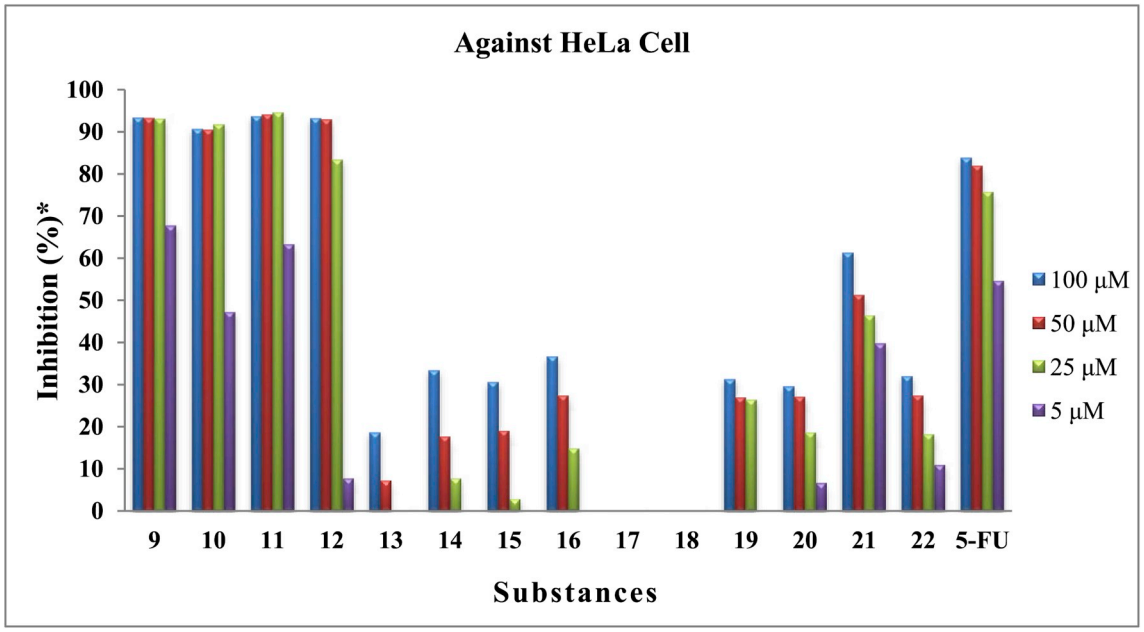


Fig. 3. Antiproliferative activity of **9–22** and 5-FU against HeLa cells line × Data are presented as mean ± SD (n = 6). Statistically significant difference ($p < 0.01$) was observed between treatments (ANOVA, Duncan).

samples **9–12** were applied in L929 fibroblast cell. As can be seen, the cell viability changed in the range of 53.01%–61.72% for all studied compounds, when at the highest concentration (0.2 mg/mL) of the compounds (Table 2). On the other hand, while the compounds **9–11** inhibited cancer cells (HeLa and PC3) at a concentration of 1 µM, no toxic effects were observed in healthy cells (L929). Therefore, it can be said that the synthesized compounds **9–12** can be tested as a cancer drug after additional tests.

3. Conclusions

Good to excellent yields of chiral ureas, thioureas and squaramides **9–22** bearing two Brønsted acidic hydrogens were obtained after simple purification or flash column. Their antiproliferative activities were tested against HeLa and PC3 cancer cell lines *in vitro*. As far as we are aware, antiproliferative screening for chiral urea, thiourea, and squaramide possessing sugar backbones has not been reported in the relevant literature yet, even though many stable examples of urea, thiourea, and squaramide as biological active compounds have been demonstrated before. In the preliminary studies, it was determined that the compounds **9–22** have dose-dependent activities against two cancer cell lines. Moreover, the results also indicated that the compounds **9–12** that including urea or thiourea have higher or comparable activities than the standard 5-FU against HeLa and PC3 cancer cell lines. In addition, the compound **10** was determined to exhibit cell-selective activity against HeLa cells.

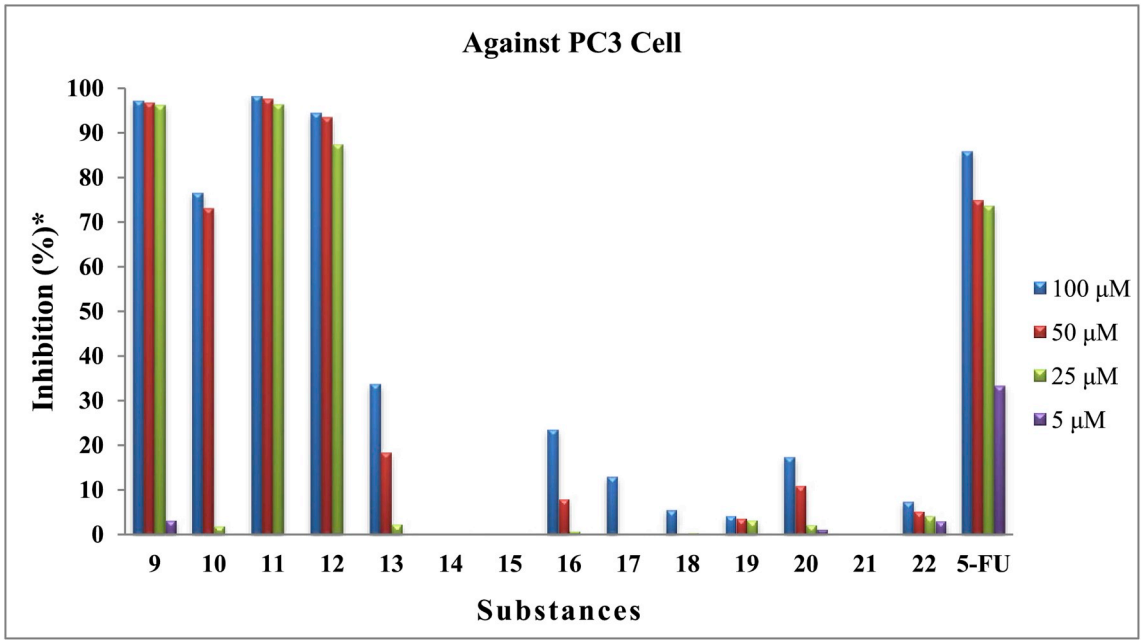


Fig. 4. Antiproliferative activity of **9–22** and 5-FU against PC3 cells line × Data are presented as mean ± SD (n = 6). Statistically significant difference ($p < 0.01$) was observed between treatments (ANOVA, Duncan).

Table 2
Viability values of L929 fibroblast cells. L929 fibroblast cells were seeded in a concentration of 5 × 10^3 cells and incubated for 24 h. All the experiments were repeated 3 times. The viability of L929 fibroblast cells were given as absorbance vs. concentration.

Entry	Concentration (mg/mL)	% Cell viability			
		9	10	11	12
1	0.2	53.01 ± 1.2	60.64 ± 1.4	57.60 ± 0,5	61.72 ± 1
2	0.1	55.08 ± 1	66.47 ± 0.7	61.47 ± 1,2	70.31 ± 3.5
3	0.05	57.00 ± 0,7	68.88 ± 2	68.28 ± 1	83.26 ± 2
4	0.025	75.38 ± 2.3	81.94 ± 1.2	86.72 ± 1,5	91.52 ± 0.7
5	0.0125	80.84 ± 2.5	83.49 ± 1.8	88.23 ± 1,4	93.39 ± 1.5
6	0.00625	86.73 ± 4	85.44 ± 3	94.77 ± 0,7	101.96 ± 2
Control		100 ± 0.7			

4. Experimental

4.1. Chemistry (General methods)

All chemicals and solvents were purchased from Sigma-Aldrich. TLC analyses were performed on 250 μm Silica Gel 60 F254 plates. Column chromatography was performed using 230–400-mesh silica gel. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Spectrospin Avance DPX400 spectrometer at 400 and 100 MHz, respectively. FT-IR spectra were obtained by Bruker Platinum ATR-IR instruments. Optical rotations were measured on a Rudolph Research Analytical Autopol III Polarimeter. High-resolution mass spectra (HRMS) were obtained on an Agilent 6224 (TOF-ESI) LC/MS spectrometer. Melting points were determined with a Stuart SMP50 apparatus.

4.1.1. 1,2:5,6-Di-O-isopropylidene-3-O-tosyl-α-D-allofuranose (2)

The synthesis procedure was adapted which has been previously reported by Richardson [22]. 1.0 g (3.8 mmol) 1,2:5,6-di-*O*-isopropylidene-α-D-*allofuranose* was dissolved in 4.3 mL pyridine. To this solution, catalytic amount DMAP and 0.73 g (3.8 mmol) of *p*-toluenesulfonyl chloride were added and stirred for 2 days at room temperature. When TLC (Hexane/Ethyl acetate, 10:1) indicated the disappearance of the starting materials, it was poured into ice. Next, it was extracted with $CHCl_3$ and purified with Hexane/Ethyl acetate (10:1) system. Finally, the amount of product was 1.399 g (57% yield). White solid: mp 175 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.28 (d, *J* = 8.2 Hz, 2H, Ar-H), 5.69 (d, *J* = 3.1 Hz, 1H, H-1), 4.58 (m, 2H, H-4, H-5), 4.19 - 4.02 (m, 2H, H-2, H-3), 3.86 (dd, *J* = 8.5, 6.7 Hz, 1H, H-6a), 3.71 (dd, J = 8.5–6.5 Hz, 1H, H-6b), 2.38 (s, 3H, Ar-$\underline{C}H_3$), 1.46 (s, 3H, -CH_3), 1.25 (s, 3H, -CH_3), 1.22 (s, 3H, -CH_3), 1.21 (s, 3H, -CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.2 ($\underline{C}$-SO_3), 133.2 (C-Ar), 129.7 (C-Ar), 128.4 (C-Ar), 113.6 ($\underline{C}(CH_3)_2$), 109.9 ($\underline{C}(CH_3)_2$), 103.8 (C-1), 77.9 (C-4), 77.0 (C-2), 76.6 (C-5), 74.7, (C-3), 65.2 (C-6), 26.7 (-CH_3), 26.6 (-CH_3), 26.1 (-CH_3), 25.1 (-CH_3), 21.7 (Ar-$\underline{C}H_3$).

4.1.2. 3-Azido-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (3)

The synthesis procedure was adapted which has been previously reported by Nayak [30]. 0.45 g (1.08 mmol) **2** was dissolved in 10 mL DMF (N,N-dimethylformamide). To this solution, 1.156 g (17.79 mmol) NaN_3 was added. The mixture was refluxed at 153 °C for 4 h. Next, 20 mL of distilled water was added to the mixture. The reaction mixture was extracted with 50 mL $CHCl_3$. Then, organic phase was concentrated under reduced pressure. 0.25 g product was obtained as light yellow oil (83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86 (d, *J* = 3.6 Hz, 1H, H-1), 4.62 (d, *J* = 3.6 Hz, 1H, H-2), 4.29–4.20 (m, 1H, H-4), 4.18–4.06 (m, 3H, H-3, H-5, H-6a), 3.98 (dd, *J* = 8.7, 4.8 Hz, 1H, H-6b), 1.51 (s, 3H, -CH_3), 1.44 (s, 3H, -CH_3), 1.37 (s, 3H, -CH_3), 1.32 (s, 3H, -CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 112.3 ($\underline{C}(CH_3)_2$), 109.6 ($\underline{C}(CH_3)_2$), 105.0 (C-1), 83.4 (C-4), 80.5 (C-2), 73.0 (C-5), 67.6 (C-3), 66.4 (C-6), 26.9 (-CH_3), 26.6 (-CH_3), 26.2 (-CH_3), 25.1 (-CH_3).

4.1.3. 3-Amino-3-deoxy-1,2:5,6-di-O-isoproylidene-α-D-glucofuranose (4)

0.5 g (1.75 mmol) **3** was dissolved in 15 mL dry THF at −10 °C under a nitrogen atmosphere. The reaction mixture was stirred for 15 min. Subsequently, 0.27 g $LiAlH_4$ (7.00 mmol) was added, which was then stirred for 4 h. Next, the mixture was hydrolysed with saturated NH_4Cl and extracted with CH_2Cl_2. The organic phase was dried with $MgSO_4$ and concentrated under reduced pressure. 0.34 g product was obtained as light yellow oil (74% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (d, *J* = 3.5 Hz, 1H, H-1), 4.30 (d, *J* = 3.5 Hz, 1H, H-2), 4.17–4.08 (m, 1H, H-3), 4.05 (dd, *J* = 8.3, 6.1 Hz, 1H, H-4), 3.90 (ddd, *J* = 13.2, 8.6, 4.1 Hz, 2H, H-5, H-6a), 3.44 (d, *J* = 3.3 Hz, 1H, H-6b), 1.41 (s, 3H, -CH_3), 1.33 (s, 3H, -CH_3), 1.27 (s, 3H, -CH_3), 1.22 (s, 3H, -CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 111.1 ($\underline{C}(CH_3)_2$), 108.9 ($\underline{C}(CH_3)_2$), 104.8 (C-1), 86.3 (C-4), 81.3 (C-2), 72.7 (C-5), 67.8 (C-3), 57.1 (C-6), 26.6 (-CH_3), 26.6 (-CH_3), 25.9 (-CH_3), 25.1 (-CH_3).

4.1.4. 1,2:3,4-Di-O-isopropylidene-6-O-tosyl-α-D-galactopyranose (6)

0.52 g (1,99 mmol) 1,2:3,4-di-*O*-isopropylidene-α-D-*galactopyranose* was dissolved in 2.0 mL pyridine. To this solution, catalytic amount DMAP and 0.46 g (2.41 mmol) *p*-toluenesulfonyl chloride were added and stirred for 24 h. The reaction was monitored with TLC (Hexane/Ethyl acetate, 10:1). When TLC indicated the disappearance of the starting materials, it was poured into ice-crash and extracted with $CHCl_3$. The solvent was removed under reduced pressure and the residue was purified by silica gel using Hexane/Ethyl acetate, (10:1) as eluent. 0.74 g product was obtained as white solid (90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.33 (d, *J* = 8.0 Hz, 2H, Ar-H), 5.45 (d, *J* = 4.9 Hz, 1H, H-1), 4.59 (dd, *J* = 7.9, 2.5 Hz, 1H, H-3), 4.29 (dd, *J* = 5.0, 2.5 Hz, 1H, H-2), 4.23–4.16 (m, 2H, H-4, H-5), 4.13–4.00 (m, 2H, H-6a, H-6b), 2.43 (s, 3H, Ar-C$\underline{H}_3$), 1.49 (s, 3H, -CH_3), 1.34 (s, 3H, -CH_3), 1.31 (s, 3H, -CH_3), 1.27 (s, 3H, -CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.8 ($\underline{C}$-SO_3), 132.9 (C-Ar) 129.7 (C-Ar), 128.1 (C-Ar), 109.5 ($\underline{C}(CH_3)_2$), 108.9 ($\underline{C}(CH_3)_2$), 96.1 (C-1) 70.5 (C-4) 70.4 (C-3), 70.3 (C-2), 68.2 (C-6), 65.9 (C-5), 25.9 (-CH_3), 25.8 (-CH_3), 24.9 (-CH_3), 24.3 (-CH_3), 21.6 (Ar-$\underline{C}H_3$).

4.1.5. 6-Azido-6-deoxy-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (7)

0.7 g (1.69 mmol) **6** was dissolved in 14 mL DMF. To this solution, 0.44 g (6.76 mmol) NaN_3 was added. The mixture was refluxed for 4 h. Then, the reaction mixture allowed to cool to room temperature and 25 mL distilled water was added. The solution was extracted with CH_2Cl_2 and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel using Hexane/Ethyl acetate (7:1). 0.4 g product was obtained as light yellow oil (83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.48 (d, *J* = 5.0 Hz, 1H, H-1), 4.56 (dd, *J* = 7.9, 2.5 Hz, 1H, H-3), 4.27 (dd, *J* = 5.0, 2.5 Hz, 1H, H-2), 4.13 (dd, *J* = 7.9, 1.9 Hz, 1H, H-4), 3.85 (ddd, *J* = 7.5, 5.3, 1.8 Hz, 1H, H-5), 3.45 (dd, *J* = 12.7, 7.9 Hz, 1H, H-6a), 3.29 (dd, *J* = 12.7, 5.3 Hz, 1H, H-6b), 1.48 (s, 3H, -CH_3), 1.39 (s, 3H, -CH_3), 1.28 (s, 3H, -CH_3), 1.27 (s, 3H, -CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 109.6 ($\underline{C}(CH_3)_2$), 108.8 ($\underline{C}(CH_3)_2$), 96.3

(C-1), 71.2 (C-4), 70.8 (C-3), 70.4 (C-2), 66.9 (C-6), 50.7 (C-5), 26.0 (-CH_3), 25.9 (-CH_3), 24.9 (-CH_3), 24.4 (-CH_3).

4.1.6. 6-Amino-6-deoxy-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (8)

0.38 g (1.33 mmol) **7** was dissolved in dry THF at −10 °C under a nitrogen atmosphere. Subsequently, 0.20 g $LiAlH_4$ (5.32 mmol) was added. Then, the reaction mixture allowed to cool to room temperature and stirred overnight. The solution was cooled to 0 °C and 1 M NaOH solution was added until gas evolution was ceased. Then the solution was extracted with $CHCl_3$ (20 mL) and removal of the solvent *in vacuo* afforded 0.29 g amino sugar **8** as light yellow oil (85% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.55 (d, J = 5.1 Hz, 1H, H-1), 4.60 (dd, J = 7.9, 2.3 Hz, 1H, H-3), 4.32 (dd, J = 5.1, 2.3 Hz, 1H, H-2), 4.23 (dd, J = 7.9, 1.9 Hz, 1H, H-4), 3.73–3.67 (m, 1H, H-5), 2.96 (dd, J = 13.2, 7.8 Hz, 1H, H-6a), 2.84 (dd, J = 13.2, 4.9 Hz, 1H, H-6b), 1.54 (s, 3H, -CH_3), 1.45 (s, 3H, -CH_3), 1.34 (s, 3H, -CH_3), 1.34 (s, 3H, -CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 109.1 ($\underline{C}(CH_3)_2$), 108.3 ($\underline{C}(CH_3)_2$), 96.3 (C-1), 71.7 (C-4), 70.7 (C-3), 70.6 (C-2), 69.4 (C-6), 42.3 (C-5), 26.0 (-CH_3), 25.9 (-CH_3), 24.9 (-CH_3), 24.3 (-CH_3).

4.2. General procedure for the synthesis of urea and thiourea based compounds

A solution of sugar amine (**4** or **8**) (1.0 mmol) in dry THF (5 mL) was treated with 3,5-bis(trifluoromethyl)phenyl isocyanate (1.1 mmol) or 3,5-bis(trifluoromethyl)phenyl isothiocyanate (1.1 mmol) at room temperature. The mixture was stirred overnight. The solvent was removed in vacuum and remaining solid was purified by column chromatography.

4.2.1. 3-[3,5-Bis(trifluoromethylphenyl)-thiourido]-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-glucofuranoside (9)

Following the general procedure from **4** (1.0 mmol) and 3,5-bis (trifluoromethyl)phenyl isothiocyanate (1.1 mmol) the compound **9** was obtained (387.6 mg, 72%). Light yellow solid: mp 67.1–70.4 °C; $[\alpha]_D^{25}$= –10.49 (c 0.01, CH_2Cl_2); FT-IR (neat): 3355, 2989, 2963, 2937, 1701, 1537, 1472, 1382, 1276, 1214, 1170, 1128, 1069, 1015, 882; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H, Thiourea N-H), 7.81 (s, 2H, Ar-H), 7.60 (s, 1H, Ar-H), 5.87 (s, 1H, Thiourea N-H), 4.77 (d, J = 3.7 Hz, 1H, H-1), 4.47–3.67 (m, 6H, H-2,3,4,5,6a,6b), 1.59–1.21 (m, 12H, -CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 131.9 (C-Ar), 123.6 (C-Ar), 118.6 ($\underline{C}(CH_3)_2$), 109.8 ($\underline{C}(CH_3)_2$), 109.7 (C-1), 71.5 (C-2), 70.7 (C-4), 70.6 (C-5), 47.2 (C-6), 45.0 (C-3), 25.9 (-CH_3), 25.8 (-CH_3), 24.9 (-CH_3), 24.3 (-CH_3); HRMS (ESI), *m/z* calcd. for $C_{21}H_{25}F_6N_2O_5S$ $[M + H]^+$ 531.1388, found: 531.1192.

4.2.2. 6-[3,5-Bis(trifluoromethylphenyl)-thiourido]-6-deoxy-1,2:3,4-di-O-isopropylidene-α-D-galactopyranoside (10)

Following the general procedure from **8** (1.0 mmol) and 3,5-bis (trifluoromethyl)phenyl isothiocyanate (1.1 mmol) the compound **10** was obtained (470.0 mg, 88%). Light yellow solid: mp 171.6–174.2 °C; $[\alpha]_D^{25}$= –39.41 (c 0.005, CH_2Cl_2); FT-IR (neat): 3303, 3101, 2986, 2932, 2861, 1548, 1468, 1384, 1274, 1210, 1168, 1128, 1061, 991, 882; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H, Thiourea N-H), 7.73 (s, 2H, Ar-H), 7.49 (s, 1H, Ar-H), 7.36 (s, 1H, Thiourea N-H), 5.51 (d, J = 5.0 Hz, 1H, H-1), 4.58–4.45 (m, 2H, H-3, H-5), 4.33 (dd, J = 5.0, 2.5 Hz, 1H, H-2), 4.24 (dd, J = 7.9, 1.7 Hz, 1H, H-4), 4.08 (s, 1H, H-6a), 3.50–3.33 (m, 1H, H-6b), 1.47 (s, 3H, -CH_3), 1.36–1.26 (m, 9H, -CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 181.7 ($\underline{C}$=O), 131.3 (-CF_3), 123.2 (C-Ar), 122.6 (C-Ar), 120.5 (C-Ar), 117.7 (C-Ar), 108.8 ($\underline{C}(CH_3)_2$), 108.7 ($\underline{C}(CH_3)_2$), 95.5 (C-1), 70.5 (C-4), 69.7 (C-3), 69.6 (C-2), 44.1 (C-6), 28.7 (C-5), 24.9 (-CH_3), 24.8 (-CH_3), 23.9 (-CH_3), 23.3 (-CH_3); HRMS (ESI), *m/z* calcd. for $C_{21}H_{25}F_6N_2O_5S$ $[M + H]^+$ 531.1388, found: 531.1436.

4.2.3. 3-[3,5-Bis(trifluoromethylphenyl)-urido]-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-glucofuranoside (11)

Following the general procedure from **4** (1.0 mmol) and 3,5-bis (trifluoromethyl)phenyl isocyanate (1.1 mmol) the compound **11** was obtained (283.2 mg, 55%). Light yellow solid: mp 89.4–92.6 °C; $[\alpha]_D^{25}$= –12.25 (c 0.01, CH_2Cl_2); FT-IR (neat): 3289, 2989, 1624, 1528, 1472, 1377, 1276, 1170, 1128, 1072, 1016, 884, 681; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H, Urea N-H), 7.82 (s, 2H, Ar-H), 7.60 (s, 1H, Ar-H), 5.83 (s, 1H, Urea N-H), 4.77 (d, J = 3.7 Hz, 1H, H-1), 4.09 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b), 1.56–1.23 (m, 12H, -CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7 ($\underline{C}$=O), 124.3 (C-Ar), 123.3 (C-Ar), 118.8 (C-Ar), 112.5 ($\underline{C}(CH_3)_2$), 110.6 ($\underline{C}(CH_3)_2$), 104.4 (C-1), 84.3 (C-2), 72.8 (C-4), 67.3 (C-5), 61.2 (C-6), 60.6 (C-3), 26.3 (-CH_3), 26.0 (-CH_3), 25.0 (-CH_3), 21.0 (-CH_3).

4.2.4. 6-[3,5-Bis(trifluoromethylphenyl)-urido]-6-deoxy-1,2:3,4-di-O-isopropylidene-α-D-galactopyranoside (12)

Following the general procedure from **8** (1.0 mmol) and 3,5-bis (trifluoromethyl)phenyl isocyanate (1.1 mmol) the compound **12** was obtained (444.3 mg, 86%). Light yellow solid: mp 159.3–162.1 °C; $[\alpha]_D^{25}$= 14.11 (c 0.01, CH_2Cl_2); FT-IR (neat): 3357, 2989, 2938, 1701, 1553, 1471, 1386, 1275, 1169, 1126, 1108, 1063, 987, 878; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (s, 2H, Ar-H), 7.50 (s, 1H, Urea N-H), 7.23 (s, 1H, Ar-H), 6.35 (s, 1H, Urea N-H), 5.56 (d, J = 4.8 Hz, 1H, H-1), 4.61 (dd, J = 7.8, 2.0 Hz, 1H, H-3), 4.43–4.30 (m, 2H, H-2, H-5), 4.20 (d, J = 7.9 Hz, 1H, H-4), 3.61–3.45 (m, 1H, H-6a), 3.20–3.03 (m, 1H, H-6b), 1.55 (s, 3H, -CH_3), 1.41–1.24 (m, 9H, -CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.2 ($\underline{C}$=O), 140.7 (C-Ar), 132.1 (-CF_3), 124.4 (C-Ar), 121.7 (C-Ar), 118.0 (C-Ar), 110.2 ($\underline{C}(CH_3)_2$), 109.8 ($\underline{C}(CH_3)_2$), 96.5 (C-1), 71.5 (C-4), 70.7 (C-3), 66.7 (C-2), 40.9 (C-6), 31.6 (C-5), 25.9 (-CH_3), 25.7 (-CH_3), 25.0 (-CH_3), 24.5 (-CH_3); HRMS (ESI), *m/z* calcd. for $C_{21}H_{25}F_6N_2O_6$ $[M + H]^+$ 515.1617, found: 515.1619.

4.3. General procedure for the synthesis of squaramide based compounds

To a solution of 3,4-dimethoxy-3-cyclobutene-1,2-dione (1.0 mmol) in methanol (3 mL), 1.0 mmol sugar amine (**4** or **8**) was added at room temperature in one portion. After 6 h aromatic amine or sugar amine (1.0 mmol) was dissolved in methanol (2 mL) and added to the reaction mixture in one portion. After 24 h, the product was purified by filtration.

4.3.1. 3,4-Bis-(3-amino-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose)cyclobut-3-ene-1,2-dione (13)

Following the general procedure from **4** (2.0 mmol) the compound **13** was obtained (250.7 mg, 42%). White solid: mp ≥ 250 °C; $[\alpha]_D^{25}$= –18.70 (c 0.01, DMSO); FT-IR (neat): 3148, 3055, 2938, 1805, 1653, 1556, 1458, 1374, 1211, 1162, 1077, 1044, 1024, 867, 836; ^{1}H NMR (400 MHz, DMSO) δ 7.51 (s, 2H, Amide-NH), 5.93 (d, J = 3.5 Hz, 2H, H-1), 4.64 (m, 4H, H-2, H-3), 4.24–3.96 (m, 6H, H-4, H-5, H-6a), 3.86 (dd, J = 7.8, 4.1 Hz, 2H, H-6b), 1.44 (s, 6H, -CH_3), 1.32 (s, 6H, -CH_3), 1.26 (s, 6H, -CH_3), 1.24 (s, 6H, -CH_3); ^{13}C NMR (101 MHz, DMSO) δ 183.1 ($\underline{C}$=O), 167.3 (C=C), 111.4 ($\underline{C}(CH_3)_2$), 108.8 ($\underline{C}(CH_3)_2$), 104.0 (C-1), 84.1 (C-4), 78.7 (C-2), 71.9 (C-5), 66.5 (C-3), 58.7 (C-6), 26.7 (-CH_3), 26.2 (-CH_3), 25.9 (-CH_3), 25.2 (-CH_3); HRMS (ESI), *m/z* calcd. for $C_{28}H_{41}N_2O_{12}$ $[M + H]^+$ 597.2659, found: 597.2629.

4.3.2. 3-(3-Amino-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose)-4-(R)-((1-phenylethyl)amino)cyclobut-3-ene-1,2-dione (14)

Following the general procedure from **4** (1.0 mmol) and (R)-(+)-α-Methylbenzylamine (1.0 mmol) the compound **14** was obtained (247.8 mg, 54%). White solid: mp 248 °C (decomposed); $[\alpha]_D^{25}$= 35.55 (c 0.01, DMSO); FT-IR (neat): 3149, 2982, 2935, 1799, 1647, 1549, 1452, 1373, 1210, 1163, 1073, 1014, 841; ^{1}H NMR (400 MHz, DMSO) δ 7.71 (s, 1H, Amide-NH), 7.49 (s, 1H, Amide-NH), 7.44–7.35 (m, 4H, Ar-H),

7.34–7.25 (m, 1H, Ar-H), 5.89 (d, J = 3.4 Hz, 1H, H-1), 5.19 (s, 1H, H-2), 4.71–4.53 (m, 2H, H-3, H-4), 4.21–3.94 (m, 3H, H-5, H-6a, H-6b), 3.84 (s, 1H, -NHCH), 1.55 (d, J = 6.9 Hz, 3H, -CH_3), 1.43 (s, 3H, -CH_3), 1.32 (s, 3H, -CH_3), 1.24 (s, 6H, -CH_3); ^{13}C NMR (101 MHz, DMSO) δ 182.9 (C=O), 182.4 (C=O), 167.1 (C=C), 166.9 (C=C), 128.7 (C-Ar), 127.4 (C-Ar), 126.0 (C-Ar), 111.3 (C-Ar), 108.7 (C(CH$_3$)$_2$), 104.0 (C(CH$_3$)$_2$), 84.1 (C-1), 72.0 (C-2), 70.0 (C-4), 66.6 (C-5), 60.2 (C-6), 58.7 (C-3), 52.7 (-NHCH), 31.3 (-CH_3), 26.6 (-CH_3), 26.3 (-CH_3), 25.9 (-CH_3), 25.2 (-CH_3); HRMS (ESI), m/z calcd. for $C_{24}H_{31}N_2O_7$ [M + H]$^+$ 459.2131, found: 459.2114.

4.3.3. 3-(3-Amino-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose)-4-(S)-((1-phenylethyl)amino)cyclobut-3-ene-1,2-dione (15)

Following the general procedure from **4** (1.0 mmol) and (S)-(−)-α-Methylbenzylamine (1.0 mmol) the compound **15** was obtained (303 mg, 66%). White solid: mp ≥ 250 °C; $[\alpha]_D^{25}$= –37.36 (c 0.01, DMSO); FT-IR (neat): 3150, 3030, 2980, 2934, 1794, 1645, 1550, 1467, 1373, 1210, 1164, 1076, 1025, 836; ^{1}H NMR (400 MHz, DMSO) δ 7.72 (s, 1H, Amide-NH), 7.48 (s, 1H, Amide-NH), 7.42–7.34 (m, 4H, Ar-H), 7.32–7.26 (m, 1H, Ar-H), 5.92 (d, J = 3.6 Hz, 1H, H-1), 5.19 (s, 1H, H-2), 4.76–4.53 (m, 2H, H-3, H-4), 4.21–3.96 (m, 3H, H-5, H-6a, H-6b), 3.89–3.73 (m, 1H, -NHCH), 1.54 (d, J = 6.9 Hz, 3H, -CH_3), 1.44 (s, 3H, -CH_3), 1.28 (s, 3H, -CH_3), 1.26 (s, 3H, -CH_3), 1.17 (s, 3H, -CH_3); ^{13}C NMR (101 MHz, DMSO) δ 182.9 (C=O), 182.4 (C=O), 167.2 (C=C), 167.0 (C=C), 128.7 (C-Ar), 127.4 (C-Ar), 125.9 (C-Ar), 111.4 (C-Ar), 108.7 (C(CH$_3$)$_2$, 104.0 (C(CH$_3$)$_2$, 84.1 (C-1), 71.9 (C-2), 66.6 (C-5), 58.7 (C-6), 52.7 (-NHCH), 26.6 (-CH_3), 26.3 (-CH_3), 25.9 (-CH_3), 25.1 (-CH_3); HRMS (ESI), m/z calcd. for $C_{24}H_{31}N_2O_7$ [M + H]$^+$ 459.2131, found: 459.2107.

4.3.4. 3-(3-Amino-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose)-4-(R)-((1-(naphthalen-2-yl)ethyl)amino)cyclobut-3-ene-1,2-dione (16)

Following the general procedure from **4** (1.0 mmol) and (R)-(+)-1-(2-Naphthyl)ethylamine (1.0 mmol) the compound **16** was obtained (162.8 mg, 32%). White solid: mp 238 °C (decomposed); $[\alpha]_D^{25}$= +31.28 (c 0.02, DMSO); FT-IR (neat): 3150, 3054, 2980, 2936, 1801, 1645, 1553, 1470, 1381, 1212, 1163, 1074, 1018, 858, 819; ^{1}H NMR (400 MHz, DMSO) δ 7.99–7.87 (m, 5H, Amide-NH, Ar-H), 7.55–7.45 (m, 4H, Ar-H), 5.89 (d, J = 2.8 Hz, 1H, H-1), 5.38 (s, 1H, H-2), 4.76–4.53 (m, 2H, H-3, H-4), 4.28–3.94 (m, 3H, H-5, H-6a, H-6b), 3.84 (s, 1H, -NHCH), 1.70–1.59 (m, 3H, -CH_3), 1.43 (s, 3H, -CH_3), 1.32 (s, 3H, -CH_3), 1.24 (s, 6H, -CH_3); ^{13}C NMR (101 MHz, DMSO) δ 183.0 (C=O), 182.4 (C=O), 167.2 (C=C), 167.0 (C=C), 132.8 (C-Ar), 132.3 (C-Ar), 128.4 (C-Ar), 127.8 (C-Ar), 127.5 (C-Ar), 126.4 (C-Ar), 126.1 (C-Ar), 124.5 (C-Ar), 124.3 (C-Ar), 108.7 (C(CH$_3$)$_2$), 104.0 (C(CH$_3$)$_2$), 84.1 (C-1), 78.7 (C-2), 71.9 (C-4), 66.6 (C-5), 58.7 (C-6), 54.9 (C-3), 52.8 (-NHCH), 26.6 (-CH_3), 26.3 (-CH_3), 25.9 (-CH_3), 25.1 (-CH_3), 22.8 (-CH_3); HRMS (ESI), m/z calcd. for $C_{28}H_{33}N_2O_7$ [M + H]$^+$ 509.2287, found: 509.2268.

4.3.5. 3-(3-Amino-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose)-4-(S)-((1-(naphthalen-2-yl)ethyl)amino)cyclobut-3-ene-1,2-dione (17)

Following the general procedure from **4** (1.0 mmol) and (S)-(−)-1-(2-Naphthyl)ethylamine (1.0 mmol) the compound **17** was obtained (247.8 mg, 54%). White solid: mp 241 °C (decomposed); $[\alpha]_D^{25}$= –34.45 (c 0.01, DMSO); FT-IR (neat): 3152, 3055, 2981, 2935, 1800, 1647, 1556, 1471, 1381, 1212, 1165, 1075, 1018, 855, 819; ^{1}H NMR (400 MHz, DMSO) δ 7.96 (s, 1H, Amide-NH), 7.94–7.89 (m, 3H, Ar-H), 7.87 (s, 1H, Amide-NH), 7.54–7.50 (m, 4H, Ar-H), 5.92 (d, J = 3.0 Hz, 1H, H-1), 5.37 (s, 1H, H-2), 4.72–4.53 (m, 2H, H-3, H-4), 4.17–3.98 (m, 3H, H-5, H-6a, H-6b), 3.85–3.79 (m, 1H, -NHCH), 1.63 (t, J = 7.1 Hz, 3H, -CH_3), 1.44 (s, 3H, -CH_3), 1.26 (s, 6H, -CH_3), 1.13 (s, 3H, -CH_3); ^{13}C NMR (101 MHz, DMSO) δ 182.5 (C=O), 167.2 (C=C), 132.8 (C-Ar), 132.3 (C-Ar), 128.4 (C-Ar), 127.8 (C-Ar), 127.5 (C-Ar), 126.4 (C-Ar), 126.0 (C-Ar), 124.5 (C-Ar), 124.2 (C-Ar), 111.4 (C-Ar), 108.7 (C(CH$_3$)$_2$), 104.0 (C(CH$_3$)$_2$), 84.1 (C-1), 71.9 (C-2), 66.6 (C-4), 58.7 (C-5), 54.9 (C-6), 52.9 (C-3), 52.8 (-NHCH), 26.6 (-CH_3), 26.3 (-CH_3), 25.9 (-CH_3), 25.1 (-CH_3), 22.8 (-CH_3); HRMS (ESI), m/z calcd. for $C_{28}H_{33}N_2O_7$ [M + H]$^+$ 509.2287, found: 509.2280.

4.3.6. 3,4-Bis-(6-amino-6-deoxy-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose)cyclobut-3-ene-1,2-dione (18)

Following the general procedure from **8** (2.0 mmol) the compound **18** was obtained (365.2 mg, 59%). White solid: mp ≥ 250 °C; $[\alpha]_D^{25}$= –19.09 (c 0.01, DMSO); FT-IR (neat): 3148, 2938, 1806, 1653, 1556, 1457, 1264, 1211, 1162, 1076, 1044, 1024, 883, 867; ^{1}H NMR (400 MHz, DMSO) δ 7.56 (s, 2H, Amide-NH), 5.48 (d, J = 4.9 Hz, 1H, H-1), 4.63 (dd, J = 7.8, 2.2 Hz, 2H, H-3), 4.37 (dd, J = 4.9, 2.3 Hz, 2H, H-5), 4.22 (d, J = 8.1 Hz, 2H, H-2), 3.86 (s, 2H, H-4), 3.69–3.61 (m, 2H, H-6a), 3.58–3.47 (m, 2H, H-6b), 1.39 (s, 3H, -CH_3), 1.37 (s, 3H, -CH_3), 1.30 (s, 3H, -CH_3), 1.27 (s, 3H, -CH_3); ^{13}C NMR (101 MHz, DMSO) δ 182.8 (C=O), 168.1 (C=C), 108.6 (C(CH$_3$)$_2$), 108.0 (C(CH$_3$)$_2$), 95.6 (C-1), 70.3 (C-4), 70.1 (C-3), 69.7 (C-2), 67.3 (C-6), 43.5 (C-5), 25.9 (-CH_3), 25.6 (-CH_3), 24.9 (-CH_3), 24.3 (-CH_3); HRMS (ESI), m/z calcd. for $C_{28}H_{40}N_2O_{12}Na$ [M + Na]$^+$ 619.2478, found: 619.2525.

4.3.7. 6-Amino-6-deoxy-3-(1,2:3,4-di-O-isopropylidene-α-D-galactopyranose)-4-(R)-(1-phenylethyl)amino)cyclobut-3-ene-1,2-dione (19)

Following the general procedure from **8** (1.0 mmol) and (R)-(+)-α-Methylbenzylamine (1.0 mmol) the compound **19** was obtained (321.3 mg, 70%). White solid: mp 248 °C (decomposed); $[\alpha]_D^{25}$= –31.27 (c 0.01, DMSO); FT-IR (neat): 3151, 3032, 2975, 2932, 1795, 1645, 1541, 1453, 1374, 1209, 1075, 1002, 905; ^{1}H NMR (400 MHz, DMSO) δ 7.86 (s, 1H, Amide-NH), 7.40–7.36 (m, 5H, Ar-H), 7.30 (s, 1H, Amide-NH), 5.48 (d, J = 5.0 Hz, 1H, H-1), 5.21 (s, 1H, H-3), 4.62 (dd, J = 7.8, 2.2 Hz, 1H, H-5), 4.37 (dd, J = 4.9, 2.3 Hz, 1H, H-2), 4.22 (d, J = 7.6 Hz, 1H, H-4), 3.86 (s, 1H, H-6a), 3.67 (s, 1H, H-6b), 3.53 (s, 1H, -NHCH), 1.57–1.49 (m, 3H, -CH_3), 1.39 (s, 3H, -CH_3), 1.35 (s, 3H, -CH_3), 1.29 (s, 3H, -CH_3), 1.27 (s, 3H, -CH_3); ^{13}C NMR (101 MHz, DMSO) δ 182.6 (C=O), 182.4 (C=O), 167.9 (C=C), 167.0 (C=C), 143.5 (C-Ar), 128.6 (C-Ar), 127.3 (C-Ar), 126.0 (C-Ar), 108.5 (C(CH$_3$)$_2$), 108.0 (C(CH$_3$)$_2$), 95.6 (C-1), 70.4 (C-4), 70.1 (C-3), 69.7 (C-2), 67.3 (C-6), 52.6 (-NHCH), 43.6 (C-5), 25.9 (-CH_3), 25.6 (-CH_3), 24.9 (-CH_3), 24.3 (-CH_3), 23.0 (-CH_3); HRMS (ESI), m/z calcd. for $C_{24}H_{31}N_2O_7$ [M + H]$^+$ 459.2131, found: 459.2159.

4.3.8. 6-Amino-6-deoxy-3-(1,2:3,4-di-O-isopropylidene-α-D-galactopyranose)-4-(S)-((1-phenylethyl)amino)cyclobut-3-ene-1,2-dione (20)

Following the general procedure from **8** (1.0 mmol) and (S)-(−)-α-Methylbenzylamine (1.0 mmol) the compound **20** was obtained (312.2 mg, 68%). White solid: mp ≥ 250 °C; $[\alpha]_D^{25}$= –73.47 (c 0.01, DMSO); FT-IR (neat): 3150, 3032, 2974, 2932, 1793, 1644, 1543, 1488, 1454, 1209, 1149, 1051, 913, 835; ^{1}H NMR (400 MHz, DMSO) δ 7.86 (s, 1H, Amide-NH), 7.42–7.34 (m, 5H, Ar-H), 7.32–7.25 (m, 1H, Amide-NH), 5.46 (d, J = 4.9 Hz, 1H, H-1), 5.21 (s, 1H, H-3), 4.63 (dd, J = 7.8, 2.3 Hz, 1H, H-5), 4.37 (dd, J = 5.0, 2.4 Hz, 1H, H-2), 4.22 (d, J = 7.5 Hz, 1H, H-4), 3.84 (s, 1H, H-6a), 3.65 (dd, J = 12.1, 6.2 Hz, 1H, H-6b), 3.54 (s, 1H, -NHCH), 1.55–1.50 (m, 3H, -CH_3), 1.37 (s, 3H, -CH_3), 1.35 (s, 3H, -CH_3), 1.30 (s, 3H, -CH_3), 1.26 (s, 3H, -CH_3); ^{13}C NMR (101 MHz, DMSO) δ 182.6 (C=O), 182.2 (C=O), 168.0 (C=C), 166.9 (C=O), 143.6 (C-Ar), 128.6 (C-Ar), 127.3 (C-Ar), 125.9 (C-Ar), 108.6 (C(CH$_3$)$_2$), 108.0 (C(CH$_3$)$_2$), 95.6 (C-1), 70.4 (C-4), 70.1 (C-3), 69.7 (C-2), 67.4 (C-6), 52.7 (-NHCH), 43.6 (C-5), 25.9 (-CH_3), 25.6 (-CH_3), 24.8 (-CH_3), 24.3 (-CH_3), 22.9 (-CH_3); HRMS (ESI), m/z calcd. for $C_{24}H_{31}N_2O_7$ [M + H]$^+$ 459.2131, found: 459.2159.

4.3.9. 6-Amino-6-deoxy-3-(1,2:3,4-di-O-isopropylidene-α-D-galacto-pyranose)-4-(R)-((1-(naphthalen-2-yl)ethyl)amino)cyclobut-3-ene-1,2-dione (21)

Following the general procedure from **8** (1.0 mmol) and (R)-(+)-1-(2-Naphthyl)ethylamine (1.0 mmol) the compound **21** was obtained (377.0 mg, 71%). White solid: mp 248 °C (decomposed); $[\alpha]_D^{25} = +19.71$ (c 0.01, DMSO); FT-IR (neat): 3180, 2978, 2931, 1803, 1649, 1564, 1488, 1374, 1211, 1168, 1071, 1003, 903, 858; ^{1}H NMR (400 MHz, DMSO) δ 8.05–7.81 (m, 4H, Ar-H), 7.86 (s, 1H, Amide-NH) 7.60–7.49 (m, 3H, Ar-H), 7.44 (s, 1H, Amide-NH), 5.48 (d, J = 4.9 Hz, 1H, H-1), 5.39 (s, 1H, H-3), 4.61 (d, J = 7.1 Hz, 1H, H-5), 4.36 (d, J = 2.6 Hz, 1H, H-2), 4.22 (s, 1H, H-4), 3.86 (s, 1H, H-6a), 3.69 (s, 1H, H-6b), 3.54 (s, 1H, -NHCH), 1.64 (d, J = 6.8 Hz, 3H, -CH_3), 1.38 (s, 3H, -CH_3), 1.34 (s, 3H, -CH_3), 1.27 (s, 6H, -CH_3); ^{13}C NMR (101 MHz, DMSO) δ 182.6 (C=O), 168.0 (C=C), 167.0 (C=C), 141.0 (C-Ar), 132.8 (C-Ar), 132.2 (C-Ar), 128.4 (C-Ar), 127.8 (C-Ar), 127.5 (C-Ar), 126.3 (C-Ar), 126.0 (C-Ar), 124.6 (C-Ar), 124.2 (C-Ar), 108.5 (C(CH_3)$_2$), 108.0 (C(CH_3)$_2$), 95.6 (C-1), 70.4 (C-4), 70.1 (C-3), 69.7 (C-2), 67.4 (C-6), 52.7 (-NHCH), 43.6 (C-5), 25.9 (-CH_3), 25.6 (-CH_3), 24.8 (-CH_3), 24.3 (-CH_3), 22.9 (-CH_3); HRMS (ESI), m/z calcd. for $C_{28}H_{32}N_2O_7Na$ $[M + Na]^+$ 531.2107, found: 531.2133.

4.3.10. 6-Amino-6-deoxy-3-(1,2:3,4-di-O-isopropylidene-α-D-galactopyranose)-4-(S)-((1-(naphthalen-2-yl)ethyl)amino)cyclobut-3-ene-1,2-dione (22)

Following the general procedure from **8** (1.0 mmol) and (S)-(−)-1-(2-Naphthyl)ethylamine (1.0 mmol) the compound **22** was obtained (351.2 mg, 69%). White solid: mp 235 °C (decomposed); $[\alpha]_D^{25} = -35.11$ (c 0.01, DMSO); FT-IR (neat): 3161, 3056, 2974, 2931, 1798, 1646, 1552, 1472, 1380, 1212, 1182, 1072, 1003, 855, 818; ^{1}H NMR (400 MHz, DMSO) δ 7.98–7.89 (m, 4H, Ar-H), 7.85 (s, 1H, Amide-NH), 7.56–7.50 (m, 3H, Ar-H), 7.49 (s, 1H, Amide-NH), 5.48 (d, J = 4.9 Hz, 1H, H-1), 5.40 (s, 1H, H-3), 4.62 (dd, J = 7.8, 1.8 Hz, 1H, H-5), 4.37 (dd, J = 4.9, 2.4 Hz, 1H, H-2), 4.23 (d, J = 7.4 Hz, 1H, H-4), 3.85 (s, 1H, H-6a), 3.74–3.64 (m, 1H, H-6b), 3.57 (s, 1H, -NHCH), 1.63 (d, J = 6.8 Hz, 3H, -CH_3), 1.37 (s, 3H, -CH_3), 1.31 (s, 3H, -CH_3), 1.30 (s, 3H, -CH_3), 1.25 (s, 3H, -CH_3); ^{13}C NMR (101 MHz, DMSO) δ 182.7 (C=O), 182.5 (C=O), 168.1 (C=C), 167.2 (C=C), 141.1 (C-Ar), 132.8 (C-Ar), 132.2 (C-Ar), 128.3 (C-Ar), 127.8 (C-Ar), 127.5 (C-Ar), 126.3 (C-Ar), 126.0 (C-Ar), 124.5 (C-Ar), 124.0 (C-Ar), 108.6 (C(CH_3)$_2$), 108.0 (C(CH_3)$_2$), 95.6 (C-1), 70.4 (C-4), 70.1 (C-3), 69.7 (C-2), 67.5 (C-6), 52.7 (-NHCH), 43.6 (C-5), 25.9 (-CH_3), 25.5 (-CH_3), 24.8 (-CH_3), 24.3 (-CH_3), 23.0 (-CH_3); HRMS (ESI), m/z calcd. for $C_{28}H_{33}N_2O_7$ $[M + H]^+$ 509.2287, found: 509.2298.

4.4. Antiproliferative/cytotoxic activity

HeLa (human cervical cancer) and PC3 (human prostate cancer cell) cancer cell lines were purchased in this study (ATCC®, Manassas, VA, USA). Cell proliferation BrdU ELISA kits were provided from Roche (Germany) while 5-fluorouracil (5-FU) and others were from Sigma and Merck. Antiproliferative activities of the compounds were investigated on the cell lines using the proliferation BrdU ELISA assay [25–27]. 5-FU was used as a positive control. 5-FU is unsymmetrical cyclic fluoride urea and it has been used for the treatment of cancer for 40 years [28,29]. The results of investigation *in vitro* are based on the means ± SD of six measurements. Differences between groups were tested with ANOVA, Duncan, and p values of < 0.01 were considered as significant. IC_{50} values were determined using ED50plus v1.0.

L929 Fibroblast cells (5×10^3 cells per well) were placed in 96-well plates containing DMEM and RPMI1640 respectively, with L-glutamine, 10% FCS, and 1% antibiotic. The plates were then kept in a CO_2 incubator (37 °C in 5% CO_2) for 24 h until the cells attached to the bottom of the plate. Then, the cell culture medium was replaced with fresh medium, and different concentrations of **9**, **10**, **11** and **12** samples (0.2 mg/mL, 0.1 mg/mL, 0.05 mg/mL, 0.025 mg/mL, 0.0125 mg/mL and 0.0625 mg/mL) were placed into the wells. Following 24 h incubation under the same conditions, 3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test was used for detecting of the cytotoxicity. MTT reagent (50 μL) was added into each well. Upon incubation for an additional 2 h, then MTT solvent (isopropanol) 100 μL was added into each well, the plates were immediately read in an Elisa Microplate Reader (BioTek, USA) at 570 nm wavelength and the percentage of cell viability of each group was calculated according to the definition of control cell viability as 100%.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carres.2020.107991.

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