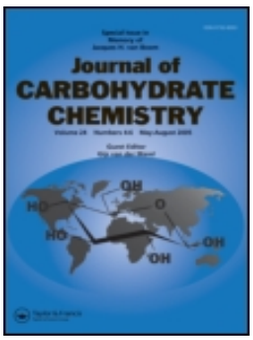

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Synthesis and Characterization of Some Novel Thiosemicarbazones of Substituted Benzaldehydes and *N*-(Hepta-*O*-Acetyl-β-D-Lactosyl)Thiosemicarbazide

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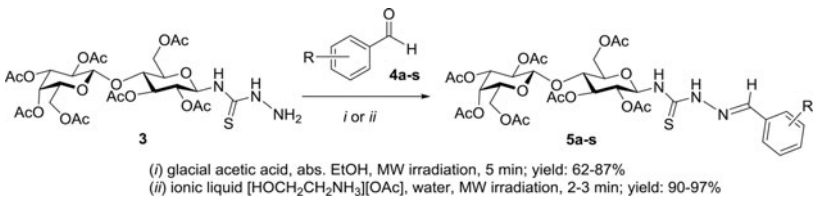
GRAPHICAL ABSTRACT



(*i*) glacial acetic acid, abs. EtOH, MW irradiation, 5 min; yield: 62-87%
(*ii*) ionic liquid [$HOCH_2CH_2NH_3$][OAc], water, MW irradiation, 2-3 min; yield: 90-97%

A set of reaction conditions, including ionic liquids as catalysts, water as solvent and microwave-assisted heating method, had been investigated for the synthesis of hepta-*O*-acetyl-β-D-lactosyl thiosemicarbazones. Based on optimized conditions, namely, [$HO(CH_2)_2NH_3$][OAc] as catalyst, water as solvent and 300 W microwave power, a series of substituted benzaldehyde hepta-*O*-acetyl-β-D-lactosyl thiosemicarbazones was synthesized by reaction of hepta-*O*-acetyl-β-D-lactosyl thiosemicarbazide with the corresponding substituted benzaldehydes. The high yields of 90–97% were achieved. Almost all of the obtained thiosemicarbazones exhibited remarkable antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Enterobacter, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella*

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pneumonia in comparison with sulphamethoxazole and trimethoprim as drug references and antifungal activity against *Aspergillus niger, Candida albicans* and *Saccharomyces cerevisiae* in comparison with clotrimazole as drug reference. MIC values of the compounds range from 39.1 to 2500 μg/mL for bacterial activity and from 0.25 to 25.6 μg/mL for fungal activity.

Keywords Antibacterial; Antifungal; Ionic liquid; β-Lactose; Thiosemicarbazone; Microwave-assisted reaction

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INTRODUCTION

Thiosemicarbazones are compounds that contain azomethine and thiourea bonds simultaneously. Thiosemicarbazone derivatives of monosaccharide and disaccharide have received considerable attentions because of their multifaceted properties. These compounds are known to possess a lot of interesting biological activities, such as antituberculosis,[1] antimicrobial,[2,3] antibacterial,[4,11] anticancer,[5,6] anticonvulsant,[7] antidiabetic,[8] antifungal,[9] antitumor,[10,11] cytotoxic,[12] antioxidant,[13–15] anti-dyslipidemic,[15] and enzyme inhibitory activities.[16] In general, thiosemicarbazone derivatives containing monosaccharide moiety have significant anti-microorganisms and antioxidant activities both *in vivo* and *in vitro*.[13,14,16] On the other hand, sugar isothiocyanates are among the most versatile synthetic intermediates in carbohydrate chemistry.[17,18] They can be converted into a broad spectrum of functional groups such as amide, isonitrile, carbodiimide, and also *N*-thiocarbonyl derivatives allowing, simultaneously, the covalent coupling of a quite unrestricted variety of structures to the saccharide moiety.

In several previous articles, the synthesis of substituted aromatic aldehyde/ketone *N*-(per-*O*-acetylated glucopyranosyl) were reported.[17,13,14,20–28] These compounds were synthesized by reaction of *N*-(per-*O*-acetylglycosyl)thiosemicarbazides with the corresponding carbonyl compounds, however, thiosemicarbazones containing simultaneously disaccharide moiety and benzene ring have not been reported yet.

In recent years, ionic liquids have been used more and more frequently in chemistry as reaction and/or extraction solvents or as catalysts. Ionic liquids with imidazole ring could solubilize carbonyl compounds, alcohols, alkyl halides, and also transition metal complexes, depending on the anion and the alkyl group of the imidazolium cation.[29–31]

It's known that the conventional catalysts for reactions between aldehydes or ketones and amines involve mainly organic and mineral acids, such as acetic acid and hydrochloric acid.[1,3,6–10,12,13,16] The use of these catalysts often suffers from the drawbacks of prolonged reaction times, harsh and harmful reaction conditions, and sometimes difficulty in product separations. This problem needs to be addressed in carbohydrate synthesis because of the susceptibility of the derivatives of carbohydrates towards acids, especially strong acids. In reactions of peracetylated glucopyranosyl thiosemicarbazides,

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this becomes extremely important due to the sensibility of acetate groups toward hydrolysis in the presence of strong acidic and basic catalysts. Therefore, we are especially interested in developing the use of inexpensive, simple and efficient catalysts in the synthesis of thiosemicarbazones having monosaccharide moiety. On the other hand, it's known that Brønsted-acidic task-specific ionic liquids (TSILs), which are the third generation of ionic liquids, are designed to replace traditional mineral or organic liquid acids as catalysts, such as sulfuric acid, hydrochloric acid, acetic acid, and *p*-toluenesulfonic acid in these chemical processes.[32] Such acidic TSILs exhibit dual role, as solvents and as catalysts, in different organic reactions.[33,44–48] In fact, the use of such Brønsted-acidic TSILs as catalysts is being developed and explored in many types of organic reactions. Our report is one of such contributions in development and exploration of ionic liquids in general and TSILs in particular.

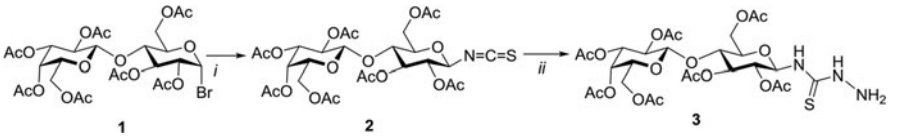
Continuing previous studies on the synthesis and the reactivity of *N*-(per-*O*-acetyl-D-glycopyranosyl)thiosemicarbazides,[13,28] in the present paper we report a systematic study on the synthesis, spectral characterization, and biological activities of a series of substituted benzaldehyde *N*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-*β*-D-lactosyl)thiosemicarbazones. The use of acids (acetic and hydrochloric) and piperidine, as well as ionic liquids, as catalysts was also investigated in this paper. The reaction was carried out by using conventional and microwave-assisted heating methods.[34] The antibacterial and antifungal activities of these thiosemicarbazones against some microorganisms were evaluated.[39–42]

RESULTS AND DISCUSSION

Chemistry

Required *N*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-*β*-lactosyl)thiosemicarbazide **3** was prepared from the corresponding isothiocyanate (Sch. 1) by a procedure similar to that used for other peracetylated glucopyranosyl thiosemicarbazides.[15,23,24] It's known that the conversion of *N*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)isothiocyanate into the corresponding thiosemicarbazide could usually be carried out at below room temperature in aprotic solvents, such as dichloromethane[15] and dioxane,[23,24] due to the higher reactivity of peracetylated glucopyranosyl isothiocyanate[13,15,23,24] in comparison with aromatic isocyanate[35] towards water. Previously, we found that the conversion of peracetylated glycopyranosyl isothiocyanates into the corresponding thiosemicarbazides could be performed in a protic solvent, such as absolute ethanol, but the reaction must be performed at lower temperature (usually 7–10°C) to prevent the transformation of isothiocyanate compounds into thiocarbamic acid derivatives.[8,35] We realized that the use of absolute ethanol as solvent for this reaction is of great advantage to work out the reaction due to low solubility of

these thiosemicarbazides in ethanol. For improvement of this reaction, we have used an 85% hydrazine solution[13] instead of 100% hydrazine hydrate.[15] In this way, *N*-(hepta-*O*-acetyl-β-lactosyl)thiosemicarbazide **3** was obtained from the corresponding isothiocyanate derivative **2** by reaction with 85% hydrazine hydrate (Sch. 1) using the similar procedure in anhydrous dichloromethane[15] or in anhydrous dioxane.[23,24] The advantage of using ethanol as a reaction solvent is that the thiosemicarbazide product is precipitated as soon as it is formed, hence the isolation of the product became easier, simply by filtering the product precipitates separated. A high yield of 96% was achieved.



Scheme 1: Synthesis of *N*-(hepta-*O*-acetyl-β-D-lactosyl)thiosemicarbazide **3**. Conditions: (*i*) NH_4SCN, TBAB, 50°C; 1 h; (*ii*) 85% hydrazine hydrate, EtOH, 7–10°C.

There are several methods for the synthesis of isothiocyanate of sugars.[23,24,36–38] In this article, the precursor of 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl isothiocyanate **2** was prepared from 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl bromide **1** by reaction with lead thiocyanate in toluene[36] or xylene.[37] In this research, we used the reaction of this bromide derivative with dry ammonium thiocyanate in anhydrous acetonitrile with tetrabutylammonium bromide as transfer catalyst for 10 min under microwave-assisted irradiation (modified the Tashpulatov's method).[23,24] This procedure is greener than the reported procedures[25,26] which used harmful lead thiocyanate in toluene or xylene.

In the literatures, peracetylated glucopyranosyl thiosemicarbazones, in particular, and other sugars thiosemicarbazones, in general, were synthesized under severe conditions: heating with acidic catalysts, such as hydrochloric and acetic acids, in organic solvents, such as methanol, ethanol, and propanol.[1–12,13–27] The reaction time of these protocols are usually lengthy (often from 2 h to 48 h). Therefore, the search for methods with smooth conditions is always laid out. First, we considered the reaction of peracetylated β-lactosyl thiosemicarbazide **3** with unsubstituted benzaldehyde **4a** (R = H), as a model reaction, under the conventional conditions for the synthesis of thiosemicarbazones (Sch. 2, Table 1, Procedure A). This procedure used absolute ethanol as solvent and glacial acetic acid as catalyst, and the reaction mixture was heated by the conventional heating method or microwave-assisted conditions. The product yield was 75% for 2 h under refluxing using conventional heating, while in the case of microwave-assisted heating, the reaction afforded a yield of 78% in only 5 min.

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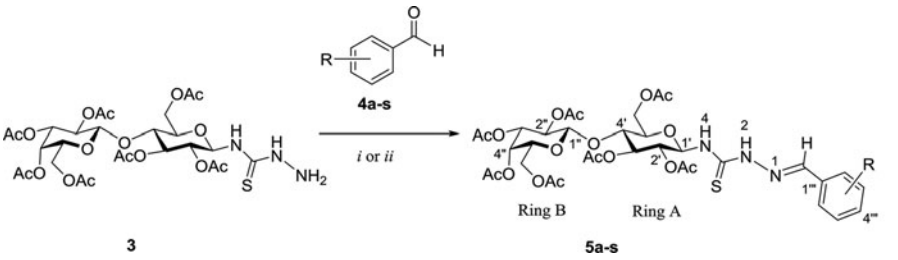

Scheme 2: Synthesis of benzaldehyde *N*-(hepta-*O*-acetyl-β-D-lactosyl)thiosemicarbazones **5a–s**. Conditions: (*i*) glacial acetic acid, EtOH, MW irradiation, 5 min; (*ii*) ionic liquid ($HOCH_2CH_2NH_3$)(OAc), water, MW irradiation, 2–3 min.

We have evaluated the effect of microwave power on reaction time and product yield for these reactions (Table 1). We found that, initially, pulses of 30 s of microwave irradiation at maximum power (800 W) were applied, but the yields were not reproducible, and that it was difficult to maintain the heating of the reaction mixture. Other high microwave powers from 600 W to 300 W were also evaluated, and the results were similar, except for at 450 W the yields were higher (78%). A similarly high yield (78%) was also achieved at microwave power of 300 W. The microwave power of 300 W was then chosen as the optimized one and continuous microwave irradiation was evaluated below.

We have also used concentrated hydrochloric acid and piperidine as catalysts in this reaction instead of glacial acetic acid (Table 2). The results obtained in Table 2 showed that the use of hydrochloric acid and piperidine did not give the desired products **5a**, even if the mixture was heated for 10 min under microwave-assisted conditions. It is possible that HCl had blocked the free amino group in thiosemicarbazide, therefore its reactivity toward carbonyl functional group was lost, whereas piperidine could not activate the carbonyl group in benzaldehyde **4a**.

Table 1: Different microwave powers used for synthesis of **5a** from **3** and **4a**

Entry	Microwave power (Watts)	Yield (%) [a]
1	800 [b]	60
2	600 [b]	70
3	450 [b]	78
4	300 [b]	78
5	100 [b]	55
6	Conventional heating [c]	75 (for 2 h)

[a]Isolated yields.
[b]Catalyst: Glacial acetic acid, 1 mmol%; Solvent: absolute ethanol; 5 min MW irradiation.
[c]Catalyst: Glacial acetic acid, 1 mmol%; Solvent: absolute ethanol; 2 h refluxing.

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Table 2: Comparison of ionic liquids used as catalysts for model reaction in Scheme 1 with **4a** (R = H) at microwave power of 300 W

Entry	Catalysts	**5a:** Yield (%) / Time (min) [a]	
		In 96% ethanol	In water
1	CH_3CO_2H [b]	78 / 5	18 / 10
2	HCl acid [c]	N.A. / 10	N.A. / 10
3	Piperidine [b]	N.A. / 10	N.A. / 10
4	$(Et_3NH)HSO_4$ [b]	50 / 2	50 / 2
5	$(Bmim)HSO_4$ [b]	88 / 2	90 / 2
6	$(HO(CH_2)_2NH_3)(OAc)$ [b]	96 / 2	97 / 2
7	$(HSO_3\text{-}Bmim)(HSO_4)$ [b]	80 / 2	82 / 2
8	$(Hmim)BF_4$ [b]	87 / 2	88 / 2

[a]Isolated yields.
[b]Molar ratio of the catalyst to benzaldehyde is according to the general procedure (see Experimental part).
[c]for Entry 2: 0.01 mL 36% HCl solution in water and absolute ethanol as solvent.

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Continuing our study, we have investigated the catalytic capacity of obtained ionic liquids, such as $[Et_3NH]HSO_4$,[51] $[Bmim][HSO_4]$,[52] $[HO(CH_2)_2NH_3][OAc]$,[53] $[HSO_3\text{-}Bmim][HSO_4]$[54] and $[Hmim][BF_4]$[55] in synthesis of thiosemicarbazones **5a** from thiosemicarbazide **3** and benzaldehyde **4a** (R = H), as the optimized microwave power (300 W) was used according to Table 2. Because some of the ionic liquids could be miscible with water, we have used water as solvent in these investigations instead of absolute ethanol. In addition, we found that the use of absolute ethanol or 96% ethanol did not change the product yields significantly, with 78% and 77% yields of **5a**, respectively. Based on these results, we decided to use 96% ethanol as solvent instead absolute ethanol in following investigations.

We found that almost all of these ionic liquids increased remarkably the yields of thiosemicarbazones and decreased the reaction time typically by 2 min. Table 2 indicated that, in almost all cases, the reaction that carried out in water gave the higher yield than the one in 96% ethanol, and that the product yields went up in the following order of these catalysts: $[Bmim][HSO_4]$ (50%) < $[HSO_3\text{-}Bmim][HSO_4]$ (82%) < $[Et_3NH][HSO_4]$ (86%) < $[Hmim][BF_4]$ (88%) < $[HO(CH_2)_2NH_3][OAc]$ (97%). Clearly, the ionic liquid $[HO(CH_2)_2NH_3][OAc]$ is the most suitable for the model reaction described above. Since then, we found that the ionic liquid $[HO(CH_2)_2NH_3][OAc]$ was the best catalyst for synthesis of thiosemicarbazones. On the other hand, when these ionic liquids were used as catalysts, the solvent change from 96% ethanol to water only alters the product yield inconsiderably. Thus, than advantage of using ionic liquids as catalysts is that the reactions could be carried out in water instead of 96% ethanol (Tables 2 and 3), making this process green.

Table 3: Different microwave powers for synthesis of **5a** using ionic liquid $(HO(CH_2)_2NH_3)(OAc)$ as catalyst in water solvent

Entry	Microwave power (Watts)	Yield (%) [a,b]
1	800	53
2	600	67
3	450	97.2
4	300	97
5	100	60

[a]Isolated yields.
[b]Reaction time: 2 min. Molar ratio of the catalyst to benzaldehyde is according to the general procedure (see Experimental part).

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Next, we have surveyed the effects of microwave power on the reaction yield when the ionic liquid $[HO(CH_2)_2NH_3][OAc]$ was used. The results listed in Table 3 indicated that, similarly to the case of using acetic acid as catalyst in the ethanol, the product yields were lower when the higher microwave powers were applied in comparison with in cases of the lower microwave powers. The yield of products **5a** were 97.2% and 97% in microwave powers of 450 W and 300 W, respectively. Therefore, the microwave power of 300 W was both energy-efficient and eco-friendly (using water as the reaction solvent).

Next, we surveyed further how the amounts of the catalyst might influence this reaction. When the amount of catalyst was reduced to 5 mol%, we found that the product performance decreased, although the reaction time was prolonged twice. When augmenting the amounts of moles of catalyst from 10 mol% to 20, 30, 40 and 50 mol%, respectively, the product yield did not change much, even if the reaction time was maintained or prolonged twice. However, the use of 40 mol%- and 50 mol%-amounts of this TSIL could raise the yield up to ~98%, so in general, the use of a large amount of the catalyst is not beneficial in this synthetic reaction. As such, the amount of catalyst 10 mol% is the most appropriate for this reaction (Table 4).

After the optimized conditions for the synthesis of thiosemicarbazone **5a** were discovered, we applied these optimized conditions, i.e., using ionic liquid $[HO(CH_2)_2NH_3][OAc]$ as catalyst, water as solvent and microwave power of 300 W, to the synthesis of other thiosemicarbazones **5b–t** (Table 5). The results from this table indicated that the obtained product yields by using TSIL as catalyst were higher than the ones obtained with acetic acid as catalyst whereas the reaction times were maintained equally or changed a little.

Next, we examined the reusability of the catalyst under microwave-assisted conditions using the model reaction of benzaldehyde **4a** with thiosemicarbazide **3** in the presence of 2-hydroxyethylammonium acetate (Table 5, Entry 1). For this purpose, the amount of reagents were raised up to 50 mmol and the amount of $[HO(CH_2)_2NH_3][OAc]$ was 50 mmol%. Once the reaction

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Table 4: Effects of catalytic amounts of $(HO(CH_2)_2NH_3)(OAc)$ on synthetic reaction of **5a** in water at microwave power of 300 W

Entry	TSIL,[a] (mmol%)	Time (min)	Yields (%) [b]
1	5	2	45
2	5	4	47
3	10	2	97
4	10	4	97.3
5	20	2	97.6
6	20	4	97.8
7	30	2	97.6
8	30	4	97.2
9	40	2	97.1
10	40	4	98
11	50	2	98
12	50	4	98.3

[a]TSIL = $(HO(CH_2)_2NH_3)(OAc)$.
[b]Isolated yields. Molar ratio of the catalyst to benzaldehyde is according to the general procedure (see Experimental part).

Table 5: Comparison of synthesis of benzaldehyde N-(peracetylated β-lactosyl)thiosemicarbazones **5a–s** with acetic acid and $(HO(CH_2)_2NH_3)(OAc)$ as catalyst in microwave-assisted conditions

			CH_3CO_2H[a]		TSIL[b]	
Entry	Compd.	R	Yield (%) [c]	Time (min)	Yield (%) [c]	Time (min)
1	**5a**	H	78	5	97	2
2	**5b**	4-NO_2	87	5	94	3
3	**5c**	3-NO_2	83	5	90	3
4	**5d**	2-NO_2	83	5	90	3
5	**5e**	4-F	70	5	91	2
6	**5f**	4-Cl	76	5	93	2
7	**5g**	3-Cl	68	5	97	3
8	**5h**	2-Cl	78	5	92	3
9	**5i**	4-Br	78	5	97	2
10	**5j**	4-Me	78	5	97	2
11	**5k**	4-iPr	72	5	97	2
12	**5l**	4-OMe	76	5	97	2
13	**5m**	3-OMe	73	5	92	2
14	**5n**	2-OMe	73	5	97	2
15	**5o**	4-OH	78	5	97	2
16	**5p**	2-OH	62	5	97	2
17	**5q**	3-OMe-4-OH	77	5	97	2
18	**5r**	3-OEt-4-OH	77	5	97	2
19	**5s**	4-NMe_2	77	5	96	2

[a]In 5 mL of 96% ethanol, otherwise, in 5 mL of water.
[b]TSIL = $(HO(CH_2)_2NH_3)(OAc)$ in water.
[c]Isolated yields. Molar ratios of the catalyst to benzaldehyde is according to the general procedure (see Experimental part).

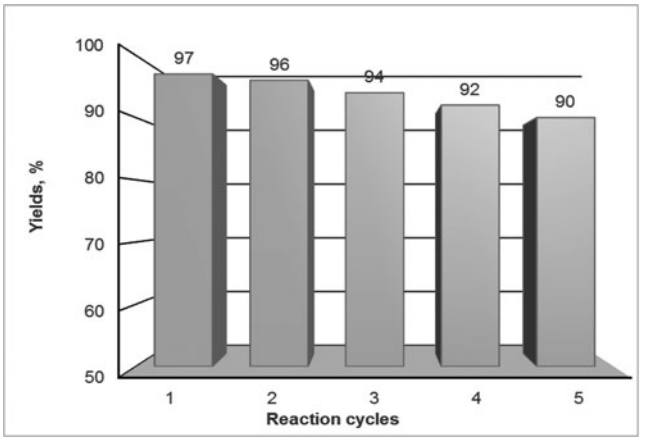

Figure 1: Recycling of $(HO(CH_2)_2NH_3)(OAc)$ as catalyst for reaction of benzaldehyde **4a** with thiosemicarbazide **3** under microwave-assisted conditions: **3** (50 mmol), **4a** (50 mmol) and $(HO(CH_2)_2NH_3)(OAc)$ (50 mmol%).

was completed, precipitates was filtered out the water solution and washed with water to remove ionic liquid out to obtain the crude product. The water-containing ionic liquid (this TSIL is soluble in water) was evaporated under reduced pressure and the ionic liquid was recovered and reused. The results of the first experiment and subsequent experiments were almost all consistent in yields after five runs without any loss of its activity (97%, 96%, 94%, 92% and 90%, Fig. 1).

The structures of the prepared substituted benzaldehyde *N*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-lactosyl)thiosemicarbazones **5a–s** were confirmed by their spectroscopic data. The IR spectra show characteristic absorptions in the range of 3540–3480 (ν_{OH}) and 3348–3159 cm^{-1} (ν_{NH}), 1737–1753 ($\nu_{C=O}$ ester), 1216–1252 and 1044–1056 cm^{-1} (ν_{COC} ester), 1367–1377 cm^{-1} ($\nu_{C=S}$), and 1580–1615 cm^{-1} ($\nu_{CH=N}$). In the ^1H NMR spectra, signal of proton NH-2 was found farther downfield in region of δ 11.74–10.69 ppm (in singlet) as this proton was deshielded by anisotropic effect of adjacent imine bond, whereas the signal in region of δ 8.72–8.44 ppm belongs to NH-4, which was split into a doublet by one neighboring proton on the carbon C-1′ of lactose moiety. The coupling constant in this case was J = 8.5–9.5 Hz. The protons of the disaccharide moiety had the signals in region of δ = 5.90–3.80 ppm. The protons in the carbons C-1″ and C-2″ magnetically interacted with each other with the coupling constant of J = 7.5–8.0 Hz. This corresponded with β-(1→4)-glycoside linkage between ring A and ring B in β-D-lactose (see Sch. 1). In addition, the spin-spin splitting pattern between H-3″ and H-4″ on ring B (D-galactose component) differed from the one between H-3′ and H-4′ on ring A (D-glucose component). The values of coupling constants were J = 2.5–3.75 Hz (in ring B) and J = 9.25–9.75 Hz (in ring A),

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respectively. The chemical shift of proton H-1′ (on ring A) was in region of $\delta =$ 5.90–5.74 ppm as triplet pattern. This proton also had similar interaction with proton H-2′ with the coupling constant of $J = 9.00$–9.25 Hz. The value of this coupling constant was consistent with a 1,2-*trans* relationship between these protons and showed that synthesized hepta-*O*-acetyl-β-D-lactosyl thiosemicarbazones had β-D-anomeric configuration. Proton in azomethine group showed a sharp peak at δ 7.97–8.18 ppm (CH = N) in singlet. The positions of resonance signals of protons NH-2, NH-4 and CH = N were affected significantly by the substituent nature on the benzene ring: the e-donating groups, such as 4-NMe_2, 4-OH, 4-OCH_3 and 4-CH_3, caused signal of proton NH-2 shifted to upfield region, and the e-withdrawing ones, such as 4-NO_2, 3-NO_2, 4-F, 4-Br, 4-Cl and 3-OCH_3 and 3-OCH_3-4-OH, caused this signal shifted to downfield region.

The ^{13}C-NMR spectrum of compound **5a–s** showed the four-parted regions of signals at δ 179.7–169.1, 159.7–111.3, 82.1–61.5 and 21.7–20.2 ppm. Signals at δ 179.1–177.8 ppm belonged to the carbon atom in the C = S group. The acetyl groups in these compounds showed resonance at δ 171.7–169.1 ppm for carbonyl carbon atom and 21.7–20.2 ppm for the methyl carbon one. The presence of imine in molecule were confirmed by signal at δ 147.8–140.2 ppm, belong to carbon atom in the azomethine group. It was observed that the NMR signal of carbon atom in the imine group was affected by these substituents in the following trend: the e-donating groups, such as 4-NMe_2, 4-OH, 4-OCH_3 and 4-CH_3, caused signal shifted to upfield region, and the e-withdrawing ones, such as 4-NO_2, 3-NO_2, 4-F, 4-Br, 4-Cl and 3-OCH_3 and 3-OCH_3-4-OH, caused the carbon signal shifted to downfield region.

Biological Evaluation

Antibacterial Activity

Antibacterial activity studies on thiosemicarbazones **5a–s** showed that these compounds had mild to moderate antibacterial activity against the tested bacteria (Table 6). Moreover, all compounds are more active (lower MIC) than the references sulphamethoxazole and trimethoprim for the majority of tested bacteria. It's known that sulfamethoxazole (SMZ or SMX**)** is a sulfonamide bacteriostatic antibiotic[43] and trimethoprim (TMP)-an antibiotic used mainly in the treatment of urinary tract infections.[44] These drugs are most often used as part of a synergistic combination with trimethoprim in a 5:1 ratio in co-trimoxazole, and known under trade names such as Bactrim, Septrin, or Septra, Biseptol . . . [45] Its primary activity is against susceptible forms of *Streptococcus*, *Staphylococcus aureus* (including MRSA-Methicillin-resistant *Staphylococcus aureus*), *Escherichia coli*, *Enterobacter* spp., *Klebsiella pneumoniae, Mycobacterium tuberculosis*, *Salmonella typhi* (typhoid fever) and oral

Table 6: Antibacterial activities of thiosemicarbazones **5a–s**

	Microorganisms/ MIC (μg/mL)							
	Gram positive			Gram negative				
Compound/Drug	*B.s.*[a]	*S.a.*[a]	*S.e.*[a]	*En.*[a]	*E.c.*[a]	*P.a.*[a]	*K.p.*[a]	log*P* (calc.)
5a	1200	225.1	2500	435	435	225.4	185.1	4.32
5b	1200	112.7	1200	225.4	225.4	185.7	175.3	4.24
5c	1200	215.3	1200	435	225.4	185.7	156.4	4.23
5d	1200	225.5	1200	225.4	225.4	185.7	183.7	4.42
5e	1200	58.3	1200	215.5	47.5	185.7	172.1	4.53
5f	1200	185.7	1200	435	63.5	58.3	175.3	5.08
5g	1200	435	435	185.4	54.5	185.7	39.1	5.15
5h	1200	433	435	187.1	85.7	185.7	185.7	5.01
5i	435	235.4	435	275.2	87.7	55.3	58.3	5.25
5j	225.4	245.8	435	435	58.3	58.8	39.1	4.78
5k	1200	255.1	435	245.9	185.2	165.7	39.1	5.66
5l	1200	175.7	1200	435	182.0	58.3	58.7	4.45
5m	1200	215.3	435	265.4	187.4	177.1	39.1	4.41
5n	1200	165.7	1200	435	175.8	59.1	175.9	4.05
5o	1200	245.4	435	215.3	165.1	155.3	39.1	4.07
5p	1200	181.7	1200	435	155.2	58.3	195.1	4.29
5q	1200	228.4	435	255.3	190.3	185.1	39.1	3.95
5r	1200	176.7	1200	435	174.7	58.3	188.4	4.48
5s	1200	235.4	435	215.6	185.7	155.5	39.1	4.49
Ref. A[b]	3200	19.5	3000	3000	3200	3000	156.2	0.89
Ref. B[c]	3000	2500	58.32	2500	2500	3000	1100	0.79

[a]*B.s.: Bacillus subtilis; S.a.: Staphylococcus aureus; S.e.: Staphylococcus epidermidis; En.:Enterobacter; E.c.: Escherchia coli; Pa.: Pseudomonas aeruginosa; K.p.: Klebsiella pneumonia*
[b]Ref. A: Sulphamethoxazole
[c]Ref. B: Trimethoprim

anaerobes.[45,46] Sulfamethoxazole is commonly used to treat urinary tract infections.[46] This drug is available as a generic medication and is not very expensive.

The corresponding MIC values of these references against tested bacteria were 3200, 19.5, 3000, 3000, 3200, 3000 and 156.2 μg/mL (for sulphamethoxazole) and 3000, 2500, 58.32, 2500, 2500, 3000 and 1100 μg/mL (for trimethoprim), respectively. The data in Table 6 also showed that thiosemicarbazones **5a–s** were more active references for almost all tested bacteria, *B. subtilis* MIC = 225.4–1200 μg/mL, *S. aureus* MIC = 176.7–435 μg/mL, *S. epidermidis* MIC = 435–2500 μg/mL, *Enterobacter* MIC = 185.4–435 μg/mL, *E. coli* MIC = 174.7–225.4 μg/mL, *P. aeruginosa* MIC = 58.3–225.4 μg/mL, *K. pneumonia* MIC = 39.1–195.1 μg/mL, except *S. aureus* and *K. pneumonia*: MIC values of sulfamethoxazole for this bacterium were 19.5 μg/mL and 156.2 μg/mL, respectively. For trimethoprim, almost all tested bacteria were inhibited by these compounds more than reference, except *S. epidermidis*: MIC value in

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Table 7: Antifungal activities of thiosemicarbazones **5a–s**

	MIC (μg/mL)				
Compound/Drug	*A. niger*	*C. albicans*	*F. oxysporum*	*S. cerevisiae*	log*P* (calc.)
5a	25.6	0.50	4.9	3.8	4.32
5b	19.5	0.53	2.7	3.8	4.24
5c	7.7	0.54	2.7	2.6	4.23
5d	25.6	0.51	4.5	2.2	4.42
5e	25.6	0.28	2.7	2.3	4.53
5f	7.8	0.45	1.2	2.2	5.08
5g	19.5	0.34	2.7	2.3	5.15
5h	7.7	0.25	4.4	2.3	5.01
5i	7.7	0.25	2.2	2.3	5.25
5j	7.7	0.57	2.7	3.5	4.78
5k	7.7	0.58	9.4	3.6	5.66
5l	7.3	0.57	4.7	3.2	4.45
5m	7.7	0.25	2.2	2.3	4.41
5n	7.7	0.57	2.7	3.5	4.05
5o	7.7	0.25	2.2	2.3	4.07
5p	7.7	0.25	2.2	2.3	4.29
5q	7.7	0.57	2.7	3.5	3.95
5r	7.7	0.58	9.1	3.6	4.48
5s	7.3	0.57	4.7	3.2	4.49
Clotrimazole	2.6	0.32	4.9	2.6	5.44

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this case was 58.32 μg/mL, whereas MIC values of thiosemicarbazones were in 425–2500 μg/mL. Especially, the most active thiosemicarbazones against each bacterium were as follows: **5i** and **5j** against *B. subtilis* (MIC = 435 and 225.4 μg/mL, respectively), **5e** against *S. aureus* (MIC = 58.3 μg/mL), **5g–k,m,o,q,s** against *S. epidermidis* (MIC = 435 μg/mL each) and **5g,i–m,q** against *K. pneumoniae* (MIC = 39.1–58.7 μg/mL), **5e–j** against *E. coli* (MIC = μg/mL), **5f,i,j** against *P. aeruginosa* (MIC = 55.3–59.1 μg/mL), and **5b,d,e,g-i,k,m,o,q,s** against *Enterobacter* (MIC = 185.4–275.2 μg/mL).The increased activity of the synthesized compound comparing to the references, trimethoprim and sulphamethoxazole, could be explained by electron delocalization over the whole molecule. The other reason could be the increased lipophilic character of thiosemicarbazones **5a–s** with log*P* in the range of 3.95 to 5.66, as compared to that of the references trimethoprim (log $P = 0.79$) and sulphamethoxazole (log $P = 0.89$), thus favoring the permeation of **5a–s** through the lipid layer of the bacterial membranes. It may be suggested that this molecule deactivate various cellular enzymes, which play a vital role in various metabolic pathways of these microorganisms.[47]

Antifungal Activity

The biological data in Table 7 showed that, notably, almost all tested thiosemicarbazones **5a–s** had more remarkable activities against these fungi

C. albicans, *F. oxysporum*, and *S. cerevisiae* but were more resistant to *A. niger* than clotrimazole (the reference compound, MIC = 2.6, 0.32, 4.9 and 2.6 μg/mL, respectively). Clotrimazole is an antifungal medication, commonly, used in the treatment of fungal infections, in both humans and other animals, such as vaginal yeast infections (candidiasis), oral thrush (oral candidiasis), and ringworm (dermatophytosis).[48] This drug is on the WHO's list of Essential Medicines (the most important medication needed in a basic health system).[49]

In case of fungus *Aspergillus niger*, all compounds were less active than clotrimazole (MIC = 2.6 μg/mL), compounds **5a**,**b**,**d**,**e**,**g**,**m** had the worst ability to inhibit to this fungus (MIC = 13.2–25.1 μg/mL), and the remaining compounds had medium activity (MIC = 7.3–7.8 μg/mL) but were less active than clotrimazole. In case of fungus *Candida albicans*, almost all compounds were less active than clotrimazole (MIC = 0.42–0.58 and 0.32 μg/mL, respectively), except **5g** is equipotent (MIC = 0.34 μg/mL), **5e**,**h**,**i**,**m**,**o**,**p** are more active (MIC = 0.25–0.28 μg/mL). Almost all thiosemicarbazones had activities similarly with clotrimazole against fungus *Fusarium oxysporum* (MIC = 2.4 g/mL), in that, the compounds **5b**,**c**,**e**,**f**,**g**,**i**,**j**,**m**,**o**,**p** have MIC = 2.2–2.4 μg/mL, except thiosemicarbazone **5f** is the most active (MIC = 1.2 g/mL) and thiosemicarbazones **5k**, **5r** had lowest activity (MIC = 9.1 μg/mL). Thiosemicarbazones **5a**,**b**,**j**,**k**,**l**,**n** were less active against *Saccharomyces cerevisiae* (MIC = 2.8–3.6 μg/mL), and the remaining compounds were more active (MIC = 2.2–2.4 μg/mL) than clotrimazole (MIC = 2.6 μg/mL).

CONCLUSION

In conclusion, we evaluated in this research the catalysis capacities of selected ionic liquids in synthetic reactions of thiosemicarbazones having β-lactose moiety under microwave-assisted conditions. We have given an interestingly simple procedure, cleaner reactions, and use of inexpensive and reusable ionic liquids as catalysts in water, allowing for economic and environmental-friendly conditions. Almost all quantitative yields and simple isolation and purification procedures of the products make it a useful procedure for the synthesis of these compounds. Almost all obtained thiosemicarbazones exhibited the remarkable antibacterial activity against *B. subtilis*, *S. aureus*, *S. epidermidis*, *Enterobacter, E. coli, P. aeruginosa* and *K. pneumonia* and antifungal activity against *A. niger*, *C. albicans* and *S. cerevisiae*.

EXPERIMENTAL

All solvents, chemicals, and reagents were obtained commercially and used without purification. Melting points were determined by open capillary method

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on STUART SMP3 instrument (BIBBY STERILIN, UK) and are uncorrected. FTIR spectra (KBr disc) were recorded on an Impact 410 FT-IR Spectrometer (Nicolet, USA). ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance Spectrometer AV500 (Bruker, Germany) at 500 MHz and 125 MHz, respectively, using DMSO-d_6 as solvent and TMS as an internal standard. Mass spectra were recorded on mass spectrometer LC-MS LTQ Orbitrap XL (ThermoScientific, USA) in methanol using ESI method or AutoSpec Premier (Water, USA) using EI method. The entire microwave heating experiments were conducted under reaction conditions of power and temperature. Thin-layer chromatography was performed on silica gel pates $60F_{254}$ No. 5715 (Merck, Germany) with ethyl acetate and light petroleum (bp 60–90°C) or toluene, and spots were visualized with UV light or iodine vapor. The synthesis of some task-specific ionic liquid was carried out by modifying procedure from a similar method in the literature, as described as below. The synthesis of $[Bmim]BF_4$ was reported previously.[50] Other ionic liquids such as $[Et_3NH]HSO_4$,[51] $[Bmim][HSO_4]$,[52] $[HO(CH_2)_2NH_3][OAc]$,[53] $[HSO_3\text{-}Bmim][HSO_4]$[54] and $[Hmim][BF_4]$[55] were prepared by the procedure modified the ones in the corresponding references as indicated. The ionic liquid was formed quantitatively and in high purity. Hepta-*O*-acetyl-β-D-lactosyl bromide **1** was prepared from β-lactose by the similar procedure by previously Lemieux's method.[56]

Synthesis of 2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-D-lactosyl isothiocyanate (2)

To the mixture of hepta-O-acetyl-β-D-lactosyl bromide **1** (7.0 g, 10 mmol) and dry ammonium thiocyanate (1.9 g, 25 mmol) in 50 mL of absolute acetonitrile was added tetrabutylammonium bromide (10 mmol%) under stirring. The mixture was then irradiated for 10 min at 600 W. Upon completion (monitored by TLC, the eluent solvent system was toluene/ethyl acetate 1:1 v/v), the precipitate was removed by filtration and the solvent was removed by distillation, the residue was dissolved in chloroform, and the solution was washed with water. The chloroform layer was dried over $CaCl_2$ and evaporated to dryness *in vacuo*, and the residue was crystallized from ether-hexane (2:1) to afford isothiocyanate **2** (6.09 g). Yield: 90%. M.p. 163–164°C; ref.[57]: 163–165°C.

Synthesis of *N*-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-D-lactosyl) thiosemicarbazide (3)

To a solution of hepta-*O*-acetyl-β-D-lactosyl isothiocyanate **2** (6.77 g, 10 mmol) in 40 mL of absolute ethanol a solution of 85% hydrazine hydrate (10 mmol, 1.2 ml) in 10 mL of absolute ethanol was added dropwise slowly with stirring in 30 minutes so that the reaction temperature is below 10°C. The white precipitate appeared immediately when several drops of hydrazine

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had added due to low solubility of this thiosemicarbazide in ethanol. The temperature of solution was maintained between 10−12°C. The mixture was continued stirring at 20°C for 30 min. The solid product then was isolated by filtering with suction. The crude product was crystallized from 96% ethanol to afford thiosemicarbazide **3** (6.81 g). Yield 96%, mp 187−188°C; FTIR (KBr): ν (cm^{-1}) 3473, 3409, 3273, 1753, 1620, 1535, 1378, 1226, 1050; ^{1}H NMR (DMSO-d_6): δ (ppm) 9.21 (s, 1H, NH-2), 8.09 (s, 1H, NH-4), 5.78 (s br, 1H, H-1′), 5.21 (d, 1H, J = 2.0 Hz, H-4″), 5.20 (s br, 1H, H-3′), 5.12 (dd, 1H, J = 10.25, 3.75 Hz, H-3″), 4.95 (t, 1H, J = 9.25 Hz, H-2′), 4.84 (t, 1H, J = 9.25 Hz, H-2″), 4.75 (d, 1H, J = 8.0 Hz, H-1″), 4.56 (s br, 2H, NH-1), 4.26 (d, 1H, J = 12.0 Hz, H-5′), 4.21 (t, 1H, J = 6.25 Hz, H-6′a), 4.01–4.00 (m, 3H, H-6′b, H-6″a, H-6″b), 3.77–3.76 (m, 2H, H-4′, H-5′), 2.09–1.93 (7s, 21H, 7×CH_3CO); ^{13}C NMR (DMSO-d_6): δ (ppm) 182.1 (C = S), 170.2–169.1 (7C, 7×COCH$_3$), 99.7 (C-1″), 80.8 (C-1′), 76.1 (C-4′), 73.3 (C-5′), 72.6 (C-3′), 70.8 (C-2′), 70.4 (C-3″), 69.7 (C-5″), 68.8 (C-2″), 67.1 (C-4″), 62.3 (C-6′), 60.9 (C-6″), 20.6–20.3 (7C, 7×COCH_3); ESI-MS: 732.16 $[M+Na]^+$, 710.18 $[M]^+$, 331.31, 317.27; calc. for $C_{27}H_{39}N_3O_{17}S$ = 709.20 Da; [M+Na] = 732.19 Da; Anal. calcd. for $C_{27}H_{39}N_3O_{17}S$: C, 45.70; H, 5.54; N, 5.92; S, 4.52. Found: C, 45.74; H, 5.51; N, 5.95; S, 4.50.

General procedure for synthesis of *N*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-lactosyl)thiosemicarbazones (5a-s)

Procedure A (under refluxing microwave-assisted conditions)

A mixture of *N*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-lactosyl)thiosemicarbazide **3** (1 mmol) and appropriate substituted benzaldehydes **4a-s** (1 mmol) in 5 mL of absolute ethanol was added the appropriate acid or base catalyst (1 mmol%, Tables 2, Entry 1–3, for evaluation of the corresponding catalysts) under stirring. The mixture was then irradiated for a certain time (Table 5, for synthesis of thiosemicarbazones **5a–s**). Upon completion (monitored by TLC, the eluent solvent system was toluene/ethyl acetate 1:1 v/v), the solvent was evaporated to one half the original volumes. The resulting colorless crystals were filtered by suction. The crude product when recrystallized from 96% ethanol to afford the title compounds **5a–s**.

Procedure B (under microwave-assisted conditions and TSIL as catalyst)

A mixture of *N*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-lactosyl)thiosemicarbazide **3** (1 mmol) and appropriate substituted benzaldehydes **4a-s** (1 mmol) in 5 mL of 96% ethanol was added the appropriate ionic liquid catalyst (1 mmol%, Tables 2 and 5, Entry 4–8, for evaluation of the corresponding catalysts) under stirring. The mixture was then irradiated for a certain

time (Table 5, for synthesis of thiosemicarbazones **5a–s**). Upon completion (monitored by TLC, the eluent solvent system was toluene/ethyl acetate 1:1, v/v), the precipitated crude product was collected by filtration, washed by water and recrystallized from 96% ethanol to afford pure thiosemicarbazones **5a–s**. After removing off the water under reduced pressure to 5 mL volume, the filtrate containing the ionic liquid could be reused directly in the next run without further purification.

The products were confirmed by FTIR, ^{1}H NMR, ^{13}C NMR, MS and physical data (m.p., $[\alpha]_D^{25}$).

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*Benzaldehyde N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl) thiosemicarbazone (5a)*

From 709 mg of **3** and 106 mg of **4a**. Recrystallized from 96% ethanol to yield white crystals of **5a**. Yield: 622 mg, 78% (reaction time: 5 min, procedure A); 773 mg, 97% (reaction time: 2 min, procedure B); mp 158–159°C; $[\alpha]_D^{25}$ +85.4 (*c* 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3469, 3304, 1750, 1605, 1369, 1222, 1051; ^{1}H NMR (DMSO-d_6): δ (ppm) 11.95 (s, 1H, NH-2), 8.67 (d, 1H, J = 9.5 Hz, NH-4), 8.10 (s, 1H, CH = N), 7.82–7.80 (m, 2H, H-2‴ & H-6‴), 7.45–7.43 (m, 3H, H-3‴, H-4‴ & H-5‴), 5.86 (t, 1H, J = 9.25 Hz, H-1′), 5.31 (t, 1H, J = 9.25 Hz, H-3′), 5.24 (d, 1H, J = 3.5 Hz, H-4″), 5.19 (t, 1H, J = 9.5 Hz, H-2′), 5.15 (dd, 1H, J = 10.5, 3.5 Hz, H-3″), 4.87 (dd, 1H, J = 10.0, 3.0 Hz, H-2″), 4.80 (d, 1H, J = 8.0 Hz, H-1″), 4.30 (d, 1H, J = 11.0 Hz, H-6′a), 4.26 (t, 1H, J = 6.75 Hz, H-5″), 4.07 (dd, 1H, J = 12.0, 5.5 Hz, H-6′b), 4.04 (dd, 1H, J = 6.25, 2.25 Hz, H-6″a), 4.03 (dd, 1H, J = 6.25, 2.25 Hz, H-6″b), 3.89 (ddd, 1H, J = 9.75, 5.75, 1.75 Hz, H-5′), 3.80 (t, 1H, J = 9.5 Hz, H-4′), 2.11–1.90 (s, 21H, 7 × CO*CH*$_3$); ^{13}C NMR (DMSO-d_6): δ (ppm) 178.3 (C = S), 170.3–169.1 (7 × *CO*CH$_3$), 143.7 (CH = N), 133.7 (C-1‴), 130.3 (C-4‴), 128.7 (C-2‴ & C-6‴), 127.6 (C-3‴ & C-5‴), 99.6 (C-1″), 81.2 (C-1′), 76.1 (C-4′), 73.3 (C-5′), 72.7 (C-3′), 71.1 (C-2′), 70.4 (C-3″), 69.7 (C-5″), 68.8 (C-2″), 67.1 (C-4″), 62.4 (C-6′), 61.0 (C-6″), 20.7–20.3 (7×CO*CH*$_3$); ESI-MS: m/z: 820.34 [M+Na]$^+$, 798.39 [M+H]$^+$, 766.59, 738.50, 658.66, 541.45, 331.48; calc. for $C_{34}H_{43}N_3O_{17}S$ = 797.23 Da. Anal. calcd. for $C_{34}H_{43}N_3O_{17}S$: C, 51.19; H, 5.43; N, 5.27; S, 4.02. Found: C, 51.23; H, 5.41; N, 5.25; S, 4.05.

*4-Nitrobenzaldehyde N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5b)*

From 709 mg of **3** and 151 mg of **4b**. Recrystallized from 96% ethanol to yield white crystals of **5b**. Yield: 732 mg, 87% (reaction time: 5 min, procedure A); 791 mg, 94% (reaction time: 3 min, procedure B); mp 172–173°C; $[\alpha]_D^{25}$ +79.8 (*c* 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3490, 3325, 1746, 1615, 1376, 1231, 1056; ^{1}H NMR (DMSO-d_6): δ (ppm) 12.16 (s, 1H, NH-2), 8.88 (d, 1H, J = 9.0 Hz, NH-4), 8.25 (d, 2H, J = 9.0 Hz, H-3‴ & H-5‴), 8.18 (s, 1H, CH = N), 8.10 (d, 2H, J = 9.0 Hz, H-2‴ & H-6‴), 5.88 (t, 1H, J = 9.0 Hz, H-1′), 5.31 (t, 1H,

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J = 9.25 Hz, H-3′), 5.24–5.21 (m, 2H, H-2′ & H-4″), 5.15 (dd, 1H, J = 10.0, 3.5 Hz, H-3″), 4.88 (t, 1H, J = 8.75 Hz, H-2″), 4.80 (d, 1H, J = 8.0 Hz, H-1″), 4.31 (d, 1H, J = 11.5 Hz, H-6′a), 4.25 (t, 1H, J = 6.5 Hz, H-5″), 4.07 (dd, 1H, J = 12.5, 5.5 Hz, H-6′b), 4.03 (d, 2H, J = 6.0 Hz, H-6″a & H-6″b), 3.90–3.87 (m, 1H, H-5′), 3.81 (t, 1H, J = 9.25 Hz, H-4′), 2.11–2.01 (s, 21H, 7×COCH_3); ^{13}C NMR (DMSO-d_6): δ (ppm) 178.8 (C = S), 170.2–169.0 (7×COCH$_3$), 147.8 (C-4‴), 141.1 (CH = N), 140.2 (C-1‴), 128.4 (C-2‴ & C-6‴), 123.8 (C-3‴ & C-5‴), 99.6 (C-1″), 81.3 (C-1′), 76.0 (C-4′), 73.4 (C-5′), 72.8 (C-3′), 71.2 (C-2′), 70.4 (C-3″), 69.7 (C-5″), 68.9 (C-2″), 67.1 (C-4″), 62.4 (C-6′), 61.0 (C-6″), 20.7–20.3 (7×COCH_3); ESI-MS: m/z: 843.80 [M+H]$^+$, 840.91, 799.01, 668.15, 443.28, 366.45; calc. for $C_{34}H_{42}N_4O_{19}S$ = 842.22 Da. Anal. calcd. for $C_{34}H_{42}N_4O_{19}S$: C, 48.45; H, 5.02; N, 6.65; S, 3.80. Found: C, 48.42; H, 5.06; N, 6.68; S, 3.83.

3-Nitrobenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5c)*

From 709 mg of **3** and 151 mg of **4c**. Recrystallized from 96% ethanol to yield white crystals of **5c**. Yield: 699 mg, 83% (reaction time: 5 min, procedure A); 758 mg, 90% (reaction time: 3 min, procedure B); mp 173–174°C; $[\alpha]_D^{25}$ +94.7 (c 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3548, 3340, 1751, 1615, 1372, 1229, 1048; ^{1}H NMR (DMSO-d_6): δ (ppm) 12.10 (s, 1H, NH-2), 8.83 (d, 1H, J = 9.0 Hz, NH-4), 8.21 (s, 1H, CH = N), 8.58 (s, 1H, H-2‴), 8.30 (d, 1H, J = 7.5 Hz, H-4‴), 7.72 (d, 1H, J = 8.0 Hz, H-5‴), 8.24 (dd, 1H, J = 8.0, 1.5 Hz, H-6‴), 5.86 (t, 1H, J = 9.0 Hz, H-1′), 5.31 (t, 1H, J = 9.0 Hz, H-3′), 5.24 (d, 1H, J = 3.5 Hz, H-4″), 5.20 (t, 1H, J = 9.5 Hz, H-2′), 5.15 (dd, 1H, J = 10.0, 3.5 Hz, H-3″), 4.88 (dd, 1H, J = 10.25, 8.25 Hz, H-2″), 4.80 (d, 1H, J = 8.0 Hz, H-1″), 4.31 (d, 1H, J = 11.0 Hz, H-6′a), 4.25 (t, 1H, J = 6.75 Hz, H-5″), 4.08 (dd, 1H, J = 12.0, 5.5 Hz, H-6′b), 4.04 (dd, 1H, J = 6.75, 2.25 Hz, 2H, H-6″a), 4.03 (dd, 1H, J = 6.75, 2.25 Hz, 2H, H-6″b), 3.91–3.84 (m, 1H, H-5′), 3.82 (t, 1H, J = 9.25 Hz, H-4′), 2.11–1.91 (s, 21H, 7×COCH_3); ^{13}C NMR (DMSO-d_6): δ (ppm) 178.7 (C = S), 170.2–69.1 (7×COCH$_3$), 141.6 (CH = N), 148.4 (C-3‴), 135.7 (C-1‴), 133.4 (C-6‴), 130.2 (C-5‴), 124.4 (C-4‴), 121.9 (C-2‴), 99.6 (C-1″), 81.3 (C-1′), 76.0 (C-4′), 73.5 (C-5′), 72.7 (C-3′), 71.1 (C-2′), 70.4 (C-3″), 69.8 (C-5″), 68.9 (C-2″), 67.1 (C-4″), 62.4 (C-6′), 61.0 (C-6″), 20.7–20.3 (7×COCH_3); ESI-MS: m/z: 865.06 [M+Na]$^+$, 843.02 [M+H]$^+$, 823.13, 658.18, 331.21; calc. for $C_{34}H_{42}N_4O_{19}S$ = 842.22 Da. Anal. calcd. for $C_{34}H_{42}N_4O_{19}S$: C, 48.45; H, 5.02; N, 6.65; S, 3.80. Found: C, 48.49; H, 5.00; N, 6.63; S, 3.78.

2-Nitrobenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5d)*

From 709 mg of **3** and 151 mg of **4d**. Recrystallized from 96% ethanol to yield white crystals of **5d**. Yield: 99 mg, 83% (reaction time: 5 min, procedure

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A); 758 mg, 90% (reaction time: 3 min, procedure B); mp 170–172°C; $[\alpha]_D^{25}$ +87.2 (*c* 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3473, 3295, 1751, 1615, 1373, 1230, 1048; ^{1}H NMR (DMSO-d_6): δ (ppm) 12.18 (s, 1H, NH-2), 8.77 (d, 1H, J = 9.0 Hz, NH-4), 8.53 (s, 1H, CH = N), 8.06 (d, 1H, J = 8.5 Hz, H-3‴), 7.68 (t, 1H, J = 7.5 Hz, H-4‴), 7.79 (t, 1H, J = 7.5 Hz, H-5‴), 8.38 (d, 1H, J = 7.5 Hz, H-6‴), 5.86 (t, 1H, J = 9.25 Hz, H-1′), 5.30 (t, 1H, J = 9.25 Hz, H-3′), 5.24 (d, 1H, J = 3.5 Hz, H-4″), 5.20 (t, 1H, J = 9.5 Hz, H-2′), 5.15 (dd, 1H, J = 10.25, 3.75 Hz, H-3″), 4.87 (dd, 1H, J = 10.25, 8.25 Hz, H-2″), 4.80 (d, 1H, J = 8.0 Hz, H-1″), 4.30 (d, 1H, J = 12.0 Hz, H-6′a), 4.25 (t, 1H, J = 6.5 Hz, H-5″), 4.07 (dd, 1H, J = 12.25, 5.75 Hz, H-6′b), 4.05–4.02 (m, 2H, H-6″a & H-6″b), 3.92–3.89 (m, 1H, H-5′), 3.80 (t, 1H, J = 9.5 Hz, H-4′), 2.11–1.90 (s, 21H, 7×CO*CH*$_3$); ^{13}C NMR (DMSO-d_6): δ (ppm) 178.8 (C = S), 170.2–69.0 (7×*CO*CH_3), 148.4 (C-2‴), 138.9 (CH = N), 133.4 (C-5‴), 130.8 (C-4‴), 128.3 (C-6‴), 128.1 (C-1‴), 124.6 (C-3‴), 99.6 (C-1″), 81.3 (C-1′), 76.0 (C-4′), 73.4 (C-5′), 72.7 (C-3′), 71.1 (C-2′), 70.4 (C-3″), 69.7 (C-5″), 68.9 (C-2″), 67.1 (C-4″), 62.3 (C-6′), 61.0 (C-6″), 20.6–20.2 (7×CO*CH*$_3$); ESI-MS: *m*/*z*: 865.19 $[M+Na]^+$, 843.28 $[M+H]^+$, 783.37, 658.41, 331.69; calc. for $C_{34}H_{42}N_4O_{19}S$ = 842.22 Da. Anal. calcd. for $C_{34}H_{42}N_4O_{19}S$: C, 48.45; H, 5.02; N, 6.65; S, 3.80. Found: C, 47.49; H, 5.05; N, 6.68; S, 3.83.

4-Fluorobenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5e)*

From 709 mg of **3** and 124 mg of **4e**. Recrystallized from 96% ethanol to yield white crystals of **5e**. Yield: 70 mg, 70% (reaction time: 5 min, procedure A); 733 mg, 91% (reaction time: 2 min, procedure B); mp 159–160°C; $[\alpha]_D^{25}$ +95.4 (*c* 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3563, 3320, 1752, 1601, 1376, 1238, 1048; ^{1}H NMR (DMSO-d_6): δ (ppm) 11.90 (s, 1H, NH-2), 8.66 (d, 1H, J = 9.0 Hz, NH-4), 8.09 (s, 1H, CH = N), 7.88 (d, 2H, J = 8.5 Hz, J_{HF} = 5.5, H-2‴ & H-6‴), 8.10 (d, 2H, J = 8.5 Hz, H-3‴ & H-5‴), 5.85 (t, 1H, J = 9.25 Hz, H-1′), 5.30 (d, 1H, J = 9.25 Hz, H-3′), 5.24 (d, 1H, J = 3.5 Hz, H-4″), 5.19 (d, 1H, J = 9.25 Hz, H-2′), 5.17 (dd, 1H, J = 9.75, 3.75 Hz, H-3″), 4.88 (dd, 1H, J = 10.5, 3.25 Hz, H-2″), 4.80 (d, 1H, J = 8.0 Hz, H-1″), 4.31 (d, 1H, J = 11.5 Hz, H-6′a), 4.25 (t, 1H, J = 6.5 Hz, H-5″), 4.07 (dd, 1H, J = 5.5, 11.0 Hz, H-6′b), 4.04–4.02 (m, 2H, H-6″a & H-6″b), 3.90–3.87 (m, 1H, H-5′), 3.81 (t, 1H, J = 9.5 Hz, H-4′), 2.11–2.01 (s, 21H, 7×CO*CH*$_3$); ^{13}C NMR (DMSO-d_6): δ (ppm) 178.4 (C = S), 170.2–169.1 (7×*CO*CH_3), 163.3 (C-4‴), 142.6 (CH = N), 130.4 (J_{CF} = 11.4 Hz, C-1‴), 129.7 (J_{CF} = 33.5 Hz, C-2‴ & C-6‴), 115.7 (J_{CF} = 87 Hz, C-3‴ & C-5‴), 99.6 (C-1″), 81.3 (C-1′), 76.0 (C-4′), 73.4 (C-5′), 72.7 (C-3′), 71.1 (C-2′), 70.4 (C-3″), 69.8 (C-5″), 68.9 (C-2″), 67.1 (C-4″), 62.4 (C-6′), 61.0 (C-6″), 20.7–20.2 (7×CO*CH*$_3$); ESI-MS: *m*/*z*: 816.07 $[M+H]^+$, 838.12 $[M+Na]^+$, 784.41, 756.21, 640.29, 598.31, 331.18; calc. for $C_{34}H_{42}FN_3O_{17}S$ = 815.22 Da. Anal. calcd. for $C_{34}H_{42}FN_3O_{17}S$: C, 50.06; H, 5.19; N, 5.15; S, 3.93. Found: C, 50.10; H, 5.16; N, 5.18; S, 3.91.

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4-Chlorobenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5f)*

From 709 mg of **3** and 140 mg of **4f**. Recrystallized from 96% ethanol to yield white crystals of **5f**. Yield: 632 mg, 76% (reaction time: 5 min, procedure A); 773 mg, 93% (reaction time: 2 min, procedure B); mp 189–190°C; $[\alpha]_D^{25}$ +100.2 (*c* 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3279, 3215, 1751, 1605, 1371, 1222, 1046; ^{1}H NMR (DMSO-d_6): δ (ppm) 11.95 (s, 1H, NH-2), 8.70 (d, 1H, J = 9.0 Hz, NH-4), 8.08 (s, 1H, CH = N), 7.85 (d, 2H, J = 8.5 Hz, H-2‴ & H-6‴), 7.49 (d, 2H, J = 8.5 Hz, H-3‴ & H-5‴), 5.85 (t, 1H, J = 9.0 Hz, H-1′), 5.30 (t, 1H, J = 9.25 Hz, H-3′), 5.24 (d, 1H, J = 3.5 Hz, H-4″), 5.20 (t, 1H, J = 9.25 Hz, H-2′), 5.16 (dd, 1H, J = 3.5, 10.0 Hz, H-3″), 4.88 (t, 1H, J = 9.0 Hz, H-2″), 4.80 (d, 1H, J = 7.5 Hz, H-1″), 4.30 (d, 1H, J = 11.5 Hz, H-6′a), 4.25 (t, 1H, J = 6.0 Hz, H-5″), 4.08–4.05 (m, 1H, H-6′b), 4.04–4.03 (m, 2H, H-6″a & H-6″b), 3.90–3.88 (m, 1H, H-5′), 3.80 (t, 1H, J = 9.5 Hz, H-4′), 2.11–1.91 (s, 21H, 7×COCH_3); ^{13}C NMR (DMSO-d_6): δ (ppm) 178.3 (C = S), 170.5–169.4 (7×$CO$$CH_3$), 142.7 (CH = N), 134.9 (C-4‴), 132.5 (C-1‴), 129.8 (C-2‴ & C-6‴), 128.8 (C-3‴ & C-5‴), 99.6 (C-1″), 81.2 (C-1′), 75.9 (C-4′), 73.5 (C-5′), 72.6 (C-3′), 70.9 (C-2′), 70.3 (C-3″), 69.7 (C-5″), 67.0 (C-4″), 68.8 (C-2″), 62.2 (C-6′), 60.9 (C-6″), 20.5–20.1 (7×COCH_3); ESI-MS: m/z: 854.17/856.17, $[M+Na]^+$/$[M+2+Na]^+$, 832.13/834.09 $[M+H]^+$/$[M+2+H]^+$, 790.18, 656.27, 640.38, 598.39, 331.30; calc. for $C_{34}H_{42}{}^{35}ClN_3O_{17}S$/$C_{34}H_{42}{}^{37}ClN_3O_{17}S$ = 831.19/833.19 Da. Anal. calcd. for $C_{34}H_{42}ClN_3O_{17}S$: C, 49.07; H, 5.09; N, 5.05; S, 3.85. Found: C, 49.11; H, 5.06; N, 5.08; S, 3.90.

3-Chlorobenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5g)*

From 709 mg of **3** and 140 mg of **4g**. Recrystallized from 96% ethanol to yield white crystals of **5g**. Yield: 565 mg, 68% (reaction time: 5 min, procedure A); 806 mg, 97% (reaction time: 3 min, procedure B); mp 152–153°C; $[\alpha]_D^{25}$ +88.7 (*c* 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3500, 3336, 1751, 1605, 1371, 1222, 1046; ^{1}H NMR (DMSO-d_6): δ (ppm) 11.99 (s, 1H, NH-2), 8.75 (d, 1H, J = 9.0 Hz, NH-4), 8.08 (s, 1H, CH = N), 7.98 (s, H-2‴), 7.48–7.44 (m, 1H, H-4‴), 7.48–7.44 (m, 1H, H-5‴), 7.70 (dd, 1H, J = 7.0, 1.5 Hz, H-6‴), 5.85 (t, 1H, J = 9.0 Hz, H-1′), 5.30 (t, 1H, J = 9.0 Hz, H-3′), 5.24 (d, 1H, J = 3.5 Hz, H-4″), 5.21 (t, 1H, J = 9.25 Hz, H-2′), 5.16 (dd, 1H, J = 3.5, 10.0 Hz, H-3″), 4.88 (dd, 1H, J = 2.0, 8.0 Hz, H-2″), 4.80 (d, 1H, J = 8.0 Hz, H-1″), 4.31 (d, 1H, J = 11.0 Hz, H-6′a), 4.25 (t, 1H, J = 6.75 Hz, H-5″), 4.07 (dd, 1H, J = 5.75, 12.25 Hz, H-6′b), 4.04–4.00 (m, 2H, H-6″a & H-6″b), 3.89 (ddd, 1H, J = 1.5, 5.5, 10.0 Hz, H-5′), 3.81 (t, 1H, J = 9.5 Hz, H-4′), 2.11–1.90 (s, 21H, 7×COCH_3); ^{13}C NMR (DMSO-d_6): δ (ppm) 178.6 (C = S), 170.1–169.0 (7×$CO$$CH_3$), 142.2 (CH = N), 135.9 (C-1‴), 133.7 (C-3‴), 130.5 (C-4‴), 129.8 (C-5‴), 126.7 (C-6‴), 126.3 (C-2‴), 99.6 (C-1″), 81.3 (C-1′), 76.0 (C-4′), 73.4

(C-5′), 72.7 (C-3′), 71.1 (C-2′), 70.4 (C-3″), 69.7 (C-5″), 68.9 (C-2″), 67.1 (C-4″), 62.3 (C-6′), 61.0 (C-6″), 20.8–20.2 (7×CO*CH*$_3$); ESI-MS: *m*/*z*: 854.22/856.12 $[M+Na]^+$/$[M+2+Na]^+$, 794.36, 732.34, 658.33, 561.70, 473.73, 331.64; calc. for $C_{34}H_{42}{}^{35}ClN_3O_{17}S/C_{34}H_{42}{}^{37}ClN_3O_{17}S$ = 831.19/833.19 Da. Anal. calcd. for $C_{34}H_{42}ClN_3O_{17}S$: C, 49.07; H, 5.09; N, 5.05; S, 3.85. Found: C, 49.05; H, 5.12; N, 5.03; S, 3.83.

2-Chlorobenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5h)*

From 709 mg of **3** and 140 mg of **4h**. Recrystallized from 96% ethanol to yield white crystals of **5h**. Yield: 648 mg, 78% (reaction time: 5 min, procedure A); 765 mg, 92% (reaction time: 3 min, procedure B); mp 187–188°C; $[\alpha]_D^{25}$ +87.2 (*c* 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3309, 1750, 1615, 1375, 1223, 1050; ^{1}H NMR (DMSO-d_6): δ (ppm) 12.08 (s, 1H, NH-2), 8.75 (d, 1H, J = 9.0 Hz, NH-4), 8.52 (s, 1H, CH = N), 7.51 (dd, 1H, J = 8.0, 0.5 Hz, H-3‴), 7.44 (td, 1H, J = 7.25, 2.0 Hz, H-4‴), 7.40 (t, 1H, J = 7.25″), Hz, H-5‴), 8.27 (dd, 1H, J = 7.5, 1.5 Hz, H-6‴), 5.86 (t, 1H, J = 9.0 Hz, H-1′), 5.30 (t, 1H, J = 9.25 Hz, H-3′), 5.24 (d, 1H, J = 3.0 Hz, H-4″), 5.23 (t, 1H, J = 9.25 Hz, H-2′), 5.15 (dd, 1H, J = 10.0, 3.5 Hz, H-3″), 4.87 (dd, 1H, J = 10.0, 3.0 Hz, H-2″), 4.80 (d, 1H, J = 8.0 Hz, H-1″), 4.33–4.30 (m, 1H, H-6′a), 4.25 (t, 1H, J = 6.75 Hz, H-5″), 4.06 (dd, 1H, J = 5.25, 12.25 Hz, H-6′b), 4.03–4.02 (m, 2H, H-6′a & H-6″b), 3.90–3.88 (m, 1H, H-5′), 3.80 (t, 1H, J = 9.5 Hz, H-4′), 2.11–1.92 (s, 21H, 7×CO*CH*$_3$); ^{13}C NMR (DMSO-d_6): δ (ppm) 179.1 (C = S), 170.7–169.5 (7×*CO*CH_3), 140.2 (CH = N), 133.9 (C-1‴), 132.1 (C-2‴), 131.6 (C-4‴), 130.3 (C-3‴), 127.8 (C-5‴), 128.0 (C-6‴), 100.1 (C-1″), 81.7 (C-1′), 76.5 (C-4′), 73.9 (C-5′), 73.2 (C-3′), 71.6 (C-2′), 70.9 (C-3″), 70.2 (C-5″), 69.3 (C-2″), 67.6 (C-4″), 62.8 (C-6′), 61.5 (C-6″), 21.2–20.8 (7×CO*CH*$_3$); ESI-MS: *m*/*z*: 832.21/834.09 $[M+H]^+$/$[M+2+H]^+$, 790.17, 656.35, 640.38, 598.39, 331.28; calc. for $C_{34}H_{42}{}^{35}ClN_3O_{17}S/C_{34}H_{42}{}^{37}ClN_3O_{17}S$ = 831.19/833.19 Da. Anal. calcd. for $C_{34}H_{42}ClN_3O_{17}S$: C, 49.07; H, 5.09; N, 5.05; S, 3.85. Found: C, 49.11; H, 5.05; N, 5.03; S, 3.88.

4-Bromobenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5i)*

From 709 mg of **3** and 185 mg of **4i**. Recrystallized from 96% ethanol to yield white crystals of **5i**. Yield: 683 mg, 78% (reaction time: 5 min, procedure A); 850 mg, 97% (reaction time: 2 min, procedure B); mp 155–156°C; $[\alpha]_D^{25}$ +78.9 (*c* 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3334, 1749, 1605, 1367, 1231, 1064; ^{1}H NMR (DMSO-d_6): δ (ppm) 12.00 (s, 1H, NH-2), 8.70 (d, 1H, J = 9.0 Hz, NH-4), 8.08 (s, 1H, CH = N), 7.82 (d, 2H, J = 8.5 Hz, H-2‴ & H-6‴), 7.66 (d, 2H, J = 8.5 Hz, H-3‴ & H-5‴), 5.90 (t, 1H, J = 9.0 Hz, H-1′), 5.24 (t, 1H, J = 9.25 Hz, H-2′), 5.33 (t, 1H, J = 9.25 Hz, H-3′), 5.28 (d, 1H, J = 3.5 Hz, H-4″), 5.16 (dd, 1H, J = 10.25, 3.75 Hz, H-3″), 4.91 (dd, 1H, J = 11.5, 3.0 Hz, H-2″),

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4.83 (d, 1H, J = 8.0 Hz, H-1″), 4.33 (d, 1H, J = 11.0 Hz, H-6′a), 4.29 (t, 1H, J = 6.5 Hz, H-5″), 4.09 (dd, 1H, J = 12.0, 5.5 Hz, H-6′b), 4.07–4.05 (m, 1H, H-6″a), 3.94–3.91 (m, 1H, H-6″b), 3.93 (ddd, 1H, J = 9.75, 5.75, 1.75 Hz, H-5′), 3.84 (t, 1H, J = 9.75 Hz, H-4′), 2.15–1.94 (s, 21H, 7×CO*CH*$_3$); ^{13}C NMR (DMSO-d_6): δ (ppm) 178.4 (C = S), 170.2–169.0 (7×*CO*CH$_3$), 142.5 (CH = N), 133.0 (C-1‴), 131.7 (C-3‴ & C-5‴), 129.4 (C-2‴ & C-6‴), 123.5 (C-4‴), 99.6 (C-1″), 81.2 (C-1′), 76.0 (C-4′), 73.4 (C-5′), 72.7 (C-3′), 71.1 (C-2′), 70.4 (C-3″), 69.7 (C-5″), 68.8 (C-2″), 67.1 (C-4″), 62.4 (C-6′), 60.9 (C-6″), 20.7–20.4 (7×CO*CH*$_3$); ESI-MS: m/z: 876.41/877.98 [M+H]$^+$/[M+2+H]$^+$, 850.47, 828.21, 640.43, 598.38, 331.59; calc. for $C_{34}H_{42}{}^{79}BrN_3O_{17}S/C_{34}H_{42}{}^{81}BrN_3O_{17}S$ = 875.14/877.14 Da. Anal. calcd. for $C_{34}H_{42}BrN_3O_{17}S$: C, 46.58; H, 4.83; N, 4.79; S, 3.66. Found: C, 46.55; H, 4.87; N, 4.82; S, 3.70.

4-Methylbenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5j)*

From 709 mg of **3** and 120 mg of **4j**. Recrystallized from 96% ethanol to yield white crystals of **5j**. Yield: 632 mg, 78% (reaction time: 5 min, procedure A); 786 mg, 97% (reaction time: 2 min, procedure B); mp 177–178°C; $[\alpha]_D^{25}$ +94.5 (c 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3212, 1748, 1615, 1373, 1242, 1046; ^{1}H NMR (DMSO-d_6): δ (ppm) 11.86 (s, 1H, NH-2), 8.59 (d, 1H, J = 9.5 Hz, NH-4), 8.06 (s, 1H, CH = N), 7.69 (d, 2H, J = 8.5 Hz, H-2‴ & H-6‴), 7.25 (d, 2H, J = 8.5 Hz, H-3‴ & H-5‴), 5.84 (t, 1H, J = 9.0 Hz, H-1′), 5.30 (t, 1H, J = 9.25 Hz, H-3′), 5.24 (d, 1H, J = 3.5 Hz, H-4″), 5.21 (t, 1H, J = 9.25 Hz, H-2′), 5.15 (dd, 1H, J = 10.25, 3.75 Hz, H-3″), 4.88 (t, 1H, J = 9.0 Hz, H-2″), 4.80 (d, 1H, J = 8.0 Hz, H-1″), 4.30 (d, 1H, J = 11.0 Hz, H-6′a), 4.25 (t, 1H, J = 6.5 Hz, H-5″), 4.07 (dd, 1H, J = 12.25, 5.75 Hz, H-6′b), 4.04–4.03 (m, 2H, H-6″a & H-6″b), 3.90–3.87 (m, 1H, H-5′), 3.81 (t, 1H, J = 9.5 Hz, H-4′), 2.34 (s, 3H, 4‴-CH$_3$), 2.11–1.90 (s, 21H, 7×CO*CH*$_3$); ^{13}C NMR (DMSO-d_6): δ (ppm) 178.2 (C = S), 170.2–169.0 (7×*CO*CH$_3$), 143.9 (CH = N), 140.2 (C-4‴), 131.0 (C-1‴), 129.3 (C-3‴ & C-5‴), 127.5 (C-2‴ & C-6‴), 99.6 (C-1″), 81.2 (C-1′), 76.0 (C-4′), 73.4 (C-5′), 72.7 (C-3′), 71.1 (C-2′), 70.4 (C-3″), 69.7 (C-5″), 68.9 (C-2″), 67.1 (C-4″), 62.3 (C-6′), 60.9 (C-6″), 20.6–20.2 (7×CO*CH*$_3$), 21.0 (4‴-CH$_3$); ESI-MS: m/z: 812.25 [M+H]$^+$, 834.31 [M+Na]$^+$, 770.31, 640.45, 598.44, 331.34; calc. for $C_{35}H_{45}N_3O_{17}S$ = 811.25 Da. Anal. calcd. for $C_{35}H_{45}N_3O_{17}S$: C, 51.78; H, 5.59; N, 5.18; S, 3.95. Found: C, 51.82; H, 5.56; N, 5.16; S, 3.99.

4-Isopropylbenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5k)*

From 709 mg of **3** and 148 mg of **4k**. Recrystallized from 96% ethanol to yield white crystals of **5k**. Yield: 604 mg, 72% (reaction time: 5 min, procedure A); 814 mg, 97% (reaction time: 2 min, procedure B); mp 148–149°C; $[\alpha]_D^{25}$ +95.4 (c 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3501, 3337, 1749, 1610, 1375, 1230, 1049; ^{1}H NMR (DMSO-d_6): δ (ppm) 11.87 (s, 1H, NH-2), 8.57 (d, 1H, J = 9.0 Hz,

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NH-4), 8.09 (s, 1H, CH = N), 7.72 (d, 2H, J = 8.0 Hz, H-2‴ & H-6‴), 7.30 (d, 2H, J = 8.0 Hz, H-3‴ & H-5‴), 5.83 (t, 1H, J = 9.25 Hz, H-1′), 5.30 (t, 1H, J = 9.25 Hz, H-3′), 5.24 (d, 1H, J = 3.5 Hz, H-4″), 5.18 (t, 1H, J = 9.5 Hz, H-2′), 5.15 (dd, 1H, J = 10.5, 3.75 Hz, H-3″), 4.88 (t, 1H, J = 9.75 Hz, H-2″), 4.80 (d, 1H, J = 8.0 Hz, H-1″), 4.30 (d, 1H, J = 11.5 Hz, H-6′a), 4.25 (t, 1H, J = 5.75 Hz, H-5″), 4.09–4.03 (m, 3H, H-6′b, H-6″a & H-6″b), 3.88–3.87 (m, 1H, H-5′), 3.81 (t, 1H, J = 9.25 Hz, H-4′), 2.92 [q, 1H, J = 7.0 Hz, 4‴-*CH*$(CH_3)_2$], 2.11–1.90 (s, 21H, 7×CO*CH₃*), 1.21 [d, 6H, J = 7.0 Hz, 4‴-CH(*CH₃*)$_2$]; ^{13}C NMR (DMSO-d_6): δ (ppm) 178.2 (C = S), 170.2–169.0 (7×*CO*CH_3), 150.9 (C-4‴), 143.8 (CH = N), 131.4 (C-1‴), 127.6 (C-2‴ & C-6‴), 126.6 (C-3‴ & C-5‴), 99.6 (C-1″), 81.1 (C-1′), 76.0 (C-4′), 73.4 (C-5′), 72.7 (C-3′), 71.0 (C-2′), 70.3 (C-3″), 69.7 (C-5″), 68.8 (C-2″), 67.1 (C-4″), 62.3 (C-6′), 60.9 (C-6″), 33.4 [4″-*CH*$(CH_3)_2$], 23.5 [4‴-CH(*CH₃*)$_2$], 20.6–20.2 (7×CO*CH₃*); ESI-MS: m/z: 862.41 $[M+Na]^+$, 848.66, 820.68, 732.72, 596.04, 351.28; calc. for $C_{37}H_{49}N_3O_{17}S$ = 839.28 Da. Anal. calcd. for $C_{37}H_{49}N_3O_{17}S$: C, 52.91; H, 5.88; N, 5.00; S, 3.82. Found: C, 52.95; H, 5.85; N, 5.03; S, 3.86.

4-Methoxybenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5l)*

From 709 mg of **3** and 136 mg of **4l**. Recrystallized from 96% ethanol to yield white crystals of **5l**. Yield: 645 mg, 78% (reaction time: 5 min, procedure A); 802 mg, 97% (reaction time: 2 min, procedure B); mp 198–199°C; $[\alpha]_D^{25}$ +97.3 (c 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3348, 3284, 1737, 1604, 1370, 1228, 1044; ^{1}H NMR (DMSO-d_6): δ (ppm) 11.81 (s, 1H, NH-2), 8.55 (d, 1H, J = 9.0 Hz, NH-4), 8.05 (s, 1H, CH = N), 7.75 (d, 2H, J = 8.5 Hz, H-2‴ & H-6‴), 6.99 (d, 2H, J = 8.5 Hz, H-3‴ & H-5‴), 5.83 (t, 1H, J = 9.25 Hz, H-1′), 5.30 (t, 1H, J = 9.0 Hz, H-3′), 5.24 (d, 1H, J = 3.0 Hz, H-4″), 5.18 (t, 1H, J = 9.25 Hz, H-2′), 5.15 (dd, 1H, J = 10.0, 3.0 Hz, H-3″), 4.88 (t, 1H, J = 9.25 Hz, H-2″), 4.80 (d, 1H, J = 7.5 Hz, H-1″), 4.30 (d, 1H, J = 11.5 Hz, H-6′a), 4.24 (t, 1H, J = 6.75 Hz, H-5″), 4.09–4.05 (m, 1H, H-6′b), 4.04–4.03 (m, 2H, H-6″a & H-6″b), 3.89–3.87 (m, 1H, H-4′), 3.82–3.79 (m, 1H, H-5′), 3.81 (s, 3H, 4‴-OMe), 2.11–1.91 (s, 21H, 7×CO*CH₃*); ^{13}C NMR (DMSO-d_6): δ (ppm) 177.8 (C = S), 170.5–169.4 (7×*CO*CH_3), 161.1 (C-4‴), 144.1 (CH = N), 129.2 (C-2‴ & C-6‴), 126.0 (C-1‴), 114.2 (C-3‴ & C-5‴), 99.6 (C-1″), 81.1 (C-1′), 75.9 (C-4′), 73.5 (C-5′), 72.6 (C-3′), 70.9 (C-2′), 70.3 (C-3″), 69.7 (C-5″), 68.8 (C-2″), 67.1 (C-4″), 62.2 (C-6′), 60.9 (C-6″), 20.5–20.1 (7×CO*CH₃*), 55.2 (4‴-OCH_3); ESI-MS: m/z: 850.28 $[M+Na]^+$, 828.23 $[M+H]^+$, 786.26, 768.35, 640.38, 540.36, 331.27; calc. for $C_{35}H_{45}N_3O_{18}S$ = 827.24 Da. Anal. calcd. for $C_{35}H_{45}N_3O_{18}S$: C, 50.78; H, 5.48; N, 5.08; S, 3.87. Found: C, 50.82; H, 5.45; N, 5.12; S, 3.85.

3-Methoxybenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5m)*

From 709 mg of **3** and 136 mg of **4m**. Recrystallized from 96% ethanol to yield white crystals of **5m**. Yield: 604 mg, 73% (reaction time: 5 min, procedure

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A); 761 mg, 92% (reaction time: 2 min, procedure B); mp 196–197°C; $[\alpha]_D^{25}$ +95.8 (*c* 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3345, 3160, 1747, 1605, 1376, 1241, 1042; ^{1}H NMR (DMSO-d_6): δ (ppm) 11.95 (s, 1H, NH-2), 8.62 (d, 1H, J = 9.0 Hz, NH-4), 8.07 (s, 1H, CH = N), 7.44 (s, H-2‴), 7.34 (d, 1H, J = 8.0 Hz, H-5‴), 7.31 (d, 1H, J = 8.0 Hz, H-6‴), 7.00 (dd, 1H, J = 8.0, 1.5 Hz, H-4‴), 5.79 (t, 1H, J = 9.0 Hz, H-1′), 5.31 (t, 1H, J = 9.25 Hz, H-3′), 5.18 (t, 1H, J = 9.25 Hz, H-2′), 5.24 (d, 1H, J = 3.5 Hz, H-4″), 5.15 (dd, 1H, J = 10.5, 3.5 Hz, H-3″), 4.88 (dd, 1H, J = 10.0, 3.0 Hz, H-2″), 4.79 (d, 1H, J = 7.5 Hz, H-1″), 4.30 (d, 1H, J = 11.0 Hz, H-6′a), 4.24 (t, 1H, J = 6.75 Hz, H-5″), 4.07 (dd, 1H, J = 12.5, 6.75 Hz, H-6′b), 4.04 (d, 1H, J = 6.5 Hz, H-6″a), 4.03 (d, 1H, J = 6.5 Hz, H-6″b), 3.88 (ddd, 1H, J = 10.0, 5.5, 3.5 Hz, H-5′), 3.83 (s, 3H, 3‴-OCH_3), 3.81 (t, 1H, J = 9.0 Hz, H-4′), 2.11–2.01 (s, 21H, 7×CO*CH*$_3$); ^{13}C NMR (DMSO-d_6): δ (ppm) 178.4 (C = S), 170.2–169.0 (7×*CO*CH_3), 159.6 (C-3‴), 143.5 (CH = N), 135.1 (C-1‴), 129.6 (C-5‴), 120.7 (C-6‴), 116.5 (C-4‴), 111.4 (C-2‴), 99.6 (C-1″), 81.2 (C-1′), 76.1 (C-4′), 73.4 (C-5′), 72.5 (C-3′), 70.9 (C-2′), 70.4 (C-3″), 69.7 (C-5″), 68.8 (C-2″), 67.1 (C-4″), 62.3 (C-6′), 60.9 (C-6″), 20.6–20.2 (7×CO*CH*$_3$), 55.2 (3″-OCH_3); ESI-MS: m/z: 850.28 $[M+Na]^+$, 828.27 $[M+H]^+$, 786.33, 744.50, 656.21, 445.47, 331.37; calc. for $C_{35}H_{45}N_3O_{18}S$ = 827.24 Da. Anal. calcd. for $C_{35}H_{45}N_3O_{18}S$: C, 50.78; H, 5.48; N, 5.08; S, 3.87. Found: C, 50.76; H, 5.50; N, 5.12; S, 3.91.

2-Methoxybenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5n)*

From 709 mg of **3** and 136 mg of **4n**. Recrystallized from 96% ethanol to yield white crystals of **5n**. Yield: 604 mg, 73% (reaction time: 5 min, procedure A); 802 mg, 97% (reaction time: 2 min, procedure B); mp 153–155°C; $[\alpha]_D^{25}$ +94.8 (*c* 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3548, 3282, 1750, 1607, 1372, 1241, 1054; ^{1}H NMR (DMSO-d_6): δ (ppm) 11.89 (s, 1H, NH-2), 8.58 (d, 1H, J = 9.0 Hz, NH-4), 8.45 (s, 1H, CH = N), 8.06 (d, 1H, J = 8.0 Hz, H-6‴), 7.08 (d, 1H, J = 8.5 Hz, H-3‴), 7.42 (t, 1H, J = 7.25 Hz, H-4‴), 6.99 (t, 1H, J = 7.5 Hz, H-5‴), 5.84 (t, 1H, J = 9.0 Hz, H-1′), 5.30 (t, 1H, J = 9.25 Hz, H-3′), 5.24 (d, 1H, J = 3.0 Hz, H-4″), 5.18 (t, 1H, J = 9.0 Hz, H-2′), 5.16 (dd, 1H, J = 10.0, 3.5 Hz, H-3″), 4.88 (t, 1H, J = 9.0 Hz, H-2″), 4.79 (d, 1H, J = 8.0 Hz, H-1″), 4.31 (d, 1H, J = 11.5 Hz, H-6′a), 4.25 (t, 1H, J = 6.25 Hz, H-5″), 4.07 (dd, 1H, J = 12.5, 5.5 Hz, H-6′b), 3.89–3.78 (m, 1H, H-5′), 4.04–4.03 (m, 2H, H-6″a & H-6″b), 3.88 (ddd, 1H, J = 10.0, 5.5, 3.5 Hz, H-4′), 3.83 (s, 3H, 2‴-OCH_3), 2.11–1.90 (s, 21H, 7×CO*CH*$_3$); ^{13}C NMR (DMSO-d_6): δ (ppm) 178.2 (C = S), 170.2–169.1 (7×*CO*CH_3), 158.1 (C-3‴), 139.5 (CH = N), 131.8 (C-1‴), 126.2 (C-5‴), 121.8 (C-6‴), 120.6 (C-2‴), 111.8 (C-4‴), 99.6 (C-1″), 81.2 (C-1′), 76.1 (C-4′), 73.4 (C-5′), 72.7 (C-3′), 71.1 (C-2′), 70.4 (C-3″), 69.8 (C-5″), 68.9 (C-2″), 67.1 (C-4″), 62.4 (C-6′), 61.0 (C-6″), 55.7 (2‴-OCH_3), 20.6–20.2 (7×CO*CH*$_3$); ESI-MS: m/z: 850.25 $[M+Na]^+$, 828.36 $[M+H]^+$,763.47, 689.58, 615.57, 429.29, 331.72; calc. for $C_{35}H_{45}N_3O_{18}S$

= 827.24 Da. Anal. calcd. for $C_{35}H_{45}N_3O_{18}S$: C, 50.78; H, 5.48; N, 5.08; S, 3.87. Found: C, 50.81; H, 5.46; N, 5.05; S, 3.86.

4-Hydroxybenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5o)*

From 709 mg of **3** and 122 mg of **4o**. Recrystallized from 96% ethanol to yield white crystals of **5o**. Yield: 634 mg, 78% (reaction time: 5 min, procedure A); 788 mg, 97% (reaction time: 2 min, procedure B); mp 161–162°C; $[\alpha]_D^{25}$ +84.8 (*c* 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3548, 3340, 3275, 1749, 1609, 1372, 1238, 1042; ^{1}H NMR (DMSO-d_6): δ (ppm) 11.76 (s, 1H, NH-2), 9.97 (s, 1H, 4‴-OH), 8.51 (d, 1H, J = 9.5 Hz, NH-4), 8.00 (s, 1H, CH = N), 7.64 (d, 2H, J = 8.5 Hz, H-3‴ & H-5‴), 6.81 (d, 2H, J = 8.5 Hz, H-2‴ & H-6‴), 5.83 (t, 1H, J = 9.25 Hz, H-1′), 5.30 (t, 1H, J = 9.25 Hz, H-3′), 5.24 (d, 1H, J = 3.5 Hz, H-4″), 5.18 (t, 1H, J = 9.25 Hz, H-2′), 5.16 (dd, 1H, J = 10.0, 4.0 Hz, H-3″), 4.87 (dd, 1H, J = 10.0, 3.0 Hz, H-2″), 4.80 (d, 1H, J = 8.0 Hz, H-1″), 4.30 (d, 1H, J = 11.0 Hz, H-6′a), 4.25 (t, 1H, J = 6.75 Hz, H-5″), 4.06 (dd, 1H, J = 9.5, 5.5 Hz, H-6′b), 4.03–4.02 (m, 2H, H-6″a & H-6″b), 3.89–3.86 (m, 1H, H-4′), 3.80 (t, 1H, J = 9.75 Hz, H-5′), 2.11–1.90 (s, 21H, 7×CO*CH*$_3$); ^{13}C NMR (DMSO-d_6): δ (ppm) 177.7 (C = S), 170.2–169.0 (7×*CO*CH_3), 159.6 (C-4‴), 144.1 (CH = N), 129.4 (C-2‴ & C-6‴), 124.6 (C-1‴), 115.6 (C-3‴ & C-5‴), 99.6 (C-1″), 81.1 (C-1′), 76.1 (C-4′), 73.3 (C-5′), 72.7 (C-3′), 71.1 (C-2′), 70.3 (C-3″), 69.7 (C-5″), 68.8 (C-2″), 67.1 (C-4″), 62.3 (C-6′), 60.9 (C-6″), 20.7–20.3 (7×CO*CH*$_3$); ESI-MS: m/z: 836.31 $[M+Na]^+$, 814.22 $[M+H]^+$, 772.33, 640.45, 598.45, 427.05, 331.45; calc. for $C_{34}H_{43}N_3O_{18}S$ = 813.23 Da. Anal. calcd. for $C_{34}H_{43}N_3O_{18}S$: C, 50.18; H, 5.33; N, 5.16; S, 3.94. Found: C, 50.22; H, 5.31; N, 5.19; S, 3.91.

2-Hydroxybenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5p)*

From 709 mg of **3** and 122 mg of **4p**. Recrystallized from 96% ethanol to yield white crystals of **5p**. Yield: 504 mg, 62% (reaction time: 5 min, procedure A); 788 mg, 97% (reaction time: 2 min, procedure B); mp 150–151°C; $[\alpha]_D^{25}$ +85.9 (*c* 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3602, 3446, 3282, 1741, 1609, 1372, 1228, 1040; ^{1}H NMR (DMSO-d_6): δ (ppm) 11.74 (s, 1H, NH-2), 9.96 (s, 1H, 2‴-OH), 8.49 (d, 1H, J = 9.5 Hz, NH-4), 8.00 (s, 1H, CH = N), 7.63 (d, 2H, J = 8.5 Hz, H-4‴ & H-6‴), 6.81 (d, 2H, J = 8.5 Hz, H-3‴ & H-5‴), 5.83 (t, 1H, J = 9.25 Hz, H-1′), 5.29 (t, 1H, J = 9.25 Hz, H-3′), 5.24 (d, 1H, J = 3.5 Hz, H-4″), 5.15 (dd, 1H, J = 3.75, 9.75 Hz, H-2′), 5.11 (t, 1H, J = 9.0 Hz, H-3″), 4.87 (dd, 1H, J = 10.0, 3.0 Hz, H-2″), 4.79 (d, 1H, J = 8.0 Hz, H-1″), 4.30 (d, 1H, J = 11.5 Hz, H-6′a), 4.24 (t, 1H, J = 6.75 Hz, H-5″), 4.06 (dd, 1H, J = 12.0, 5.5 Hz, H-6′b), 4.04–4.02 (m, 2H, H-6″a & H-6″b), 3.89–3.86 (m, 1H, H-5′), 3.80 (t, 1H, J = 9.5 Hz, H-4′), 2.11–1.90 (s, 21H, 7×CO*CH*$_3$); ^{13}C NMR (DMSO-d_6): δ (ppm) 177.8 (C = S), 170.2–169.1 (7×*CO*CH_3), 159.7 (C-2‴), 144.2 (CH = N), 129.4 (C-4‴ & C-6‴), 124.7 (C-5‴), 115.6 (C-1‴ & C-3‴), 99.6 (C-1″), 81.1 (C-1′), 76.1 (C-4′),

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73.4 (C-5′), 72.7 (C-3′), 71.1 (C-2′), 70.4 (C-3″), 69.8 (C-5″), 68.9 (C-2″), 67.2 (C-4″), 62.4 (C-6′), 61.0 (C-6″), 20.7–20.3 (7×CO*CH*$_3$); ESI-MS: *m*/*z*: 836.39 $[M+Na]^+$, 814.50 $[M+H]^+$, 794.62, 659.65, 606.01, 561.97, 430.45, 342.45; calc. for $C_{34}H_{43}N_3O_{18}S$ = 813.23 Da. Anal. calcd. for $C_{34}H_{43}N_3O_{18}S$: C, 50.18; H, 5.33; N, 5.16; S, 3.94. Found: C, 50.15; H, 5.31; N, 5.19; S, 3.98.

3-Methoxy-4-hydroxybenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5q)*

From 709 mg of **3** and 152 mg of **4q**. Recrystallized from 96% ethanol to yield white crystals of **5q**. Yield: 649 mg, 77% (reaction time: 5 min, procedure A); 817 mg, 97% (reaction time: 2 min, procedure B); mp 187–188°C; $[\alpha]_D^{25}$ +101.4 (*c* 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3480, 3337, 1749, 1603, 1377, 1224, 1051; ^{1}H NMR (DMSO-d_6): δ (ppm) 11.84 (s, 1H, NH-2), 9.57 (s, 1H, 4‴-OH), 8.50 (d, 1H, J = 9.0 Hz, NH-4), 7.98 (s, 1H, CH = N), 7.46 (d, J = 1.5 Hz, H-2‴), 7.10 (dd, 1H, J = 8.25, 1.5 Hz, H-6‴), 6.81 (d, 1H, J = 8.0 Hz, H-5‴), 5.74 (t, 1H, J = 9.25 Hz, H-1′), 5.33 (t, 1H, J = 9.25 Hz, H-3′), 5.24 (d, 1H, J = 3.5 Hz, H-4″), 5.17–5.13 (m, 2H, H-2′ & H-3″), 4.87 (dd, 1H, J = 10.0, 3.0 Hz, H-2″), 4.78 (d, 1H, J = 8.0 Hz, H-1″), 4.29 (d, 1H, J = 11.0 Hz, H-6‴), 4.25 (t, 1H, J = 6.75 Hz, H-5″), 4.07 (dd, 1H, J = 12.0, 5.5 Hz, H-6‴), 4.04–4.02 (m, 2H, H-6″a & H-6″b), 3.86 (s, 3H, 3‴-OMe), 3.85–3.81 (m, 2H, H-4′ & H-5′), 2.11–1.91 (s, 21H, 7×CO*CH*$_3$); ^{13}C NMR (DMSO-d_6): δ (ppm) 177.8 (C = S), 170.2–169.0 (7×*CO*CH_3), 149.5 (C-4‴), 147.1 (C-3‴), 144.1 (CH = N), 125.0 (C-1‴), 122.4 (C-6‴), 115.4 (C-5‴), 111.1 (C-2‴), 99.6 (C-1″), 81.0 (C-1′), 76.2 (C-4′), 73.3 (C-5′), 72.4 (C-3′), 70.8 (C-2′), 70.4 (C-3″), 69.7 (C-5″), 68.8 (C-2″), 67.1 (C-4″), 62.3 (C-6′), 60.9 (C-6″), 55.7 (3‴-OCH_3), 20.7–20.3 (7×CO*CH*$_3$); ESI-MS: *m*/*z*: 865.88 $[M+Na]^+$, 843.79 $[M+H]^+$, 824.00, 806.09, 701.09, 659.10, 424.06, 331.02; calc. for $C_{35}H_{45}N_3O_{19}S$ = 843.24 Da. Anal. calcd. for $C_{35}H_{45}N_3O_{19}S$: C, 49.82; H, 5.38; N, 4.98; S, 3.80. Found: C, 49.86; H, 5.35; N, 4.96; S, 3.83.

3-Ethoxy-4-hydroxybenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5r)*

From 709 mg of **3** and 166 mg of **4r**. Recrystallized from 96% ethanol to yield white crystals of **5r**. Yield: 659 mg, 77% (reaction time: 5 min, procedure A); 831 mg, 97% (reaction time: 2 min, procedure B); mp 140–142°C; $[\alpha]_D^{25}$ +104.3 (*c* 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3539, 3277, 1757, 1734, 1604, 1371, 1240, 1036; ^{1}H NMR (DMSO-d_6): δ (ppm) 11.80 (s, 1H, NH-2), 9.45 (s, 1H, 4‴-OH), 8.45 (d, 1H, J = 9.0 Hz, NH-4), 7.97 (s, 1H, CH = N), 7.40 (d, 1H, J = 1.5 Hz, H-2‴), 7.11 (dd, 1H, J = 8.5, 1.5 Hz, H-6‴), 6.82 (d, 1H, J = 8.5 Hz, H-5‴), 5.75 (t, 1H, J = 9.0 Hz, H-1′), 5.31 (t, 1H, J = 9.25 Hz, H-3′), 5.24 (d, 1H, J = 3.5 Hz, H-4″), 5.15 (dd, 1H, J = 10.25, 3.75 Hz, H-2′), 5.13 (t, 1H, J = 9.5 Hz, H-3″), 4.87 (dd, 1H, J = 8.0, 10.5 Hz, H-2″), 4.78 (d, 1H, J = 8.0 Hz, H-1″), 4.29 (d, 1H, J = 10.5 Hz, H-6′a), 4.25 (t, 1H, J = 6.5 Hz, H-5″), 4.11 (q, 2H, J = 7.0 Hz, 3‴-O*CH*$_2$$CH_3$), 4.08 (dd, 1H, J = 12.0, 5.5 Hz,

H-6′b), 4.04–4.01 (m, 2H, H-6″a & H-6″b), 3.86 (ddd, 1H, J = 10.0, 5.5, 1.5 Hz, H-5′), 3.81 (t, 1H, J = 9.25 Hz, H-4′), 2.11–1.90 (s, 21H, 7×COCH_3), 1.35 (t, 3H, J = 7.0 Hz, 3‴-OCH$_2$$CH_3$); ^{13}C NMR (DMSO-$d_6$): δ (ppm) 177.8 (C = S), 170.2–169.0 (7×COCH$_3$), 149.6 (C-4‴), 148.1 (C-3‴), 144.3 (CH = N), 125.1 (C-1‴), 122.6 (C-6‴), 115.3 (C-5‴), 109.5 (C-2‴), 99.6 (C-1″), 81.6 (C-1′), 76.1 (C-4′), 73.3 (C-5′), 72.4 (C-3′), 70.8 (C-2′), 70.3 (C-3″), 68.8 (C-2″), 69.7 (C-5″), 67.1 (C-4″), 63.9 (3‴-OCH_2CH$_3$), 62.2 (C-6′), 60.9 (C-6″), 20.6–20.2 (7×COCH_3), 14.7 (3‴-OCH$_2$$CH_3$); ESI-MS: m/z: 880.10 [M+Na]$^+$, 858.08 [M+H]$^+$, 803.08, 540.62, 512.74, 429.44, 413.40, 318.51; calc. for $C_{36}H_{47}N_3O_{19}S$ = 857.25 Da. Anal. calcd. for $C_{36}H_{47}N_3O_{19}S$: C, 50.40; H, 5.52; N, 4.90; S, 3.74. Found: C, 50.43; H, 5.50; N, 4.93; S, 3.72.

4-Dimethylaminobenzaldehyde

*N-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-*D*-lactosyl)thiosemicarbazone (5s)*

From 709 mg of **3** and 149 mg of **4s**. Recrystallized from 96% ethanol to yield white crystals of **5s**. Yield: 655 mg, 78% (reaction time: 5 min, procedure A); 804 mg, 96% (reaction time: 2 min, procedure B); mp 187–188°C; $[\alpha]_D^{25}$ +112.5 (c 1.0, $CHCl_3$); FTIR (KBr) ν / cm^{-1}: 3320, 1753, 1604, 1372, 1220, 1054; ^{1}H NMR (DMSO-d_6): δ (ppm) 11.67 (s, 1H, NH-2), 8.41 (d, 1H, J = 9.5 Hz, NH-4), 7.97 (s, 1H, CH = N), 7.59 (d, 2H, J = 8.5 Hz, H-2‴ & H-6‴), 6.72 (d, 2H, J = 8.5 Hz, H-3‴ & H-5‴), 5.82 (t, 1H, J = 9.0 Hz, H-1′), 5.30 (t, 1H, J = 9.0 Hz, H-3′), 5.25 (d, 1H, J = 2.5 Hz, H-4″), 5.17–5.14 (m, 2H, H-2′ & H-3″), 4.88 (t, 1H, J = 9.0 Hz, H-2″), 4.79 (d, 1H, J = 7.5 Hz, H-1″), 4.30 (d, 1H, J = 11.0 Hz, H-6′a), 4.25 (t, 1H, J = 6.0 Hz, H-5″), 4.09–4.05 (m, 1H, H-6′b), 4.04–4.03 (m, 2H, H-6″a & H-6″b), 3.88–3.85 (m, 1H, H-5′), 3.81 (t, 1H, J = 9.25 Hz, H-4′), 2.97 (s, 6H, 4‴-N(CH$_3$)$_2$], 2.11–1.91 (s, 21H, 7×COCH_3); ^{13}C NMR (DMSO-d_6): δ (ppm) 177.3 (C = S), 170.2–169.0 (7×COCH$_3$), 151.7 (C-4‴), 144.8 (CH = N), 128.9 (C-2‴ & C-6‴), 120.8 (C-1‴), 111.6 (C-3‴ & C-5‴), 99.6 (C-1″), 81.1 (C-1′), 76.1 (C-4′), 73.4 (C-5′), 72.7 (C-3′), 71.0 (C-2′), 70.4 (C-3″), 69.7 (C-5″), 68.9 (C-2″), 67.1 (C-4″), 62.3 (C-6′), 60.9 (C-6″), 40.0 [4‴-N(CH$_3$)$_2$], 20.6–20.2 (7×COCH_3); ESI-MS: m/z: 863.39 [M+Na]$^+$, 841.45 [M+H]$^+$, 821.57, 799.47, 577.88, 536.38, 433.16, 331.38; calc. for $C_{36}H_{48}N_4O_{17}S$ = 840.27 Da. Anal. calcd. for $C_{36}H_{48}N_4O_{17}S$: C, 51.42; H, 5.75; N, 6.66; S, 3.81. Found: C, 51.46; H, 5.72; N, 6.64; S, 3.84.

Biological Activity

Antimicrobial activities

All the synthesized compounds **5a–s** were screened for their in vitro antibacterial and antifungal activities by the twofold serial dilution technique using the principles of Clinical and Laboratory Standards Institute.[58] Antimicrobial activity *in vitro* against *Staphylococcus aureus* (ATCC 11632),

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Escherchia coli (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 25923), *Klebsiella pneumonia* (ATCC 4352), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 11774), *Enterobacter aerogenes* (ATCC 13048) were investigated by minimum inhibitory concentration (MIC) using agar dilutions technique using Mueller–Hinton agar medium. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and applied to plates with serially diluted compounds to be tested. The inoculum was prepared using a 4–6-h broth adjusted to a turbidity equivalent of an 0.5 McFarland standard, diluted in broth media to give a final concentration of 5×10^5 CFU/mL in the test tray; they were covered and placed in plastic bags to prevent evaporation. The plates were incubated at 35°C for 18–20 h. The MIC was defined as the lowest concentration of compound giving complete inhibition of visible growth. Sulphamethoxazole and trimethoprim were used as reference (positive control) for bacteria. Also as negative control, antimicrobial effects of the solvents (DMSO) were determined. According to the values of the controls, the results were evaluated. Minimal inhibitory concentrations for each compound were investigated against standard bacterial strains. The MIC values for all test compounds and reference drug are shown in Table 1.

The compounds **5a–s** were also evaluated for their *in vitro* antifungal activity against *Aspergillus niger* (439), *Candida albicans* (ATCC 7754), *Fusarium oxysporum* (M42), *Saccharomyces cerevisiae* (SH20) using agar dilution method with Saburoud's dextrose agar (Hi-Media). Suspensions of each microorganisms were prepared to contain 10 CFU/mL and applied to agar plates which have been serially diluted with compounds to be tested. The plates were incubated at 26°C during 48 h and MIC's were determined.[58] The MIC values for all test compounds and reference drug are shown in Table 2.

The calculated logP of all synthesized thiosemicarbazones have been obtained by using ACD/Log*P* in Advanced Chemistry Development (ACD/Labs) Software (*in silico* Prediction of Physicochemical, ADME, and Toxicity Properties, Release 12, 2015).

Statistical analysis

All data on antioxidant activities were the average of triplicate analyses and one-way analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's Multiple Range tests and P values <0.05 were regarded as significant and p values <0.01 were very significant.

SUPPLEMENTARY MATERIAL

Supplemental data for this article can be accessed on the publisher's website.

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