Synthesis and antibacterial and antifungal activities of \(N\)-(tetra-O-acetyl yl-\(\beta\)-D-glucopyranosyl)thiosemicarbazones of substituted 4-formylsydnones

Nguyen Dinh Thanh\(^1\), Hoang Thanh Duc\(^2\), Vu Thi Duyen\(^1\), Phan Manh Tuong\(^1\) and Nguyen Van Quoc\(^3\)

**Abstract**

**Background:** Sydnone is a heterocycle that exhibits remarkable pharmacological activities, including antimicrobial, anti-inflammatory, analgesic, antipyretic and antioxidant activities. Thiosemicarbazones are of compounds that contain the \(\text{–NHCSNH} = \text{C} < \) linkage group and are considerable interest because they exhibit important chemical properties and potentially beneficial biological activities. Similarly, thiosemicarbazones having carbohydrate moieties also exhibit various significant biological activities.

**Results:** The compounds of 3-formyl-4-phenylsydnones were obtained by Vilsmeyer-Haack’s formylation reaction and were transformed into thiosemicarbazones by condensation reaction with \(N\)-(2,3,4,6-tetra-O-acetyl-\(\beta\)-D-glucopyranosyl)thiosemicarbazide. Reaction were performed in the presence glacial acetic acid as catalyst using microwave-assisted heating method. Reaction yields were 43–85\%. The antimicrobial activities of these thiosemicarbazones were screened in vitro by using agar well diffusion and MIC methods. Among these thiosemicarbazones, compounds 4k, 4l, 4m and 4n were more active against all tested bacterial strains, especially against \(S. \text{epidermidis, B. subtilis and E. coli}\). The MIC values in these cases are 0.156, 0.156 and 0.313 \(\mu\text{g/mL}\), respectively. All compounds showed weak to moderate antifungal activity against \(C. \text{albicans and A. niger}\) than nystatin (MIC = 0.156–0.625 \(\mu\text{g/mL}\) vs. MIC = 0.078 \(\mu\text{g/mL}\) of nystatin), and thiosemicarbazones 4l, 4m and 4n exhibited significant activity with MIC = 0.156 \(\mu\text{g/mL}\). These compounds also had good antifungal activity against \(F. \text{oxysporum}\) similarly to nystatin (MIC = 0.156 \(\mu\text{g/mL}\)). Among the tested compounds having halogen group 4k, 4l, 4m and 4n showed highest activity against three strains of fungal organisms.

**Conclusions:** In summary, we have developed a clean and efficient methodology for the synthesis of novel thiosemicarbazone derivatives bearing sydnone ring and D-glucose moiety; the heterocyclic and monosaccharide system being connected via \(\text{–NH–C(=S)NH–N=C}<\) linker using molecular modification approach. The methodology could be further extended and used for the synthesis of other thiosemicarbazones of biological importance. 4-Formyl-3-aryl sydnone \(N\)-(2,3,4,6-tetra-O-acetyl-\(\beta\)-D-glucopyranosyl)thiosemicarbazones have been synthesized under microwave-assisted heating conditions. Almost all obtained compounds showed remarkable activities against the tested microorganisms. Among the tested compounds having halogen group 4k, 4l, 4m and 4n showed highest activity against all tested strains of bacterial and fungal organisms.

**Keywords:** Antibacterial, Antifungal, D-Glucose, Microwave-assisted synthesis, Sydnones, Thiosemicarbazones

\(^{*}\)Correspondence: nguyendinhthanh@hus.edu.vn

\(^1\) Faculty of Chemistry, VNU University of Science, 19 Le Thanh Tong, Hoan Kiem, Ha Noi, Vietnam

© 2015 Thanh et al. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Background
Sydnone is a mesoionic aromatic system, which could be described with some polar resonance structures [1]. Several compounds containing a sydnone ring exhibit remarkable pharmacological activities, including antimicrobial, anti-inflammatory, analgesic, antipyretic and antioxidant activities [2–5].

Thiosemicarbazones are compounds that contain the –NHCNSNHN=–C< linkage group. This class of compounds is of considerable interest because thiosemicarbazones exhibit the important chemical properties and potentially beneficial biological activities [6–9]. Some thiosemicarbazones of 3-aryl-4-formylsydnones were synthesized in good yields by the reactions of 3-aryl-4-formylsydnones with 4′-phenylthiosemicarbazide and thiosemicarbazide, respectively [3, 4]. On the other hand, some monosaccharide thiosemicarbazides are of interested because these derivatives could be used as versatile intermediates for synthesis of various derivatives (especially heterocycles) as well as be used for making complex formations of metallic ions [11, 12].

Thiosemicarbazones having carbohydrate moieties also exhibit various significant biological activities. In recent times, a number of thiosemicarbazones derivatives containing monosaccharide moiety have not yet been synthesized more. In general, thiosemicarbazones derivatives containing monosaccharide moiety have showed remarkable anti-microorganism and antioxidant activity both in vivo and in vitro [13–15]. Some articles have been reported about the synthesis of substituted aromatic aldehyde/ketone N-(per-O-acetylated glucopyranosyl)thiosemicarbazones in the past [10, 13–15]. These compounds have been synthesized by reaction of N-(per-O-acetylglycosyl)thiosemicarbazides with the corresponding carbonyl compounds [10, 13, 16–24], but the thiosemicarbazones containing both monosaccharide and sydnone moieties have not been reported yet. Continuing the previous studies on the synthesis and the reactivity of N-(per-O-acetyl-d-glucopyranosyl)thiosemicarbazides [15, 24], we report in the present paper a study on the synthesis, spectral characterization, antibacterial and antifungal activity of a series of N-(tetra-O-acetyl-β-d-glucopyranosyl)thiosemicarbazones having sydnone moiety by using microwave-assisted heating method [25].

Results and discussion
Chemistry
Required substituted 4-aryl sydnones 1a–o [26, 27] and 3-aryl-4-formylsydnone 2a–o [28, 29] were prepared with some modifications. 3-Arylsydnones were obtained in 43–85 % yields. These sydnones are solid with yellow colour and high melting temperature. By Vilsmeier-Haack’s reaction, starting from these sydnones we obtained the corresponding substituted 3-phenyl-4-formylsydnones in 17–50 % yield (Scheme 1). This reaction has been modified by Shih and Ke’s method [30].

Condensation reaction of substituted 3-phenyl-4-formylsydnones 2a–o with N-(tetra-O-acetyl-β-d-glucopyranosyl)thiosemicarbazide 3 was carried out on refluxing in the presence of glacial acetic acid as catalyst. These reactions were executed under microwave-assisted heating. All the microwave heating experiments were conducted under optimized reaction conditions of power and temperature in reflux-heating conditions that were investigated below (Scheme 2).

It’s known that peracetylated glucopyranosyl thiosemicarbazones, in particular, and thiosemicarbazones containing other sugars, in general, were sometimes synthesized in severe conditions, in the presence of acidic catalysts, such as hydrochloric or acetic acids in organic solvent, such as methanol, ethanol, propanol under conventional heating conditions [10, 13–24]. The reaction time of these protocols are usually lengthy (2–48 h). Therefore the search for methods of smooth conditions are always laid out. Initially, we prepared a typical peracetylated (β-d-glucopyranosyl)thiosemicarbazone 4a from 4-formyl-3-phenylsydnone 2a (R=H) and thiosemicarbazide 3 under the usual conditions in our procedure for synthesis of these thiosemicarbazones (Scheme 2). This procedure used absolute ethanol as solvent, glacial acetic acid as catalyst, and the reaction mixture was heated under conventional heating method or microwave-assisted conditions. We have evaluated the irradiation time and the effect of microwave power on reaction time and product yield for these reactions (Table 1).

In the process of synthesizing the compounds of 3-aryl-4-formylsydnones N-(2,3,4,6-tetra-O-β-d-glucopyranosyl)thiosemicarbazones 4a–o, the reaction times were monitored by the thin-layer chromatography with eluent
system ethyl acetate-toluene (2:1 v/v). In the case of conventional heating method, product was obtained in yield of 50 % for 120 min under refluxing, while in the case of microwave-assisted heating method, this reaction afforded the yield of 71 % in only 25-min irradiation (The reaction time of 25 min was fixed in order to investigate the microwave power). We found that, initially, the pulses of 1 min of microwave irradiation at maximum power (800 W) were applied, but the yields were not reproducible, and it was difficult to maintain the heating of the reaction mixture. On the other hand, the pulses of 1 min allow to monitor when the reaction is complete by TLC, especially, in cases of the compound 4n which reaction time was 45 min.

The other high microwave power (from 600 to 300 W) were evaluated and the results were similar, except at 450 W the yields were higher (71 %). This higher yield was also achieved at microwave power of 300 W (71 % yield). The influence of irradiation to isolated yield of 4a was also examined. The results showed that the isolated yields of 4a were 68, 71, 71.5 and 70 % with irradiation time of 20, 25, 27 and 30 min, respectively. This microwave power (300 W) was chosen as optimized condition, and was applied for synthesis of other thiosemicarbazone 4b–o (Table 2). In the reaction process, products usually separated as colour solid after cooling to room temperature. The structure of 4-aryl-3-formylsydnone N-(tetra-O-acetyl-β-d-glucopyranosyl)thiosemicarbazones 4a–o were confirmed by spectroscopic methods. We found that, in general, the electronic nature of the substituents R on the benzene ring of 4-arylsydnone does not affect significantly the reaction yields. However, the strong electron-withdrawing substituents such as NO2, Cl, Br, I slow down the reaction and prolong reaction time more than the electron-donating groups such as CH3, C2H5, OCH3, OC2H5 (Table 2). The yields of obtained thiosemicarbazones is quite high, from 63 to 85 %, except the compound 4o, in this case the yield reached only 43 % after 45 min irradiation. As the result, compounds of 3-aryl-4-formylsydnone N-(2,3,4,6-tetra-O-acetyl-β-d-glucopyranosyl)thiosemicarbazones (4a–o) have been synthesized with yields of 43–85 %. Meanwhile, the conventional heating method only gave the yields of 50–60 % during prolonged reaction time from 100 min to 150 min.

IR spectra show the characteristic absorption bands for two molecular components: sydnone and monosaccharide. IR spectral regions are 3476–3343 and 3334–3164 cm⁻¹ (νNH thiosemicarbazone), 1777–1746 cm⁻¹ (νC=O ester), 1624–1599 cm⁻¹ (νCH=N), 1228–1222 and 1056–1043 cm⁻¹ (νCOC ester), 1092–1090 cm⁻¹ (νC=C aromatic). The absorbance of carbonyl-lactone group of the sydnone ring was sometimes superposed partially by carbonyl-ester group in the range 1777–1746 cm⁻¹. The presence of the characteristic spectral regions for two moieties, 3-arylsydnone and monosaccharide, and characteristic
absorbance band in the range 1624–1600 cm\(^{-1}\) belong to azomethine bond in IR spectra indicated that the reaction of 3-aryl-4-formylsydnones and \(N\)-(tetra-O-acetyl-\(\beta\)-d-glucopyranosyl)thiosemicarbazide was occurred.

The \(^1\)H NMR spectra of these thiosemicarbazones showed the characteristic resonance signals of the protons present in the molecule, which are located in the region of \(\delta = 7.83–6.40\) ppm for aromatic protons, \(\delta = 5.87–3.98\) ppm for glucopyranose ring. Methyl groups in acetates had signals at \(\delta = 2.07–1.87\) ppm. The interaction of protons on neighbour carbons in molecules could be shown in \(^1\)H–\(^1\)H COSY spectrum of compound \(4i\) (Fig. 1). The \(^13\)C NMR spectral data showed the carbon of the aromatic ring with the signals in the \(\delta = 135.5–125.3\) ppm, the carbon C-4\(^{\prime}\) and C-5\(^{\prime}\) of the sydnone ring has characteristic signal is in the range \(\delta = 105.6–104.6\) ppm and 165.9–164.6 ppm, respectively. The carbon in the glucopyranose had chemical shifts at \(\delta = 81.3–61.2\) ppm. Carbon atoms in acetyl groups had signals at \(\delta = 21.5–20.1\) ppm (for methyl group) and 170.5–169.2 ppm (for carbonyl group).

From the structure of thiosemicarbazones \(4a–o\) above we can confirm that the presence of sydnone round cannot be used \(^1\)H NMR spectrum, because the unique C–H bond of sydnone ring substituted by the other group. So the presence of the sydnone ring could be recognized by the presence of resonance signal lying in region at \(\delta = 105.6–104.6\) ppm. The HMBC spectral results of compound \(4i\) showed the long-ranged interaction that appeared in this spectrum (Fig. 2). Some typical ones are below: Carbon atom C-1\(^{\prime}\) (\(\delta = 80.4\) ppm) interacts with proton H-2\(^{\prime}\) (\(\delta = 4.55\) ppm), carbon C-2\(^{\prime}\) (\(\delta = 70.9\) ppm) with protons H-1\(^{\prime}\) (\(\delta = 5.86\) ppm) and H-3\(^{\prime}\) (\(\delta = 5.41\) ppm), carbon C-3\(^{\prime}\) (\(\delta = 72.1\) pm) with protons H-2\(^{\prime}\) and H-4\(^{\prime}\) (\(\delta = 5.12\) ppm), carbon C-4\(^{\prime}\) with protons H-3\(^{\prime}\) and H-6\(^{\prime}\)b (\(\delta = 4.00\) ppm).

**Antimicrobial screening**

**Antibacterial activities**

Bacterium *Staphylococcus epidermidis* an cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis foliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia,… It is not a known human pathogen.

| Entry | R          | Reaction time (min) | Yield (%) |
|-------|------------|---------------------|-----------|
|       |            | Conventional heating | MW heating | Conventional heating | MW heating |
| 4a    | H          | 100                 | 25        | 50                  | 71         |
| 4b    | 2-Me       | 120                 | 28        | 55                  | 75         |
| 4c    | 3-Me       | 130                 | 30        | 55                  | 73         |
| 4d    | 4-Me       | 130                 | 30        | 56                  | 76         |
| 4e    | 2,3-diMe   | 130                 | 35        | 55                  | 70         |
| 4f    | 2,4-diMe   | 130                 | 35        | 50                  | 68         |
| 4g    | 4-Et       | 120                 | 28        | 60                  | 83         |
| 4h    | 3-OMe      | 130                 | 30        | 60                  | 78         |
| 4i    | 4-OEt      | 130                 | 30        | 60                  | 81         |
| 4j    | 4-OEt      | 130                 | 25        | 60                  | 82         |
| 4k    | 4-F        | 130                 | 30        | 55                  | 65         |
| 4l    | 4-Br       | 150                 | 35        | 55                  | 63         |
| 4m    | 4-I        | 130                 | 35        | 57                  | 68         |
| 4n    | 2-Me-5-Cl  | 140                 | 45        | 50                  | 43         |
| 4o    | Cyclohexyl  | 130                 | 30        | 60                  | 85         |

\(^{a}\) Cyclohexyl group is attached directly to sydnone ring at position 4
or disease causing agent. *Bacillus subtilis* produces the enzyme subtilisin, which has been reported to cause dermal allergic or hypersensitivity reactions in individuals repeatedly exposed to this enzyme. The bacteria *Salmonella* is commonly associated with food poisoning in countries all over the world, and the species that most people refer to when they talk about *Salmonella* is *S. enterica*. *Salmonella* infections can originate from household pets containing the bacteria, particularly reptiles, improperly prepared meats and seafood, or the surfaces of raw eggs, fruits, or vegetables that have not been adequately disinfected. As their name suggests *Salmonella enterica* are involved in causing diseases of the intestines (enteric means pertaining to the intestine). The three main serovars of *Salmonella enterica* are Typhimurium, Enteritidis, and Typhi.

The ability of thiosemicarbazones 4a–o to inhibit the bacterial growth were screened in vitro at 500 μg/mL concentration against *Staphylococcus epidermidis* and *Bacillus subtilis* as Gram positive bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* as Gram negative bacteria using ciprofloxacin as standard antibacterial reference. The obtained results of testing antimicrobial activities of 3-aryl-4-formylsydnone N-(2,3,4,6-tetra-O-β-D-glucopyranosyl)thiosemicarbazones 4a–o shows that some substances have significant bacterial inhibitory effects, but are less active than ciprofloxacin. The data from Table 3 revealed that almost all thiosemicarbazones have insignificant activity against *Staphylococcus epidermidis* except compounds 4i, 4m and 4n that medium one. Almost all compounds are remarkable active to *Bacillus subtilis* except thiosemicarbazones 4b, 4c, 4g, and 4h. In general, thiosemicarbazone 4a–o are more active to Gram negative bacteria, namely *Escherichia coli* and *Salmonella enterica* (Table 3), except compounds 4j and 4o.

The MIC data in Table 4 indicated that almost all the compounds 4a–o showed good antibacterial activity, and some of them had the one similar to the standard drug ciprofloxacin, determined through the serial tube dilution method. Thiosemicarbazone 4k–n were more active against *S. epidermidis* than other ones with MIC.
Table 3 Antibacterial activity (paper disc diffusion method) of thiosemicarbazones 4a–o

| Entry | Gram positive bacteria | Gram negative bacteria |  |
|-------|-------------------------|------------------------|---|
|       |                         | S. epidermidis | B. subtilis | E. coli | S. enterica |
| 4a    | 14                      | 25               | 26          | 27 |
| 4b    | 13                      | 16               | 25          | 26 |
| 4c    | 14                      | 17               | 26          | 27 |
| 4d    | 14                      | 27               | 28          | 29 |
| 4e    | 14                      | 28               | 28          | 30 |
| 4f    | 14                      | 19               | 29          | 31 |
| 4g    | 13                      | 20               | 30          | 31 |
| 4h    | 14                      | 20               | 29          | 30 |
| 4i    | 14                      | 27               | 31          | 32 |
| 4j    | 14                      | 28               | 14          | 13 |
| 4k    | 14                      | 32               | 32          | 33 |
| 4l    | 14                      | 34               | 34          | 33 |
| 4m    | 24                      | 34               | 34          | 35 |
| 4n    | 19                      | 32               | 31          | 30 |
| 4o    | 14                      | 25               | 13          | 14 |
| Ciprofloxacin | 43                   | 44               | 42          | 45 |
| Control | –                      | –                | –           | – |

Table 4 Antibacterial activity (minimum inhibitory concentration, μg/mL) of thiosemicarbazones 4a–o

| Entry | Gram positive bacteria | Gram negative bacteria |  |
|-------|-------------------------|------------------------|---|
|       |                         | S. epidermidis | B. subtilis | E. coli | S. enterica |
| 4a    | 0.313                   | 0.313               | 0.313       | 0.625 |
| 4b    | 0.313                   | 0.313               | 0.313       | 0.313 |
| 4c    | 0.313                   | 0.625               | 0.313       | 0.313 |
| 4d    | 0.313                   | 0.313               | 0.313       | 0.625 |
| 4e    | 0.313                   | 0.313               | 0.625       | 0.625 |
| 4f    | 0.313                   | 0.625               | 0.313       | 0.625 |
| 4g    | 0.313                   | 0.313               | 0.313       | 0.313 |
| 4h    | 0.313                   | 0.313               | 0.313       | 0.625 |
| 4i    | 0.156                   | 0.313               | 0.313       | 0.625 |
| 4j    | 0.313                   | 0.313               | 0.313       | 0.625 |
| 4k    | 0.156                   | 0.313               | 0.313       | 0.313 |
| 4l    | 0.156                   | 0.156               | 0.156       | 0.313 |
| 4m    | 0.156                   | 0.156               | 0.156       | 0.313 |
| 4n    | 0.156                   | 0.156               | 0.156       | 0.313 |
| 4o    | 0.313                   | 0.313               | 0.313       | 0.625 |
| Ciprofloxacin | 0.078              | 0.156               | 0.078       | 0.156 |
| Control | –                      | –                | –           | – |

Zone diameter of growth inhibition (mm) after 24 h: 50 μL of stock solution was applied in each hole of each paper disk, i.e. 25 μg/hole. Ciprofloxacin is used as a standard antibacterial reference. Control sample is 10 % DMSO solution in water.

Fig. 2 HMBC spectrum of thiosemicarbazone 4i
values of 0.156 μg/mL. All compounds showed significant activities for all bacterial strains used. Among these thiosemicarbazones, compounds 4k, 4l, 4m and 4n were more active against all tested bacterial strains, especially against S. epidermidis, B. subtilis and E. coli. The MIC values in these cases are 0.156, 0.156 and 0.313 μg/mL, respectively. Compounds 4k, 4l, 4m and 4n contain fluorine, bromine, iodine and chlorine group, respectively, whereas the remained thiosemicarbazones contains no halogen group in benzene ring. Overall most of the compounds exhibit excellent antibacterial activity against the both tested Gram positive and Gram negative bacteria as compared to standard drug ciprofloxacin.

**Antifungal activities**

There are over 20 species of Candida yeasts that can cause infection in humans, the most common of which is Candida albicans. Candida yeasts normally live on the skin and mucous membranes without causing infection; however, overgrowth of these organisms can cause symptoms to develop. Symptoms of candidiasis vary depending on the area of the body that is infected. Fungus Fusarium oxysporum plays the role of a silent assassin—the pathogenic strains of this fungus can be dormant for 30 years before resuming virulence and infecting a plant. F. oxysporum is infamous for causing a condition called Fusarium wilt. Furthermore, F. oxysporum can be harmful to both humans and animals, with its mycotoxins causing the diseases fungal keratitis, Onychomycosis, and Hyalohyphomycosis. Aspergillus niger is a fungus and one of the most common species of the genus Aspergillus. It causes a disease called black mould on certain fruits and vegetables such as grapes, apricots, onions, and peanuts, and is a common contaminant of food, but may also infect humans through inhalation of fungal spores.

The thiosemicarbazones 4a–o were screened against three fungal strains, namely Candida albicans, Fusarium oxysporum and Aspergillus niger. Tested concentration of these thiosemicarbazones is 500 μg/mL using nystatin as standard antifungal reference. Almost all tested compounds have remarkable activities against these three fungal strains, but are less active than nystatin (Table 5). All compounds are significantly active to two first fungi, except substances 4b, 4c, 4g, 4h (against C. albicans) and 4j, 4o (against F. oxysporum). Almost all thiosemicarbazones are resistant to fungus A. niger, except compound 4j.

The MIC values listed in Table 6 showed that all thiosemicarbazones had good antibacterial activity, but almost all compounds were equal or less active than the standard drug nystatin, determined through the serial tube dilution method. All compounds showed weak to moderate antifungal activity against C. albicans and A. niger than nystatin (MIC = 0.156–0.625 μg/mL vs. MIC = 0.078 μg/mL of nystatin), and thiosemicarbazones 4l, 4m and 4n exhibited significant activity with MIC = 0.156 μg/mL. These compounds also had good antifungal activity against F. oxysporum similarly to
nystatin (MIC = 0.156 μg/mL). Among the tested compounds having halogen group 4k, 4l, 4m and 4n showed highest activity against three strains of fungal organisms.

Conclusions
The authors have developed an effective method for synthesis of 4-formyl-3-arylsydnone N-(2,3,4,6-tetra-O-acetyl-β-d-glucopyranosyl)thiosemicarbazones under microwave-assisted conditions. These thiosemicarbazones have been obtained in good to excellent yields, except compound 4o, and fully characterized on the basis of their detailed spectral studies. Among the tested compounds having halogen group 4k, 4l, 4m and 4n showed highest activity against all tested strains of bacterial and fungal organisms. This heating method is advantageous in having a smaller solvent volume and a shorter reaction time. We also believe that the procedural simplicity, the efficiency and the easy accessibility of the reaction components give access to a wide array of possible products with high activity against bacterial and fungal organisms. This method is simple, easy to perform, and advantageous over the classical conditions from the reaction components give access to a wide array of heterocyclic frameworks bearing monosaccharide moiety. Almost all synthesized compounds had their antibacterial and antifungal activities evaluated and showed remarkable results. In summary, we have developed a clean and efficient methodology for the synthesis of novel thiosemicarbazone derivatives bearing sydnone ring and d-glucose moiety; the heterocyclic and monosaccharide system being connected via \(-\text{NH}-\text{C(=S)}\text{NH}-\text{N=C}< \text{linker using molecular modification approach. The methodology could be further extended and used for the synthesis of other thiosemicarbazones of biological importance.}

Experimental section
General methods
All chemicals used for the synthesis of the desired compounds were obtained from Merck chemicals. All other commercial reagents were used as received without additional purification. Melting points were measured on STUART SMP3 (BIBBY STERILIN, UK). The FTIR spectra was recorded on Impact 410 FT-IR Spectrometer (Nicole, USA), as KBr discs. The \(^1\)H NMR and \(^{13}\)C NMR spectra were recorded on an Avance Spectrometer AV500 (Bruker, Germany) at 500.13 and 125.77 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) or Agilent 6310 Ion Trap (Agilent Technologies, USA) in methanol, using ESI method. Thin-layer chromatography was performed on silica gel plates 60F\(254\) No. 5715 (Merck, Germany) with toluene: ethyl acetate = 1:2 (by volume) as solvent system, and spots were visualized with UV light or iodine vapour. \(N\)-(Tetra-O-acetyl-β-d-glucopyranosyl)thiosemicarbazide was synthesised using the method which described in Ref. [24] from corresponding thiocarbamate. Tetra-O-acetyl-β-glucopyranosyl thiocarbamate were prepared by the reaction of tetra-O-acetyl-β-glucopyranosyl bromide with dry ammonium thiocyanate in absolute acetonitrile using tetrabutylammonium bromide as transfer catalyst (modifying the Tashpulatov’s method [19, 20]). This bromide derivative was prepared from \(\alpha\)-glucose using Lemieux’s procedure [31]. The obtained thiosemicarbazones were yellow or orange solids, insoluble in water, but easily soluble in ethanol, methanol, benzene, dichloromethane, chloroform, ethyl acetate.

Synthesis of \(N\)-(tetra-O-acetyl-β-\(\alpha\)-glucopyranosyl) thiosemicarbazide (3)
To a solution of 2,3,4,6-tetra-O-acetyl-β-\(\alpha\)-glucopyranosyl thiocarbamate (3) (3.89 g, 10 mmol) in 25 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. The precipitate appears immediately when several drops of hydrazine are added due to low solubility of this thiosemicarbazide in ethanol. The temperature of solution was maintained between 10 and 12 °C. The mixture was continuously stirred 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 yield 3.75 g of white product 3. Yield 85 %, mp 156–158 °C; Ref. [19]: 169–171 °C. IR (KBr, cm\(^{-1}\)): \(\nu\) 3322, 3129 \((\nu\text{NH})\), 1752 \((\nu\text{C=O ester})\), 1355 \((\nu\text{C=S})\), 1242, 1043 \((\nu\text{COC ester})\); \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) (ppm): 12.77 (s, 1H, NH\(_b\)), 9.23 (s, 1H, NH), 8.17 (s, 1H, NH), 4.58 (s, 2H, NH\(_2\)), 5.80 (m, 1H, H-1), 5.07 (t, \(J = 9.5\) Hz, 1H, H-2), 5.34 (t, \(J = 9.75\) Hz, 1H, H-3), 4.91 (t, \(J = 9.75\) Hz, 1H, H-4), 4.14 (dd, \(J = 12.25, 4.75\) Hz, 1H, H-6a), 3.98–3.93 (m, 2H, H-5 & H-6b), 1.98–1.94 (s, 12H, 4 \(\times\) \(\text{CH}_2\text{CO}\)); \(^{13}\)C NMR (DMSO-\(d_6\)) \(\delta\) (ppm): 182.1 \((\text{C-6})\), 20.4–20.2 \((4 \times \text{CH}_3\text{CO})\), 169.9–169.2 \((4 \times \text{COCH}_3\)) 124.2, 104.3, 46.28, 81.0 (C-1), 70.5 (C-2), 72.5 (C-3), 68.1 (C-4), 72.1 (C-5), 61.8 (C-6), 20.4–20.2 (4 \(\times\) \(\text{CH}_3\text{CO}\)) ; MS (+ESI): \(m/z\) (%) = 422.42 (45) \([\text{M+H}]^+\), 462.28 (100) \([\text{M+K}]^+\); calcd. for \(\text{C}_{15}\text{H}_{23}\text{N}_3\text{O}_9\text{S}\) = 422.41 Da.

General procedure for synthesis of 3-aryl-4-formylsydnone \(N\)-(tetra-O-acetyl-β-d-glucopyranosyl)thiosemicarbazones (4a–o)
To a solution of \(N\)-(tetra-O-acetyl-β-d-glucopyranosyl) thiosemicarbazide 3 (2 mmol) in absolute ethanol (5 mL) was added substituted 3-aryl-4-formylsydnone 2a–o (2 mmol). Glacial acetic acid (2 mmol%) as catalyst was added dropwise with stirring. The obtained mixture was then irradiated in microwave oven for 25–45 min (Tables 1, 2), cooled to room temperature, the separated
precipitate was filtered and recrystallized from 96% ethanol to afford 4a–o.

3-Phenyl-4-formylsydnone N-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)thiosemicarbazone (4a)

Pale yellow crystals, mp 137–138 °C (from 96% ethanol), R_f = 0.57; [α]_D^20 +44.0 (c = 0.21, CHCl_3); FTIR (KBr): ν/cm⁻¹ 3343, 3122 (ν(CH)), 1750 (ν(C=O ester and sydnone), 1600 (ν(CH=O)), 1541 (ν(C=C)), 1080 (ν(C=O)), 1235, 1037 (ν(COC ester)); 1H NMR (500 MHz, DMSO-d_6): δ 12.96 (s, 1H, NH-2), 7.83–7.74 (m, 5H, H-2″, H-3″, H-4″, H-5″, H-6″), 7.79 (s, 1H, CH=CH-N), 7.05 (d, 1H, J = 9.5 Hz, NH-4), 5.88 (t, 1H, J = 9.5 Hz, H-1″), 5.40 (t, 1H, J = 9.5 Hz, H-3″), 5.02 (t, 1H, J = 9.75 Hz, H-4″), 4.81 (t, 1H, J = 9.5 Hz, H-2″), 4.23 (dd, 1H, J = 4.5, 12.25 Hz, H-6″a), 4.09 (ddd, 1H, J = 1.75, 3.75, 9.75 Hz, H-5″), 3.99 (dd, 1H, J = 1.0, 12.25 Hz, H-5″b), 2.06–1.90 (s, 12H, 4×CH_3CO); 13C NMR (125 MHz, DMSO-d_6): δ 177.7 (C-S), 170.5–169.8 (4×CH_3CO), 165.6 (C-5″), 134.4 (C-1″), 132.8 (C-3″, C-4″, C-5″), 130.1 (CH = CH = N), 126.0 (C-2″, C-6″), 105.6 (C-4″), 81.3 (C-1″), 72.9 (C-3″), 72.7 (C-5″), 71.3 (C-2″), 68.3 (C-4″), 61.2 (C-6″), 21.0–20.6 (4×CH_3CO); ESI–MS (+MS): m/z (%) 594.01 (M + H, 47), 407.12 (25), 390.21 (10), 348.17 (20), 331.28 (8), 218.28 (5), 190.37 (8), 173.69 (60), 132.56 (7), 114.71 (100), 102.78 (60), 76.75 (10), 74.59 (33), 59.47 (55); calc. for C_{24}H_{27}N_5O_{11}S = 593.14 Da.

3-(2-Methylphenyl)-4-formylsydnone N-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)thiosemicarbazone (4b)

Pale yellow crystals, mp 119–121 °C (from 96% ethanol), R_f = 0.58; [α]_D^20 +52.3 (c = 0.25, CHCl_3); FTIR (KBr): ν/cm⁻¹ 3329, 3215 (ν(C=O ester and sydnone), 1601 (ν(CH=O)), 1510, 1537 (ν(COC ester), 1083 (ν(COC ester)), 1226, 1043 (ν(COC ester)); 1H NMR (500 MHz, DMSO-d_6): δ 12.04 (s, 1H, NH-2), 7.70 (s, 1H, CH = CH-N), 7.75 (d, 2H, J = 9.0 Hz, H-3″, H-5″), 7.27 (d, 2H, J = 9.0 Hz, H-2″, H-6″), 6.73 (d, 1H, J = 10.0 Hz, NH-4), 5.85 (t, 1H, J = 9.5 Hz, H-1″), 5.41 (t, 1H, J = 9.75 Hz, H-3″), 5.12 (t, 1H, J = 9.75 Hz, H-4″), 4.54 (t, 1H, J = 9.5 Hz, H-2″), 4.27 (dd, 1H, J = 4.5, 12.5 Hz, H-6″a), 4.11 (dd, 1H, J = 2.0, 4.5, 10.0 Hz, H-5″), 3.99 (d, 1H, J = 12.5 Hz, H-6″b), 3.97 (s, 3H, 4″-CH_3), 2.06–1.87 (s, 12H, 4×CH_3CO); 13C NMR (125 MHz, DMSO-d_6): δ 177.2 (C-S), 170.1–169.2 (4×CH_3CO), 165.9 (C-5″), 161.5 (C-4″), 129.9 (CH=CH-N), 126.9 (C-3″, C-5″), 126.8 (C-1″), 115.1 (C-2″, C-6″), 104.6 (C-4″), 80.4 (C-1″), 72.3 (C-5″), 72.1 (C-3″), 70.8 (C-2″), 67.5 (C-4″), 61.6 (C-6″), 55.8 (4″-CH_3), 20.5–20.1 (4×CH_3CO); ESI–MS (+MS): m/z (%) 608.00 (M + H, 55), 536.00 (10), 412.11 (14), 407.15 (20), 390.19 (7), 348.13 (10), 321.36 (25), 290.20 (8), 218.32 (5), 204, 138.30 (55), 139.18 (37), 117.32 (95), 102.45 (100), 81.37 (18), 74.58 (35), 59.45 (55); calc. for C_{25}H_{29}N_5O_{11}S = 607.16 Da.

3-(2,3-Dimethylphenyl)-4-formylsydnone N-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)thiosemicarbazone (4e)

Pale yellow crystals, mp 138–140 °C (from 96% ethanol), R_f = 0.53; [α]_D^25 +47.0 (c = 0.23, CHCl_3); FTIR (KBr): ν/cm⁻¹ 3525, 3164 (ν(C=O ester and sydnone), 1624 (ν(CH=O)), 1532 (ν(COC ester), 1237, 1041 (ν(COC ester)); 1H NMR (500 MHz, DMSO-d_6): δ 11.98 (s, 1H, NH-2), 7.78 (s, 1H, CH=CH-N), 7.63–7.60 (m, 4H, H-2″, H-3″, H-4″, H-5″, H-6″), 7.00 (d, 1H, J = 10.0 Hz, NH-4), 5.87 (t, 1H, J = 9.5 Hz, H-1″), 5.41 (t, 1H, J = 9.5 Hz, H-3″), 5.01 (t, 1H, J = 9.75 Hz, H-2″), 4.72 (t, 1H, J = 9.5 Hz, H-2″), 4.24 (dd, 1H, J = 4.5, 12.5 Hz, H-6″a), 4.10 (dd, 1H, J = 2.0, 4.5, 10.0 Hz, H-5″), 3.98 (dd, 1H, J = 1.5, 12.0 Hz, H-6″b), 2.46 (s, 3H, 3″-CH_3), 2.05–1.90 (s, 12H, 4×CH_3CO); 13C NMR (125 MHz, DMSO-d_6): δ 177.2 (C-S), 170.1–169.3 (4×CH_3CO), 129.5 (CH=CH-N), 80.7 (C-1″), 70.9 (C-2″), 72.2 (C-3″), 67.8 (C-4″), 72.3 (C-5″), 61.7 (C-6″), 104.9 (C-4″), 165.1 (C-5″), 140.2 (C-1″), 122.6 (C-2″), 133.9 (C-3″), 129.9 (C-4″), 132.9 (C-5″), 125.6 (C-6″), 20.7–20.16 (4×CH_3CO), 20.7 (3″-CH_3); ESI–MS (+MS): m/z (%) 606.1 (M – H, 100); calc. for C_{25}H_{29}N_5O_{11}S = 607.16 Da.
cm⁻¹ 1750 (νC=O ester and sydnone), 3338, 3124 (νN-H), 1610 (νC-H=O), 1490, 1450 (νC-C), 1085 (νC=O), 1039, 1229 (νC=O ester); ¹H NMR (500 MHz, DMSO-d₆): δ 11.97 (s, 1H, NH-2), 7.70 (s, 1H, CH=N), 7.39 (t, 2H, J = 7.0 Hz, H-4"'), 7.61 (s, 1H, H-6"'), 6.33 (dd, 1H, J = 9.5 Hz, NH-4), 5.81 (m, 1H, H-1), 5.36 (t, 2H, J = 9.5 Hz, H-3"), 4.77 (m, 1H, H-2), 4.33 (t, 1H, J = 11.5 Hz, H-5), 4.09 (d, 1H, J = 6.0 Hz, H-6'a, H-6'b), 2.45–2.39 (s, 3H, 2"-CH₃), 2.39–2.09 (s, 12H, 4 × CH₂CO₂H), 1.89 (s, 3H, 3"-CH₃); ¹³C NMR (125 MHz, DMSO-d₆): δ 177.1 (C-S), 170–169.3 (4 × CH₂CO₂H), 165.6 (C-5"'), 139.0 (C-1"'), 133.7 (C-2"'), 133.6 (C-3"'), 132.5 (C-4"'), 128.5 (CH=N), 127.1 (C-6"'), 123.7 (C-5"'), 105.1 (C-4'), 80.6 (C-1), 72.1 (C-5), 71.7 (C-3'), 71.4 (C-2', 66.7 (C-4'), 61.6 (C-6'), 20.5–20.11 (4 × CH₂CO₂H), 13.2 (2"-CH₂), 19.7 (3"-CH₃); ESI-MS (+MS): m/z (%) 622.03 (M⁺, 87), 600.44 (5), 590.29 (10), 556.47 (8), 473.51 (10), 407.29 (10), 390.41 (6), 348.25 (12), 330.44 (6), 218.39 (12), 202.42 (40), 132.44 (8), 122.33 (10), 117.36 (100), 102.59 (38), 74.43 (25), 59.18 (53); calc. for C₂₈H₃₁N₂O₁₃S: 621.17 Da.

3-(2,4-Dimethylphenyl)-4-formylsydnone N-(2,3,4,6-tetra-O-acetyl-β-glucopyranosyl)thiosemicarbazone (4f)

Pale yellow crystals, mp 119–121 °C (from 96 % ethanol), Rₛ = 0.55; [α]D₂₀ +56.0 (c = 0.22, CHCl₃), FTIR (KBr): ν/cm⁻¹ 3476, 3334 (νN=H), 1756 (νC=O ester and sydnone), 1609 ν(CH=O), 1528 (νC=O), 1093 (νC=O), 1040, 1040 (νCOC ester); ¹H NMR (500 MHz, DMSO-d₆): δ 11.97 (s, 1H, NH-2), 7.81 (s, 1H, CH=N), 7.64 (t, 1H, J = 7.5 Hz, H-5"'), 7.47 (t, 1H, J = 2.0 Hz, H-6"'), 7.38 (dd, 1H, J = 1.0, 7.5 Hz, H-4"), 7.34 (dd, 1H, J = 2.0, 7.5 Hz, H-6"'), 7.18 (d, 1H, J = 9.5 Hz, NH-4), 5.88 (t, 1H, J = 9.5 Hz, H-1), 5.42 (t, 1H, J = 9.5 Hz, H-3), 5.00 (t, 1H, J = 9.5 Hz, H-4), 4.80 (t, 1H, J = 9.5 Hz, H-2'), 4.21 (dd, 1H, J = 5.0, 12.25 Hz, H-6'a), 4.10 (ddd, 1H, J = 2.0, 4.5, 10.0 Hz, H-5'), 3.99 (dd, 1H, J = 1.5, 12.25 Hz, H-6'b), 3.86 (s, 3H, 3"-OCH₃), 2.05–1.90 (s, 12H, 4 × CH₂CO₂H); ¹³C NMR (125 MHz, DMSO-d₆): δ 177.2 (C-S), 170.1–169.3 (4 × CH₂CO₂H), 164.8 (C-5"'), 160.0 (C-3"'), 134.8 (C-1"'), 131.0 (C-5"'), 129.7 (CH = N), 118.4 (C-6"'), 117.5 (C-4"'), 111.0 (C-5'), 105.1 (C-4'), 80.8 (C-1), 72.3 (C-5'), 72.3 (C-3), 71.0 (C-2'), 67.9 (C-4'), 61.8 (C-6), 55.8 (3"-OCH₃), 20.5–20.2 (4 × CH₂CO₂H); ESI-MS (+MS): m/z (%) 622.3 (M⁺, 100); calc. for C₂₉H₃₂N₂O₁₃S: 623.15 Da.

3-(4-Methoxyphenyl)-4-formylsydnone N-(2,3,4,6-tetra-O-acetyl-β-glucopyranosyl)thiosemicarbazone (4i)

Light yellow crystals, mp 160–162 °C (from 96 % ethanol), Rₛ = 0.58; [α]D₂₀ +56.0 (c = 0.26, CHCl₃), FTIR (KBr): ν/cm⁻¹ 3344, 3260 (νN=H), 1746 (νC=O ester and sydnone), 1599 ν(CH=O), 1549, 1505 (νC=O), 1093 (νC=O), 1223, 1043 (νCOC ester); ¹H NMR (500 MHz, DMSO-d₆): δ 12.02 (s, 1H, NH-2), 7.77 (s, 1H, CH=N), 7.74 (d, 2H, J = 8.75 Hz, H-3"'), 7.27 (d, 2H, J = 8.75 Hz, H-2"'), 6.75 (d, 1H, J = 10.0 Hz, NH-4), 5.86 (t, 1H, J = 9.5 Hz, H-1), 5.41 (t, 1H, J = 9.5 Hz, H-3), 5.12 (t, 1H, J = 9.75 Hz,
3-(4-Ethoxyphenyl)-4-formylsydnone N-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)thiosemicarbazone (4j)

Light yellow crystals, mp 159–161 °C (from 96% ethanol), \( R_f = 0.60; \alpha [\delta^2]_D +54.0 (c = 0.22, CHCl_3); \) FTIR (KBr): \( \nu (\text{cm}^{-1}) = 3214, 3202 (\text{ν NH}), 1737 (\nu C=O \text{ ester}), 1478, 1520 (\nu C=O \text{ sydnone}); \) \( \delta 177.2 (C=S), 170.1–169.3 (4 \times CH_2CO), 165.5 (C-5'), 161.5 (C-4'), 129.2 (CH=N), 126.9 (C-1'), 127.0 (C-3', C-6'), 115.1 (C-2', C-6'), 104.6 (C-4'), 80.4 (C-1'), 72.2 (C-5), 72.1 (C-2), 67.5 (C-4), 61.6 (C-6), 55.8 (4''-OCH_3), 20.5–20.1 (4 \times CH_2CO); \) ESI–MS (+MS): \( m/z (%) = 624.01 (M + H, 100), 556.02 (7), 407.11 (15), 391.21 (5), 348.17 (8), 331.25 (5), 204.21 (75), 122.42 (8), 117.15 (80), 102.25 (95), 84.25 (12), 74.18 (50), 59.08 (67); \) calc. for \( C_{23}H_{23}N_5O_{12}S = 623.15 \) Da.

3-(4-Bromophenyl)-4-formylsydnone N-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)thiosemicarbazone (4I)

Dark yellow crystals, mp 157–159 °C (from 96% ethanol), \( R_f = 0.53; \alpha [\delta^2]_D +57.3 (c = 0.26, CHCl_3); \) FTIR (KBr): \( \nu (\text{cm}^{-1}) = 1746 (\nu C=O \text{ ester and sydnone}), 3083, 3289 (\nu (\text{cm}^{-1}) = 1478, 1520 (\nu C=O \text{ sydnone}), 1036, 1222 (\nu C=O \text{ ester}); \) \( \nu (\text{cm}^{-1}) = 177.4 (C=S), 170.1–169.3 (4 \times CH_2CO), 165.5 (C-5'), 161.5 (C-4'), 129.2 (CH=N), 126.9 (C-1'), 115.4 (C-2', C-6'), 104.6 (C-4'), 80.5 (C-1'), 72.3 (C-5), 72.2 (C-6'), 67.4 (C-4''), 66.1 (C-6'), 20.5–20.2 (4 \times CH_2CO); \) ESI–MS (+MS): \( m/z (%) = 624.01 (M + H, 100), 556.02 (7), 407.11 (15), 391.21 (5), 348.17 (8), 331.25 (5), 204.21 (75), 122.42 (8), 117.15 (80), 102.25 (95), 84.25 (12), 74.18 (50), 59.08 (67); \) calc. for \( C_{24}H_{26}BrN_5O_{11}S = 661.4 \) Da.
3.0 Hz, H-6′b), 2.06–1.90 (s, 12H, 4 × CH$_3$(CO)); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ 177.3 (C-S), 170.0–169.2 (4 × CH$_3$(CO)), 165.1 (C-5′), 138.8 (C-1′), 132.5 (C-3′, C-5′), 129.8 (CH=N), 127.4 (C-2′, C-6′), 119.3 (C-4′), 104.9 (C-4′), 80.7 (C-1′), 72.5 (C-5′), 72.0 (C-2′), 70.7 (C-3′), 68.0 (C-4′), 61.7 (C-6′), 20.6–20.1 (4 × CH$_3$(CO)); ESI–MS (−MS): $m/z$ (%) 717.7 (M−2H, 100); calc. for C$_{24}$H$_{30}$N$_5$O$_{11}$S = 719.04 Da.

3-(2-Methyl-5-chlorophenyl)-4-formylsydnone N-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)thiosemicarbazone (4n)

Dark yellow crystals, mp 122–123°C (from 96 % ethanol), $R_f$ = 0.53; [α]$^D_{25} = +$43.2 ($c = 0.22$, CHCl$_3$); FTIR (KBr): v/$\nu$/cm$^{-1}$ 1754 (v$_{\text{C}=\text{O}}$ ester and sydnone), 3341, 3249 (v$_{\text{NH}}$), 1524, 1450 (v$_{\text{CO}}$), 1040, 1227 (v$_{\text{CO}}$ ester); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 12.20 (s, 1H, H, Hz, N-2), 8.03 (d, 1H, $J = 9.0$ Hz, H-4), 7.56 (s, 1H, H, CH = N), 7.70–7.47 (m, 3H, H-3′, H-4′, H-6′), 7.70–7.47 (m, 2H, H-5′, H-6′), 5.97–5.90 (m, 1H, H-1), 5.29 (t, 1H, $J = 9.7$ Hz, H-3), 5.12 (t, 1H, $J = 9.7$ Hz, H-4), 5.08–5.02 (m, 1H, H-2), 4.30 (dd, 1H, $J = 12.5, 4.5$ Hz, H-5), 4.10–4.07 (m, 1H, H-6′b), 3.84–3.80 (m, 1H, H-6′a), 2.21–1.96 (s, 12H, 4 × CH$_3$); 13C NMR (125 MHz, DMSO-$d_6$): $\delta$ 179.6 (C = S), 170.9–169.4 (4 × CH$_3$(CO)), 166.4 (C-5′), 139.8 (C-1′), 131.9 (C-2′), 132.4 (C-3′), 126.4 (C-4′), 123.9 (C-5′), 129.9 (CH = N), 127.3 (C-6′), 104.3 (C-4′), 82.1 (C-1′), 82.0 (C-2′), 74.0 (C-5′), 70.0 (C-3′), 68.5 (C-4′), 62.0 (C-6′), 20.8–20.4 (4 × CH$_3$(CO)), 16.6 (2′-CH$_3$); ESI–MS (−MS): $m/z$ (%) 642.02/644.03 (M−H, 100); calc. for C$_{25}$H$_{28}$S$_2$Cl$_2$N$_5$O$_{11}$S, 641.12/643.11 Da.

Antimicrobial screening

Antibacterial activity

The synthesized compounds 4a–o were screened in vitro for their antibacterial activities against bacteria namely Staphylococcus epidermidis (ATCC 12228) and Bacillus subtilis (ATCC 6633) as Gram positive bacteria, Escherichia coli (ATCC 25922) and Salmonella enterica (ATCC 15442) as Gram negative bacteria, were tested by using agar well diffusion (cup–plate) method [32]. The sterilized nutrient agar medium was distributed 100 mL each and allowed to cool to room temperature. The 24 h old Mueller–Hinton broth cultures of test bacteria were swabbed on sterile Mueller–Hinton agar plates in sterilized Petri dishes using sterile cotton swab followed by punching wells of 6 mm with the help of sterile cork borer. The standard drug (ciprofloxacin, 1 mg/mL of sterile distilled water), compounds 4a–o (500 μg/mL in 10 % DMSO, prepared by dissolving 2.5 mg of substance in 5 mL of 10 % DMSO solution in water), and control sample (10 % solution of DMSO in water) were added to the respectively labelled 6 mm diameter wells. The plates were allowed to stand for 30 min and then incubated at 37°C for 72 h in upright position. When growth inhibition diameter zones were developed surrounding each cup, their diameter in mm was measured and compared with that of ciprofloxacin (Table 3).

The antibacterial activities against above bacteria of all the synthesized derivatives also were evaluated in vitro by serial tube dilution method [33]. The compounds and standard drug ciprofloxacin were dissolved in DMSO to give a concentration of 5 μg/mL (stock solution). A set of test tubes of capacity 5 mL was washed, cleaned and dried completely. Double strength nutrient broth was used as a growth/culture media for all bacteria. The culture media was made by dissolving 15 g of nutrient broth No. 2 in 1 L of distilled water. Approximately 1 mL of this culture media was prepared and transferred to each test tube by micropipette and capped with non-adsorbent cotton plugs. A set of test tubes containing 1 mL culture media was sterilized in an autoclave at 15 psi pressure at 121°C for 20 min. Sub-culturing of bacteria was done by transferring a loopful of particular bacterial strain from standard bacterial agar slant to 10 mL sterilized nutrient broth.
broth aseptically in a laminar air flow cabinet. It was then incubated for a period of 24 h at 37 °C in an incubator. After 24 h incubation the bacterial stain suspension was prepared by aseptically inoculating 0.2 mL of revived bacterial colony into 100 mL of 0.9 % m/v saline. The study involved a series of five assay tubes for each compound against each strain. A stock solution of each test compound at concentration 5 μg/mL was serially diluted in series of 5 assay test tubes (containing 1 mL nutrient broth) to give concentration of 2.5, 1.25, 0.625, 0.313 and 0.156 μg/mL. Then, 0.1 mL of normal saline suspension of revived bacteria was added to each test tube. The inoculated tubes were incubated at 37 °C for 24 h. The MIC (minimum inhibitory concentration) values were determined by subsequently checking for the absence of visible turbidity (Table 4).

Experiments were repeated three times, and the results were expressed as average values.

**Antifungal activity**
The synthesized compounds 4a–o were screened for their antifungal activity against three fungal strains [34], namely *Aspergillus niger* 439, *Candida albicans* ATCC 7754, *Fusarium oxysporum* M42, at the concentration levels of 500 μg/mL (Table 4) by agar well diffusion (cup-plate) method, using nystatin as the standard and control sample is a 10 % solution of DMSO in water. The sterilized potato dextrose agar medium incubated at 30 °C for 48 h, then the subculture of fungus were added, and shaken thoroughly to ensure uniform distribution. After that, this was poured into previously sterilized and labelled Petri dishes and allowed to solidify. Two cups were filled with 0.1 mL of two test dilutions and the other two cups with respective concentrations of standard dilutions. The plates were left as it is for 2–3 h for diffusion and then they were kept for 24 h at 37 °C for incubation. Then the diameter of the zones of growth inhibition was measured and compared with that of standard (nystatin).

Similarly, the antifungal activities against above fungi of all thiosemicarbazone derivatives also were evaluated in vitro by serial tube dilution method [33, 34]. Experiments were repeated three times, and the results were expressed as average values.

**Abbreviations**

OAc: acetyl; DMF: N,N-dimethylformamide; DMSO: dimethyl sulfoxide; dMe: dimethyl; FTIR: Fourier-transformed infrared spectroscopy; MS: mass spectrometry; NMR: nuclear magnetic resonance spectroscopy; ESI: electron-spray ionization.

**Authors’ contributions**

NDT developed the synthesis, NDT, HDT, VTD, PMT and NVQ undertook synthesis, purification and analytical studies; carried out the acquisition of data, analysis and interpretation of data collected and involved in drafting of manuscript, revision of draft for important intellectual content and give final approval of the version to be published. All authors read and approved the final manuscript.

**Author details**

1 Faculty of Chemistry, VNU University of Science, 19 Le Thanh Tong, Hoan Kiem, Ha Noi, Vietnam. 2 Faculty of Chemistry, Hanoi University of Industry, Minh Khai, Tu Liem, Ha Noi, Vietnam. 3 Faculty of Chemistry, Vinh University, 182 Le Duan, Vinh, Nghe An, Vietnam.

**Acknowledgements**

Financial support for this work was provided by Vietnam’s National Foundation for Science and Technology Development (NAFOSTED), code 104.01–2013.26.

**Competing interests**

The authors declare that they have no competing interests.

**Received:** 8 July 2015  **Accepted:** 12 October 2015  **Published online:** 19 October 2015

**References**

1. Browne DL, Harity JPA (2010) Recent developments in the chemistry of sydones. Tetrahedron 66:553–568
2. Satyanarayana K, Rao MNA (1995) Synthesis and antiinflammatory, analgesic, and antiarthritic testing of 4-[1-oxo-(3-substituted aryl)-2-prophenyl]-3-phenylsydones and of 3-[1-(3-substituted aryl)-1-oxo-2-prophenyl]phenylsydones. J Pharm Sci 84:263–266
3. Kavali JR, Badami BV (2000) 1,5-Benzodiazepine derivatives of 3-arylsydnones: synthesis and antimicrobial activity of 3-aryl-4-[2'-aryl-2'-A',6',7'-tetrahydro-1'H,1'-5'-benzodiazepine-4'-yl]sydones. II Farmaco 55:406–409
4. Shih M-H, Su Y-S, Wu C (2007) Syntheses of aromatic substituted hydrazo-thiazole derivatives to clarify structural characterization and antioxidant activity between 3-arylsydnoril and aryl substituted hydrazino-thiazoles. Chem Pharm Bull 55:1126–1135
5. Hegde JC, Girisha KS, Adhikari A, Kalluraya B (2008) Synthesis and antimicrobial activities of a new series of 4-[4'-[4-aminom-4'-oxo-b'-substituted benzyl]-5'-dihydro-1',2',4'-triazin-3-yl]-mercaptoacetyl-3-arylsydnones. Eur J Med Chem 43:2831–2834
6. Dilworth JR, Hueting R (2012) Metal complexes of thiosemicarbazones for imaging and therapy. Inorg Chim Acta 389:1–15
7. Hassan AA, Shawky AM, Shehata HS (2012) Chemistry and heterocyclization of thiosemicarbazones. J Heterocycl Chem 49:211–35
8. Casas JS, Garcia-Tasende MS, Sordo J (2000) Main group metal complexes of semicarbazones and thiosemicarbazones. A structural review. Coord Chem Rev 209:197–261
9. Tarasconi P, Capacchi S, Pelosi G, Cornia M, Albertini R, Bonati A, Dall’Aglio PP, Lunghi P, Pinelli S (2000) Synthesis, spectroscopic characterization and biological properties of new natural aldehydes thiosemicarbazones. Bioorg Med Chem 8:157–162
10. Alho MAM, d’Accorso NB (2000) Behavior of free sugar thiosemicarbazones toward heterocyclization reactions. Carbohydr Res 328:481–488
11. Gyurcsik B, Nagy L (2000) Carbohydrates as ligands: coordination equilibria and structure of the metal complexes. Coord Chem Rev 203:81–149
12. Iskander MF, Shaban MAE, El-Badry SM (2003) Sugar hydrazine-metal complexes: transition- and non-transition metal complexes of monosaccharide S-alkylhydrazonecarbodithioates and dehydro-L-ascorbic acid bis(S-alkylhydrazonecarbodithioates). Carbohydr Res 338:2341–2347
13. Ghosh S, Misra AK, Bhatia G, Khan MM, Khanna AK (2009) Syntheses and evaluation of glucosyl aryl thiosemicarbazides and glucosyl thiosemicarbazone derivatives as antioxidant and anti-dyslipidemic. Bioorg Med Chem 18:7911–7922
14. Alexacou K-M, Tzouhiu A-C, Chrysa ED, Charavgi M-D, Kostas ID, Zographos SE, Oikonomakos NG, Leonidas DD (2010) The binding of β-gluco- pyranosyl-thiosemicarbazone derivatives to glycogen phosphorylase: a new class of inhibitors. Bioorg Med Chem 18:7911–7922
15. Nguyen DT, Le TH, Bui TTT (2013) Antioxidant activities of thiosemicarbazones from substituted benzaldehydes and...
16. van de Kamp F-P, Micheell F (1956) Über α-glucose-derivat von thiosemicarbazonen und ihre biologische Wirksamkeit. Chem Ber 89:133–140
17. Bogdán R, Somogyi L, Sallágy L, Györgydeák Z (1967) N-Glykosyl-Derivate: Teil XIII. Der nachtragliche Ausbau des glykokons. Synthese von N-glykosyl-derivaten des 2-amino-thiazols, 2-amino-1,3,4-thiadiazols und 5-amino-1,2,3,4-thiatriazols. Carbohydrat Res 5:320–328
18. Wójtowicz M, Gmernicka-Haftek C, Wieniawski W (1975) Synthesis of 4-β-d-glucopyranosyl-3-thiosemicarbazones of some aromatic aldehydes. Acta Pol Pharm 32:49–52
19. Tashpulatov AA, Ismailov N (1988) Synthesis and some reactions of glycosyl isocyanate. Zh Org Khim 24:1893–1897 (Chem Abstr 1989, 111:39684m)
20. Shih MH, Ke FY (2004) Syntheses and evaluation of antioxidant activity of sydnonyl substituted thiazolidinone and thiazoline derivatives. Bioorg Med Chem 12:4633–4643
21. Yang B, Zhang SS, Li HX (2006) Synthesis and characterization of novel thiosemicarbazones bearing sugar moieties. Chem Res Chin Univ 22:738–741
22. Garnak BK, Behera RK (1988) Synthesis, antimicrobial, antifungal activities of some 2-arylimino-4-tetra-O-acetyl-β-d-glucopyranosyl-4-thiazolidinones. Indian J Chem 27B:1157–1158
23. Tenchiu AC, Kostas ID, Kovala-Demertzis D, Terzis A (2009) Synthesis and characterization of new aromatic aldehyde/ketone 4-(β-d-glucopyranosyl)thiosemicarbazones. Carbohydr Res 344:1352–1364
24. Thanh ND, Giang NTK, Hoai LT (2010) Microwave-assisted synthesis of acetophenone (per-O-acetylated-β-d-glucopyranosyl)thiosemicarbazones. E-J Chem 7:899–907
25. Corsaro A, Chiacchio U, Pistorà V, Romeo G (2006) Microwave-assisted chemistry of carbohydrates. In: Loupy A (ed) Microwave in organic synthesis, vol 1, 2nd edn. WILEY-VCH Verlag, Weinheim, pp 579–594
26. Thoman CJ and Voaden DJ (1973) 3-Phenylsydnone. In: Organic syntheses, coll. Wiley and sons, New York, vol. 5, pp 962–965
27. Azafar D, Borsa HG, Zolfigol M-A, Tajbaksh M (2006) Microwave-assisted synthesis of N-arylglycosyl derivatives of 4-formyl-3-arylsydnone. J Chin Chem Soc 53:29–37
28. Šarkanj B, Molnar M, Čačić M, Gille L (2013) 4-Methyl-7-hydroxycoumarin antifungal and antioxidant activity enhancement by substitution with thiosemicarbazide and thiazolidinone moieties. Food Chem 139:488–495

Open access provides opportunities to our colleagues in other parts of the globe, by allowing anyone to view the content free of charge.*

* W. Jeffery Hurst, The Hershey Company.