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Research paper

Synthesis and evaluation of *in vivo* antioxidant, *in vitro* antibacterial, MRSA and antifungal activity of novel substituted isatin *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazones

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ABSTRACT

Some new isatin *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazones **4a-t** with different substituents at 1-, 5- and 7-positions of isatin ring have been synthesized by reaction of *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazide **2** with corresponding isatins **3a-t**. Compounds **4a-t** were evaluated *in vivo* for antioxidant activity and *in vitro* for anti-microorganism activities. The MIC values were found for Gram positive bacteria (MIC = 1.56–6.25 μM), for Gram negative bacteria (MIC = 12.5 μM), and for fungi *Aspergillus niger* (MIC = 3.12–12.5 μM), *Fusarium oxysporum* (MIC = 6.25–12.5 μM) and *Saccharomyces cerevisiae* (MIC = 6.25–12.5 μM). Regarding the antioxidant activity, the SOD, GHS-Px and catalase activities of **4c-i** and **4m-r** were MIC = 10.57–10.85, 0.27–0.93 and 345.45–399.75 unit/mg protein, respectively. Compounds **4e-h** had MIC values of 0.78, 1.56, and 3.12 μM for three clinical MRSA isolates. Compound **4e** showed the selective cytotoxic effects against some cancer (LU-1, HepG2, MCF7, P338, SW480, KB) cell lines and normal fibroblast cell line NIH/3T3.

1. Introduction

The ring of isatin, 1*H*-indole-2,3-dione, is a heterocyclic compound of significant importance in organic chemistry and also in medicinal chemistry. Thiosemicarbazone molecules bearing isatin moiety have diverse types of biological activity, including antiviral, antibacterial, anticancer, anticonvulsant and antidepressant activity [1,2]. On the other hand, isatin-β-thiosemicarbazone and *N*-methylisatin-β-thiosemicarbazone (methisazone, marboran) are extensively studied thiosemicarbazones that demonstrate an inhibitory effect against the replication of pox-viruses [3,4]. Methisazone has been described as being used in prophylaxis since at least 1965 [5]. Methisazone (See Fig. 1), which works by inhibiting mRNA and protein synthesis, especially in pox viruses, was one of the first clinically used synthetic antiviral agent for treatment of smallpox [6], also of other groups of viruses, such as adenovirus, herpesvirus, picornavirus, reovirus, arbovirus, myxo- and paramyxovirus, and retrovirus [7]. Some isatin derivatives also have antifungal and anti-mycotoxin activities [8].

It is known that methicillin-resistant *Staphylococcus aureus* (MRSA) causes serious public health problems. MRSA is defined as difficult-to-treat strain of Staphylococcus aureus which resists to almost all antibiotics [9]. However, infections caused by MTSA have been a major threat to public health in hospitals and the community during the past decade. Some substituted isatin-β-thiosemicarbazones (IBTs) of substituted benzaldehydes have been synthesized and shown the inhibit the growth of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) [10].

On the other hand, the chemistry of monosaccharide thiosemicarbazides is interesting because these derivatives could be

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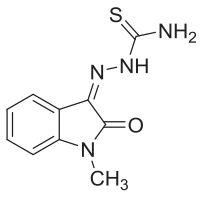
used as versatile intermediates for preparing various compounds (e.g., heterocycles [11]) as well as be used for making complex formation of several metallic ions [12]. Thiosemicarbazones exhibit various biological activities, such as antituberculosis [11], antimicrobial [13], antibacterial [14], anticancer [15], anticonvulsant [16], antidiabetic [17], antifungal [18], antitumor [19], cytotoxic activities [20], and some of them were used as antioxidant [22–24], antidyslipidemic [24] agents.

Thiosemicarbazone derivatives containing monosaccharide moiety have shown the remarkable anti-microorganisms and antioxidant activity both *in vivo* and *in vitro* [23,24]. Some articles in the past reported about the synthesis of substituted aromatic aldehyde/ketone *N*-(per-*O*-acetylated glucopyranosyl)thiosemicarbazones [11,23,24–27]. These compounds have been synthesized by reaction of *N*-(per-*O*-acetyl-β-D-glycosyl) thiosemicarbazides with the corresponding carbonyl compounds, but the thiosemicarbazones containing simultaneously monosaccharide and isatin moieties have not been reported yet. Continuing our previous studies on synthesis and reactivity of *N*-(per-*O*-acetyl-D-glycopyranosyl)thiosemicarbazides [22,26], in the present paper we have reported a study for the synthesis and spectral characterization of a series of substituted isatin *N*-(tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazones using microwave-assisted method. The biological activities of synthesized compounds have been estimated.

2. Results and discussion

2.1. Chemistry

Unsubstituted *N*-(tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazide, in particular, and other thiosemicarbazides linked to carbohydrate (*N*-glycosylthiosemicarbazides), to the best of our knowledge, could only be prepared from corresponding isothiocyanate. It's known that the conversion of *N*-(tetra-*O*-acetyl-β-D-glucopyranosyl)isothiocyanate into corresponding thiosemicarbazide usually could take place in aprotic solvent (such as dichloromethane [24], dioxane [27]) due to the higher reactivity towards water of peracetated glucopyranosyl isothiocyanate in comparing with aromatic isocyanate [21,27,28]. We found that a protic solvent, such as absolute ethanol, could be used for this reaction, but the reaction must be performed at a low temperature (<10 °C) to prevent the decomposition of isothiocyanate derivative [27]. The use of absolute ethanol as solvent allowed to avoid the reaction of this isocyanate to water, while 96% ethanol isn't a good solvent for that reaction as above mentioned. Reaction outcome was improved by using an 85% solution of hydrazine solution [22] instead of 100% hydrazine hydrate [24] by dropping into the solution of this isocyanate. In this way, *N*-(tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazide was synthesized from corresponding isothiocyanate derivative by reaction with 85% hydrazine hydrate (Scheme 1) by similar method for synthesis of *N*-(tetra-*O*-acetyl-β-D-galactopyranosyl)thiosemicarbazide using 85% hydrazine hydrate in dichloromethane [22]. In case of the latter, thiosemicarbazide formed in reaction is well dissolved in dichloromethane, then the solvent must be removed completely and 96% ethanol must be added to isolate the product. The advantage of using absolute ethanol as a reaction solvent is that the thiosemicarbazide product is precipitated when formed, isolating the product more easily, just by the filtering the precipitate. The high yield of 95% is achieved.



Methisazone

Fig. 1. Methisazone.

The condensation reaction of substituted isatins **3a-l** and *N*-alkylisatins **3m-t** with *N*-(tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazide was carried out in absolute ethanol in the presence of glacial acetic acid as catalyst under microwave irradiation (Scheme 1) [22,26]. Reaction solvent is absolute ethanol, the solvent 5 volume is usually from 5 mL to 10 mL depending on solubility of substituted isatins or *N*-alkylisatins, for example, ethanol volume is 5 mL for isatin **3a-l**, while for *N*-alkylisatins **3m-t** is 10 mL due to lower solubility of the latter. The reaction mixture in suspension type became a clear solution after irradiating for several minutes, and at the end of reaction process, the precipitate appeared. Reaction products usually separated as yellow or orange solids after cooling to room temperature. The products are soluble in almost common organic solvents, such as toluene, DMF, acetone, and ethyl acetate. The reaction yields were 70–88% for substituted isatin series **4a-l** and 74–89% for *N*-alkylisatin series **4m-t**. The synthesized products were characterized by elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral data.

2.2. The in vitro antimicrobial activity evaluation

The antimicrobial activities *in vitro* against *Staphylococcus aureus* (ATCC 11632), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 4352), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 11774), and *Enterobacter aerogenes* (ATCC 13048) were screened. A series of methicillin resistant *Staphylococcus aureus* (MRSA) strains was isolated from 198th hospital of Ministry of Public Security (in Ha Noi), numbered MRSA198-1, MRSA198-2 and MRSA198-3, was also included in these tests. The compounds **4a-t** were also evaluated for their antifungal activity *in vitro* against *Aspergillus niger* (439), *Candida albicans* (ATCC 7754), *Fusarium oxysporum* (M42), and *Saccharomyces cerevisiae* (SH20).

The results of antibacterial activity study for thiosemicarbazones **4a-t** showed that the new molecule exhibited antibacterial activity against the studied bacteria at low and high concentrations (Table 1). It has been observed that all the compounds tested showed mild to moderate activity against tested bacteria in comparison with the MIC values of the reference compound (ciprofloxacin, MIC = 3.12 and 1.56 μM, respectively). For Gram positive bacteria, *B. subtilis*, *Staphylococcus aureus* and *S. epidermidis*, thiosemicarbazones **4c-i** had the higher ability to inhibit to these bacteria with MIC values of 1.56–6.25 μM, respectively. The order of inhibitory activity against these bacteria was **4e** > **4c**,**4d**,**4f** > **4g**,**4h**,**4i** > **4j**,**4k** > **4l** > **4a**,**4b**,**4m**,**4n**,**4o**,**4p**,**4q**,**4s** > **4r**,**4t**. The compound **4e** with two bromine atoms at 5th and 7th positions is most active amongst these thiosemicarbazones. The compounds **4j-l** bearing alkyl group at 5th or 7th positions had medium activity against these bacteria (MIC = 12.5–25 μM). The compounds **4a**,**b**,**m-t** bearing alkyl groups at 1st position had lowest activity against these bacteria (MIC = 100–200 μM). For Gram negative bacteria, *Enterobacter* spp., *E. coli*, *P. aeruginosa* and *K. pneumoniae*, thiosemicarbazones **4m-q** having alkyl substituents at 1st position exhibited higher activity at all (MIC = 12.5 μM), whereas compounds **4c**,**4d**,**4j**,**4k** bearing alkyl group at 5th or 7th positions were less active (MIC = 25 μM). The substitutions of allylic, benzylic or phenethylic groups at 1st position in compounds **4r-t** decreased the inhibitory ability of these derivatives; in these