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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

N-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazones of 6-alkoxy-2-oxo-2*H*-chromene-4-carbaldehydes: synthesis, evaluation of their antibacterial, anti-MRSA, antifungal activity, and docking study

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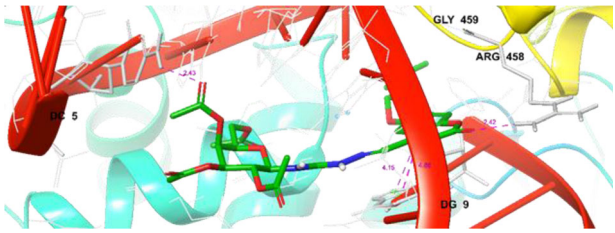
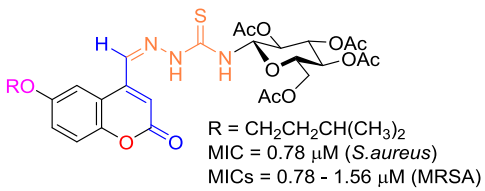
Abstract

Reaction of 6-alkoxy-2-oxo-2*H*-chromen-4-carbaldehydes with *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazide yielded corresponding thiosemicarbazones having 2*H*-chromen-2-one ring. In vitro evaluations showed that these 2*H*-chromen-2-one compounds exhibited remarkable antibacterial and antifungal activities against some typical bacteria and fungi. Representative compounds with MIC values of 0.78 − 1.56 μg/mL were **6c**, **6g** (against *S. aureus*), **6a**, **6f** (against *S. epidermidis*) (Gram-positive bacterial strains), **6e**, **6g** (against *E. coli*), **6b**, **6e** (against *K. pneumoniae*), and **6d–f** (against *S. typhimurium*) (Gram-negative bacterial strains). Almost all thiosemicarbazones **6a–g** had no activity against Gram-positive bacterial strain *B. subtilis* at these MIC values. Some compounds had strong inhibitory activity against several bacteria, such as **6b** (for *K. pneumoniae* and *S. typhimurium*), **6d**, **6e** (for *E. coli*, *K. pneumoniae*, and *S. typhimurium*), **6f** (for *S. aureus*, *E. coli*, and *S. typhimurium*), and **6g** (for *B. subtilis*, *S. aureus*, *E. coli*, and *K. pneumoniae*). Some compounds had remarkable inhibitory activity against three clinical MRSA isolates with MIC values of 0.78–6.25 μg/mL. Docking study showed that compound **6g** is compatible with the active site of *S. aureus* DNA gyrase 2XCT, which suggested that the tested compounds inhibited the synthesis of this enzyme.

Graphical Abstract





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Keywords 6-Alkoxy-2-oxo-2*H*-chromene-4-carbaldehydes · Antibacterial · Antifungal · Anti-MRSA · Thiosemicarbazones · Molecular docking

Introduction

Formyl functional group that presented in 2*H*-chromen-2-one ring (coumarin ring) could contribute to extend the reactivity of this ring. Various compounds that carry this ring could be formed based on the properties of formyl functional group, such as thiosemicarbazones [1–8], 2*H*-chromen-2-one substituted benzothiazole derivatives [9], Schiff's bases of 2*H*-chromen-2-one [7, 10], etc. The formyl group could be attached to benzene ring or to 2*H*-pyran-2-one ring at any position of those moieties of coumarin ring [11–16]. Coumarin (2*H*-chromen-2-one), a compound of cinnamon, was a family of benzopyrone (1,2-benzopyrone or 2*H*-1-benzopyran-2-ones) that widely distributed in the nature in many plants [17, 18]. Coumarin itself and its derivatives possessed various pharmacological activities, such as antibacterial [19], antioxidant [20], anti-inflammatory [21], anticancer [22], antifungal [23] activity, etc. Nowadays these compounds are still of interest to the scientific due to these biological effects [24, 25]. For instances, compound **A** (Fig. 1) exhibited potent anticancer activity with IC_{50} value of 5.18 mM against KB cell lines [26]. Compound **B** presented the highest oxygen radical absorbance capacity value of 14.1, good scavenging capacity, low cytotoxicity, and high cytoprotecting values (almost 100% cell viability at 20 µg/mL) [27]. Hydroxycoumarin **C** was used as selective MAO-B inhibitor with $IC_{50} = 2.79 \pm 0.19$ µg/mL [28].

Some study results demonstrated that the action of the coumarin derivative was against the structure of the fungal cell wall [29, 30]. Coumarin derivatives inhibited cytochrome P450 lanosterol 14α-demethylase (CYP51) [31]. Molecular docking studies showed that some most active coumarin compounds against *Staphylococcus aureus* tyrosyl-*t*RNA synthetase and topoisomerase II DNA gyrase [32]. Maxwell found that three glycoside compounds derived from different *Streptomyces* species with coumarin structure, including novobiocin (**D**, Fig. 1, also known as albamycin or cathomycin, an aminocoumarin antibiotic), clorobiocin (**E**), and coumermycin A1, displayed antibiotic activity as potent inhibitors of DNA topoisomerase type II (DNA gyrase) [33]. Kayser and Kolodziej found that both lipophilic characteristics as well as a planar structure were necessary for high antibacterial effects [34]. Sardari et al. indicated that 6- and 7-hydroxyl groups in coumarin ring played an important role for antifungal and antibacterial activity [35]. In fact, coumarins with 6-, 7-, or 8-hydroxyl/alkoxyl moiety had antibacterial effect against a broad spectrum of bacteria [18, 19, 36].

Thiosemicarbazone derivatives containing monosaccharide moiety had synthesized due to their remarkable anti-microorganisms and antioxidant activity both in vivo and in vitro [1, 3, 4, 6, 37, 38], including antibacterial [2, 37], antifungal [37], antioxidant [6], antidyslipidemic [3], antituberculosis [1], and anticancer [38] activities. Figure 2 displayed some examples of bioactive thiosemicarbazones-bearing sugar moiety. Sugar-based thiosemicarbazone of peracetylated glucoside (compound **F**) was discovered to be a potent Rhodesian (major cysteine protease from *Trypanosoma brucei*) inhibitor ($IC_{50} = 1.2$ µg/mL) [39]. Thiosemicarbazones **G** containing simultaneously monosaccharide and isatin moieties exhibited in vitro antibacterial and in vivo antioxidant activities. When R′=Br, R=H the compound showed selective cytotoxic effects against some cancer (LU-1, HepG2, MCF7, P338,

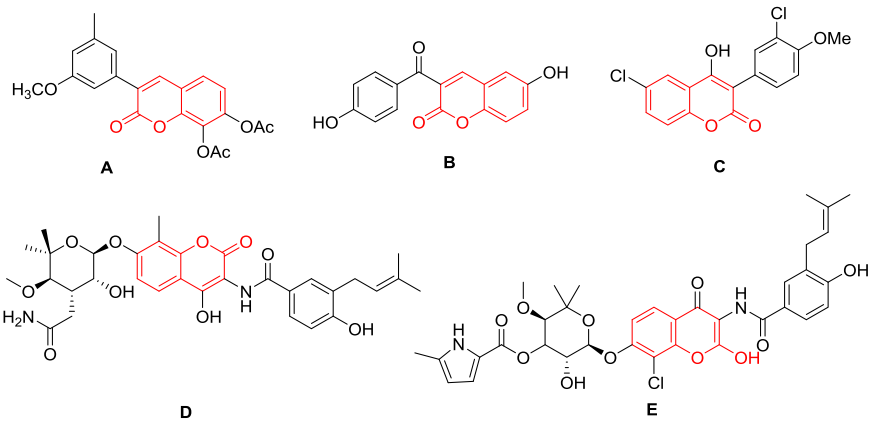


Fig. 1 Some bioactive and typical clinically well-known drugs or naturally occurring compounds containing coumarin ring

SW480, KB) cell lines and normal fibroblast cell line NIH/3T3. This compound had good inhibitory activity with minimum inhibitory concentration (MIC) of 1.56 µg/mL against *Bacillus subtilis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* [37].

Based on above-mentioned literatures, it is possible to see that the hybridization between coumarin ring and monosaccharide moiety could bring new activity or enhance the inherent activity of coumarin rings. In this article we reported the design and the synthesis of some 6-alkoxy-2-oxo-2*H*-chromene-4-carbaldehydes thiosemicarbazones-bearing D-glucose (Schemes 1 and 2). The inhibitory activity of these compounds against some typical bacterial and fungal strains was evaluated and docking studies were performed on the most potent compounds to investigate their binding mode with the active site of the representative enzyme.

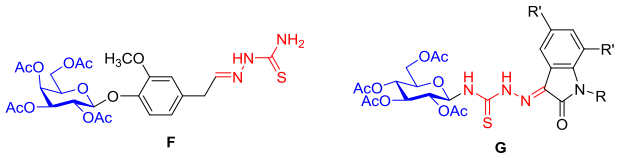
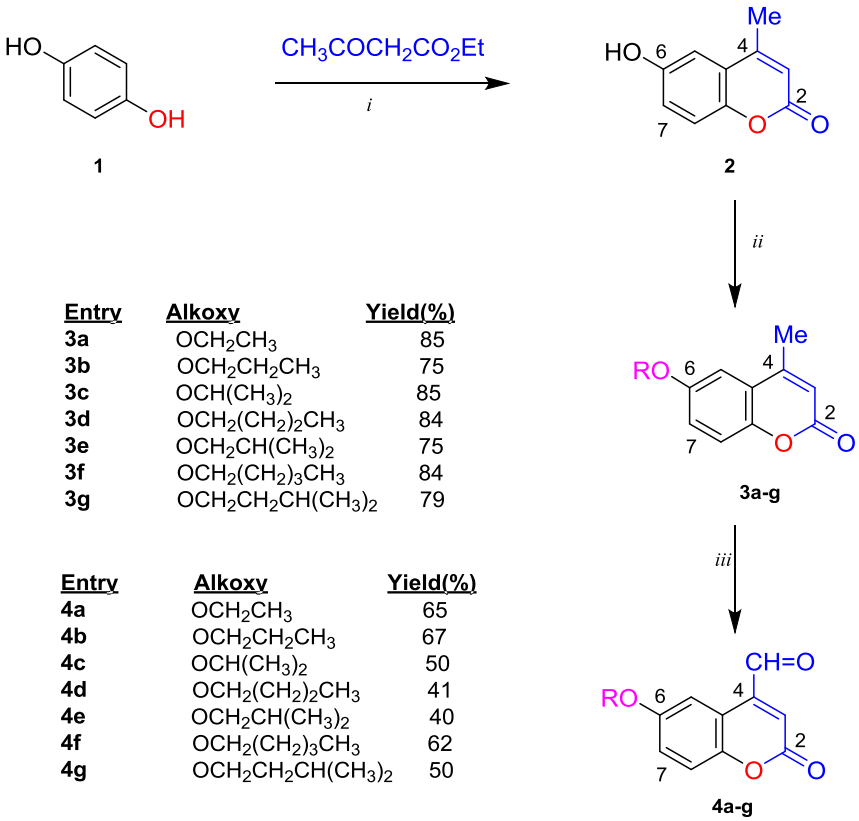


Fig. 2 Some bioactive thiosemicarbazones-bearing sugar moiety

Results and discussion

Chemistry

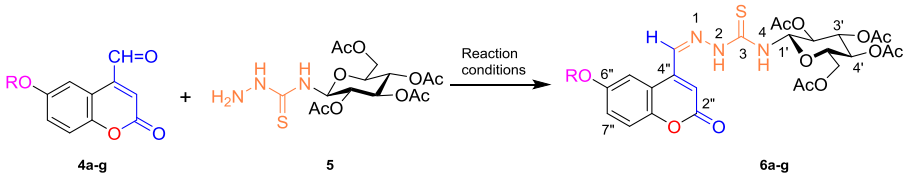
We have provided the synthetic pathway of 6-alkoxy-2*H*-chromen-2-ones (**3a–g**) that prepared from hydroquinone **2** (Scheme 1). These hydroxy derivatives were readily obtained from corresponding dihydroxy benzene (hydroquinone and resorcinol); the formers were transformed into ether by using Williamson ether synthesis, followed by oxidation reaction when using selenium dioxide as an oxidant. Almost all obtained 6-alkoxy-2-oxo-2*H*-chromen-2-ones in this study are new compounds. Yields of these ethers were 75–85%. The IR spectra of compounds **3a–g** displayed characteristic absorption bands at $\nu =$ 1737–1708 cm^{-1} (C=O lactone), 1293–1242 cm^{-1} and 1170–1142 cm^{-1} (C–O–C group in lactone and ether functional groups). The ^{1}H NMR spectra showed chemical shifts at $\delta = 7.66–6.17$ ppm (protons on 2*H*-chromen-2-one ring), 4.03–0.88 ppm (protons in the alkoxy group). Methylene group attached to oxygen atom had signal at about $\delta = 4.07–4.00$ ppm. The ^{13}C NMR spectra displayed resonance signals of carbon atoms in molecules, for examples, at $\delta = 160.6 - 101.1$ ppm (carbon atoms in the 2*H*-chromen-2-one component, except C=O lactone atom), 162.2–159.9 ppm (carbon atom in C=O lactone group), and 30.7–10.7 ppm (carbon atoms in the alkoxy group, except



Entry	Alkoxy	Yield(%)
3a	OCH_2CH_3	85
3b	$OCH_2CH_2CH_3$	75
3c	$OCH(CH_3)_2$	85
3d	$OCH_2(CH_2)_2CH_3$	84
3e	$OCH_2CH(CH_3)_2$	75
3f	$OCH_2(CH_2)_3CH_3$	84
3g	$OCH_2CH_2CH(CH_3)_2$	79

Entry	Alkoxy	Yield(%)
4a	OCH_2CH_3	65
4b	$OCH_2CH_2CH_3$	67
4c	$OCH(CH_3)_2$	50
4d	$OCH_2(CH_2)_2CH_3$	41
4e	$OCH_2CH(CH_3)_2$	40
4f	$OCH_2(CH_2)_3CH_3$	62
4g	$OCH_2CH_2CH(CH_3)_2$	50

Scheme 1 Synthetic path for 6-alkoxy-2*H*-chromene-4-carbaldehydes from 6-hydroxy-2*H*-chromen-2-ones. Reagents and conditions: (i) 1.70% H_2SO_4, 10 °C (for 20 min) to 25 °C (for 24 h); 2.5% NaOH solution to pH 9, then 20% H_2SO_4 solution to pH 5 [44]; (ii) R−Br and KI or R−I, K_2CO_3, dried acetone, or DMF, 12 −16 h, under reflux (for chloride and bromide derivatives: KI (1 mol%) was added); (iii) activated SeO_2, xylene, for 24 h under reflux



Entry	Alkoxy	Yield(%)
6a	OCH_2CH_3	64
6b	$OCH_2CH_2CH_3$	74
6c	$OCH(CH_3)_2$	68
6d	$OCH_2(CH_2)_2CH_3$	62
6e	$OCH_2CH(CH_3)_2$	74
6f	$OCH_2(CH_2)_3CH_3$	70
6g	$OCH_2CH_2CH(CH_3)_2$	70

Scheme 2 Synthetic path for substituted 2-oxo-2*H*-chromene-4-carbaldehyde *N*-(tetra-*O*-acetyl-β-D-glucopyranosyl) thiosemicarbazones. Reaction conditions: glacial acetic acid (cat.), MeOH (solv.) under MW-assisted conditions at microwave power of 600 W

methylene carbon atom attached to oxygen) depending on their positions in carbon alkyl chain, and $\delta = 74.3–64.2$ ppm (methylene carbon atom attached to oxygen).

Oxidation of 4-methyl group on 2*H*-chromen-2-one ring of compounds **3a–g** performed by using activated selenium dioxide as selective oxidant in xylene (Riley's oxidation) according to modified literature procedure (Scheme 1) [40]. Yields of corresponding 4-formyl derivatives **4a–g** achieved 41–67%. The IR spectra of these aldehydes displayed the characteristic absorption bands for functional groups that are present in the molecule. Intense absorption bands characterizing stretching vibration for functional groups, for examples, in region at $\nu = 1737–1712\ cm^{-1}$ ($\nu_{C=O}$ lactone), $1710–1700\ cm^{-1}$ ($\nu_{C=O}$ aldehyde), $1270–1243\ cm^{-1}$, and $1151–1145\ cm^{-1}$ (ν_{COC} lactone and ether). Benzene ring of 2*H*-chromen-2-one had some characteristic absorption bands for aromatic C=C bonds in region at $\nu = 1540–1480\ cm^{-1}$. ^1H NMR spectra exhibited chemical shifts in accordance with the structure of compounds **4a–g**. Protons of 2*H*-chromen-2-one ring had chemical shifts in region at $\delta = 8.00–6.33$ ppm with the splitting pattern factions conforming with the substitution on benzene ring of 2*H*-chromen-2-one. Proton of formyl group gave resonance signal at $\delta = 10.16–10.08$ ppm. Alkyl protons had chemical shifts in region at $\delta = 4.10–0.84$ ppm. The ^{13}C NMR spectra displayed resonance signals of carbon atoms that are present in the molecule. The carbon atoms in the aldehyde group have a resonance signal of nearly $\delta = 194.1–193.6$ ppm, the carbon atoms of 2*H*-chromen-2-one ring are in region at $\delta = 161.9–100.9$ ppm, and C-alkyl atoms had signals at $\delta = 69.6–10.2$ ppm.

Synthesis of target molecules, 6-alkoxy-2-oxo-2*H*-chromene-4-carbaldehyde *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-thiosemicarbazones, performed as following. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl thiosemicarbazide (**5**) reacted with above-synthesized 6-alkoxy-2-oxo-2*H*-chromene-4-carbaldehydes (**4a–g**) to yield corresponding thiosemicarbazones **6a–g**. The condensation reaction was carried out in absolute methanol in the presence of glacial acetic acid as catalyst using microwave-assisted heating method (Scheme 2). Reaction mixture was irradiated for 9–13 min at microwave power of 600 W. We found that strong inorganic acids, such as sulfuric or perchloric acids, did not apply to catalyze this reaction, although these acids usually were used for this reaction type [41]. Weak organic acids, such as acetic acid, even trichloroacetic acid were sufficient to activate the carbonyl group without protonation of amin group of thiosemicarbazide.

The IR spectra of 6-alkoxy-2-oxo-2*H*-chromene-4-carbaldehyde *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazones (**6a–g**) showed characteristic absorptions in the range of $\nu = 3354–3447$ and $3328–3234\ cm^{-1}$ belonging to stretching vibrations of N–H groups of thiosemicarbazone linkage group (–NHCSNHN=C<); this linkage group had chemical shift at $\delta = 8.53–8.44$ ppm. Chemical shifts appeared at $\delta = 12.21–12.20$ (singlet, proton NH-2) and $\delta = 9.27–9.16$ ppm (doublet, proton NH-4) confirmed the presence of N–H groups in molecular structure of thiosemicarbazone **6a–g**. Proton NH-4 had coupling interaction to proton H-1 on pyranose ring with coupling constant $J = 9.5–9.0$ Hz. β-Anomeric configuration of these thiosemicarbazones was confirmed by the values of coupling constants of $J = 10.0–9.0$ Hz between protons H-1 and H-2 in the ^1H NMR spectra. An absorption bands with medium intensities lying in region at $\nu = 1373–1327\ cm^{-1}$ belonged to the stretching vibration of C=S group of thiosemicarbazone linkage. Resonance of carbon atom C-1 on pyranose ring was easily recognized, at $\delta = 82.2–82.0$ ppm, which is the signal lying in the weakest field in resonance region of pyranose's carbon. Chemical shift at $\delta = 5.98–5.94$ ppm was assigned to proton H-1. The ^{13}C NMR spectra of these thiosemicarbazones showed resonance signals at $\delta = 179.1–178.9$ ppm (for carbon atom in C=S group) and 170.0–169.3 ppm (for carbon atoms in C=O bond of acetyl groups). Carbon atoms in 2*H*-chromen-2-one ring had chemical shifts at $\delta = 155.5–107.4$ ppm, whereas resonance signals at $\delta = 82.2–61.3$ ppm belonged carbon atoms on D-glucopyranose ring and chemical shifts at $\delta = 20.5–20.3$ ppm belonged methyl carbons in acetyl groups, except for the carbon attached to oxygen atom, which had chemical shift at $\delta = 74.3–63.8$ ppm. Carbon atom in lactone carbonyl group on 2*H*-chromen-2-one ring had chemical shift at $\delta = 160.3–160.0$ ppm.

Table 1 Antibacterial activity of thiosemicarbazones **6a–g**

Entry	Alkoxy groups	Microorganisms/MIC (μg/mL)						
		Gram-positive bacteria			Gram-negative bacteria			
		B. subtilis	*S. aureus*	*S. epidermidis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. typhimurium*
6a	OCH_2CH_3	400	100	0.78	12.5	50	100	12.5
6b	$OCH_2CH_2CH_3$	200	400	25	25	0.78	100	3.125
6c	$OCH(CH_3)_2$	400	12.5	25	25	400	200	6.25
6d	$OCH_2(CH_2)_2CH_3$	25	400	12.5	3.125	3.125	25	1.56
6e	$OCH_2CH(CH_3)_2$	25	1.56	50	1.56	1.56	400	1.56
6f	$OCH_2(CH_2)_3CH_3$	50	3.125	1.56	0.78	25	50	0.78
6g	$OCH_2CH_2CH(CH_3)_2$	3.125	0.78	50	3.125	3.125	50	6.25
	Ciprofloxacin	3.125	3.125	3.125	1.56	1.56	1.56	1.56
	Vancomycin	1.56	3.125	0.78	–	–	–	–
	Methicillin	400	400	400	400	400	400	400

Pharmacology

Antibacterial assays

The evaluations of biological activity of compounds **6a–g** against some above-mentioned typical bacteria were represented in Table 1. In general, almost all compounds had remarkable biological activities against tested microorganisms. The results in Table 1 showed that almost all novel molecule exhibited antibacterial activity against the tested bacteria at low and high concentrations. In general, it has been observed that almost all tested compounds showed mild-to-moderate activity against the tested bacteria in comparison with the MIC values of the references compound. MIC values of these reference drugs are as follows: Ciprofloxacin, 3.12 μg/mL (for Gram-positive bacteria), 1.56 μg/mL (for Gram-negative bacteria); vancomycin, 0.78–3.12 μg/mL (for Gram-positive bacteria). Methicillin had the least inhibitory activity for all tested bacteria (all MICs = 400 μg/mL). In ranges of MIC below 6.25 μg/mL, these thiosemicarbazones exhibited strong inhibitory activity against bacteria *S. aureus*, *E. coli*, *K. pneumoniae*, and *S. typhimurium*, medium activity against *S. epidermidis*, weak activity against *B. subtilis*, and had no activity against *P. aeruginosa*.

In general, almost all synthesized thiosemicarbazones were similar or more inhibitory active than ciprofloxacin but less than vancomycin. In vitro evaluations in Table 1 showed that these 2*H*-chromen-2-one compounds exhibited remarkable antibacterial and antifungal activities against some typical bacteria and fungi. Some thiosemicarbazones had higher ability to inhibit to Gram-positive bacteria (*B. subtilis*, *S. aureus*, and *S. epidermidis*) with MIC values of 1.56–6.25 μg/mL. Amongst these thiosemicarbazones were compound **6g** (MIC = 3.125 μg/mL) against *B. subtilis*; compounds **6e** (MIC = 1.56 μg/mL), **6f** (MIC = 3.125 μg/mL), **6g** (MIC = 0.78 μg/mL) against *S. aureus*; **6a** (MIC = 0.78 μg/mL), **6f** (MIC = 1.56 μg/mL) against *S. epidermidis*; **6d**, **6g** (MIC = 3.125 μg/mL), **6e** (MIC = 1.56 μg/mL), and **6f** (MIC = 0.78 μg/mL) against *E. coli*; **6d**, **6g** (MIC = 3.125 μg/mL), **6e** (MIC = 1.56 μg/mL), and **6b** (MIC = 0.78 μg/mL) against *K. pneumoniae*), **6c**, **6g** (MIC = 6.25 μg/mL), **6b** (MIC = 3.125 μg/mL), **6d**, **6e** (MIC = 1.56 μg/mL), and **6f** (MIC = 0.78 μg/mL) against *S. typhimurium*. Almost all thiosemicarbazones **6a–g** had no activity against strain *B. subtilis* (except compound **6g** with 6-isopentoxy, MIC = 3.125 μg/mL) and *P. aeruginosa* (MIC values were 25–400 μg/mL). Thus, the synthesized thiosemicarbazone 2*H*-chromen-2-one−D-glucose conjugates were more inhibitory active against Gram-negative bacteria than Gram-positive ones. Gram-positive bacterium *B. subtilis* inhibited only by thiosemicarbazone **6g** with MIC value of 3.125 μg/mL.

Some compounds had strong inhibitory activity against several bacteria simultaneously in MIC ranges of 0.78–6.25 μg/mL. Compound **6b** had activity against *K. pneumoniae* and *S. typhimurium* with MIC values of 0.78 and 3.125 μg/mL, respectively. Compound **6d** exhibited inhibitory activity against Gram-negative bacterial strains, including *E. coli*, *K. pneumoniae*, and *S. typhimurium* with MIC values of 3.125, 3.125, and 1.56 μg/mL, respectively. Compound **6e** had stronger activity against *S. aureus*, *E. coli*, *K. pneumoniae*, and *S. typhimurium* with all MIC values of 1.56 μg/mL. Compound **6f** had also stronger inhibition against *S. aureus*, *S. epidermidis*, *E. coli*, and *S. typhimurium* with MIC values of 3.125, 1.56, 0.78, and 0.78 μg/mL. Especially, compound **6g** expressed remarkable inhibitory activity against five bacterial strains, including *B. subtilis*, *S. aureus*, *E. coli*, *K. pneumoniae*, and *S. typhimurium*, with MIC values of 3.125, 0.78, 3.125, 3.125, and 6.25 μg/mL, respectively. The remaining thiosemicarbazones had MIC values of 25–400 μg/mL. Several thiosemicarbazones had inhibitory activity against each Gram-negative

Table 2 MRSA antibacterial activity of thiosemicarbazones **6a–g**

Entry	Alkoxy groups	MRSA198-1	MRSA198-2	MRSA198-3
6a	OCH_2CH_3	400	100	6.25
6b	$OCH_2CH_2CH_3$	12.5	200	6.25
6c	$OCH(CH_3)_2$	50	6.25	12.5
6d	$OCH_2(CH_2)_2CH_3$	200	50	100
6e	$OCH_2CH(CH_3)_2$	25	400	25
6f	$OCH_2(CH_2)_3CH_3$	12.5	25	1.56
6g	$OCH_2CH_2CH(CH_3)_2$	12.5	1.56	0.78
	Ciprofloxacin	400	400	400
	Vancomycin	1.56	1.56	1.56
	Methicillin	NE[a]	NE	NE

NE not evaluated

bacterium similar or stronger than ciprofloxacin, with MIC value of 0.78 or 1.56 μg/mL, such as **6e** (MIC = 1.56 μg/mL) and **6f** (MIC = 0.78 μg/mL) against *E. coli*, **6b** (MIC = 0.78 μg/mL) and **6e** (MIC = 1.56 μg/mL) against *K. pneumoniae*, **6d**, **6e** (MIC = 1.56 μg/mL), **6f** (MIC = 0.78 μg/mL) against *S. typhimurium*. Thus, the compounds mentioned herein were more active than vancomycin (MIC = 3.12 μg/mL) and methicillin (MIC = 400 μg/mL) for all tested Gram-negative bacteria. The presence 6-alkoxy substituents on coumarin ring also caused the similar changes on antibacterial activity ò compounds bearing coumarin ring [18, 19, 36].

We have evaluated the antibacterial activities of these thiosemicarbazones against a series of MRSA strains, signed MRSA198-1, MRSA198-2, and MRSA198-3 from clinical sources [42]. Among the tested compounds (Table 2), **6g** exerted excellent anti-MRSA activity against all tested MRSA strains with MIC values of 1.56, 1.56, and 0.78 μg/mL, respectively. Compound **6f** expressed good inhibitory activity against MRAS198-3 with MIC value of 1.56 μg/mL. In addition, the inhibitory effects of thiosemicarbazones **6a** and **6b** were obviously observed by treating it against strain MRSA198-3 with MIC values of 6.25 μg/mL. Thiosemicarbazone **6c** had better inhibition activity against strain MRAS198-2 with MIC value of 6.25 μg/mL. Compounds that showed mild anti-MRSA effect with MIC values of 12.5–25 μg/mL included **6b**, **6e**, **6f** (against MRSA198-1), **6f** (against MRSA198-2), and **6c**, **6e** (against MRSA198-3). Other compounds did not show any significance in anti-MRSA activity (MIC = 50–100 μg/mL).

In vitro kinase assessment

To order, understand the preliminary mechanism of the compound **6g** with potent antibacterial activity and to confirm the results of docking study, an enzyme inhibitory assay was performed toward *S. aureus* DNA gyrase and Topoisomerase IV enzymes. The obtained results as IC_{50} were presented in Table 3. The suitable positive controls were used, including ciprofloxacin and methotrexate.

Table 3 Inhibitory assessment (IC_{50} in μM) of compound **6g** on *S. aureus* DNA gyrase, Topoisomerase IV, and Dihydrofolate reductase enzymes

Compound	IC_{50} (μM)		
	S. aureus DNA gyrase	Topoisomerase IV	Dihydrofolate reductase
6g	1.82 ± 0.11	79.52 ± 1.20	0.12 ± 0.05
Ciprofloxacin	1.65 ± 0.13	26.12 ± 1.01	–
Methotrexate	–	–	0.34 ± 0.07

Table 3 showed that compound **6g** exhibited an equipotent inhibitory activity toward DNA gyrase (IC_{50} = 1.82 ± 0.11 μM vs. IC_{50} = 1.65 ± 0.13 μM for ciprofloxacin) in comparison with the reference ciprofloxacin, and weak activity against Topoisomerase IV (IC_{50} = 79.52 ± 1.20 μM vs. IC_{50} = 26.12 ± 1.01 μM for ciprofloxacin). In addition, compound **6g** displayed twofolds increase in the suppression effect toward dihydrofolate reductase comparing with methotrexate (IC_{50} = 0.08 ± 1.15 μM and 0.14 ± 1.07 μM, respectively).

Antifungal assay

We evaluated the antifungal activities of the above thiosemicarbazones **6a–g** against some fungi, such as *Aspergillus niger* (ATCC 439), *Aspergillus flavus* (ATCC 204304), *Candida albicans* (ATCC 7754), and *Saccharomyces cerevisiae* (SH 20). Miconazole and fluconazole were used as references. Miconazole is mainly used externally for the treatment and prophylactic treatment of *Candida* infection in the oral cavity and the digestive tract. Fluconazole is a first-generation triazole antifungal medication. MIC values of miconazole were 1.56, 1.56, 3.12, and 3.12 μg/mL for each fungus, respectively, and of fluconazole were 1.56, 0.78, 0.78, and 0.78 μg/mL for each fungus, respectively. The obtained results were given in Table 4.

Results in Table 4 showed that in ranges of MIC values of 0.78–6.25 μg/mL almost all of the tested thiosemicarbazones **6a–g** were stronger active against fungi *A. niger* and A. *flavus*, but medium active against *S. cerevisiae*, and weak active against *C. albicans*. Thiosemicarbazones **6b**, **6f**, and **6g** were more resistant to *A. niger* and *A. flavus* than miconazole and fluconazole against *A. niger*. Almost all of the compounds were less active than miconazole and fluconazole against *C. albicans* and *S. cerevisiae*, except compound **6a** (MIC = 1.56 μg/mL) that was more active than miconazole and less active than fluconazole (against *C. albicans*), and compounds **6c**, **6d** (MIC = 3.125 and 6.25 μg/mL, respectively) were less active than fluconazole. Notable active compounds included

Table 4 Antifungal activity of thiosemicarbazones **6a–g**

Entry	Alkoxy groups	Fungi/ MIC (μg/mL)			
		A. niger	*A. flavus*	*C. albicans*	*S. cerevisiae*
6a	OCH_2CH_3	200	200	1.56	400
6b	$OCH_2CH_2CH_3$	3.125	100	50	100
6c	$OCH(CH_3)_2$	0.78	0.78	25	3.125
6d	$OCH_2(CH_2)_2CH_3$	50	6.25	50	6.25
6e	$OCH_2CH(CH_3)_2$	25	25	50	50
6f	$OCH_2(CH_2)_3CH_3$	6.25	3.125	25	200
6g	$OCH_2CH_2CH(CH_3)_2$	6.25	25	100	25
	Miconazole	1.56	1.56	3.125	3.125
	Fluconazole	1.56	0.78	0.78	0.78

6b (MIC = 3.125 μg/mL) and **6c** (MIC = 0.78 μg/mL) against *A. niger*, **6c** (MIC = 0.78 μg/mL) and **6f** (MIC = 3.125 μg/mL) against *A. flavus*, **6a** (MIC = 1.56 μg/mL) against *C. albicans*, **6c** (MIC = 3.125 μg/mL) against *S. cerevisiae*. Remarkably, compound **6c** was more active against *A. niger*, *C. albicans*, and *S. cerevisiae* with MIC values of 0.78, 0.78, and 3.125 μg/mL, respectively. Two other compounds that had notable activity included **6d** (against *A. flavus* and *S. cerevisiae*) and **6f** (against *A. niger* and *A. flavus*) with MIC values of 3.125–6.25 μg/mL. These such influents of 6-alkoxy substituents on antifungal activity also observed in some compounds having coumarin ring [35].

Molecular docking

As above discussion, the newly synthesized compounds have exhibited good antibacterial activity, in particular against Gram-positive bacterium *S. aureus*. To understand the mechanism of antibacterial activity of newly synthesized compounds, molecular docking study was performed on X-ray crystal structure of *S. aureus* DNA gyrase with its bound inhibitor ciprofloxacin and the protein-ligand complex was constructed based on the X-ray structure of this enzyme [43]. The most active compound **6g** was docked into the empty binding site of this enzyme. The docking pose of **6g** in the active site of 2XCT is presented in Figs 3 and 4. The figures revealed the disposition of protein side-chains and DNA that formed the drug binding pockets. Our docking study showed that compound **6g** bonds to DNA cleavage as similar as ciprofloxacin did [43]. As shown in Fig. 3, this compound formed some hydrogen-bonding interactions at the active site of enzyme 2XCT. Oxygen atom of acetate ester in position 4 of pyranose ring formed hydrogen-bonding interaction with amino-hydrogen of DNA base cytosine (DC11 on chain H). Other hydrogen-bonding interactions included the ones of amino acid residue arginine ARG1122 (on chain D) with carbonyl-oxygen of coumarin ring and of amino-hydrogen of DNA base guanine (DG9 on chain G). There was two π–π stacking interactions of 2*H*-pyran ring of coumarin core with DNA base thymine (DT8 on chain E) of ribonucleotide 2-deoxythymidine-5-monophosphate and with DNA base guanine (DG9 on chain G) of ribonucleotide 2-deoxyguanosine-5-monophosphate. As a result of docking study, it can be declared that compound **6g** is compatible with the active site of *S. aureus* DNA gyrase.

Conclusions

Various 6-alkoxy-4-methyl-2*H*-chromen-2-ones were prepared using Williamson ether synthesis starting from 6-hydroxy-2*H*-chromen-2-ones. These ethers were converted into corresponding 4-carbaldehydes using Riley's oxidation procedure. Microwave-assisted heating method was efficient and convenient method for synthesis of thiosemicarbazones of 6-alkoxy-4-methyl-2-oxo-2*H*-chromene-4-carbaldehydes with *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazide. These compounds displayed significant inhibition in vitro against bacteria *S. aureus*, *E. coli*, *K. pneumoniae*, and *S. typhimurium* and fungi *A. niger*, A. *flavus*. The compound **6g** exhibited good inhibitory activity against five tested bacterial strains with MIC values of 0.78–6.25 μg/mL. Compounds **6a–c**, **6f**, and **6g** were active against three clinical MRSA isolates with MIC values of 0.78–6.25 mM, especially **6g** exerted excellent anti-MRSA activity against all tested MRSA strains with MIC values of 0.78–1.56 μg/mL. Molecular docking study was performed to observe binding efficiency and steric interactions of the lead compound **6g** with isopentoxy substituent at C-6 position of 2*H*-chromene moiety. Docking results showed that compound **6g** is compatible with the active site of *S. aureus* DNA gyrase 2XCT with some hydrogen-bonding interaction and two π–π stacking interaction, which suggested that the tested compounds inhibited the synthesis of this enzyme in *S. aureus*.

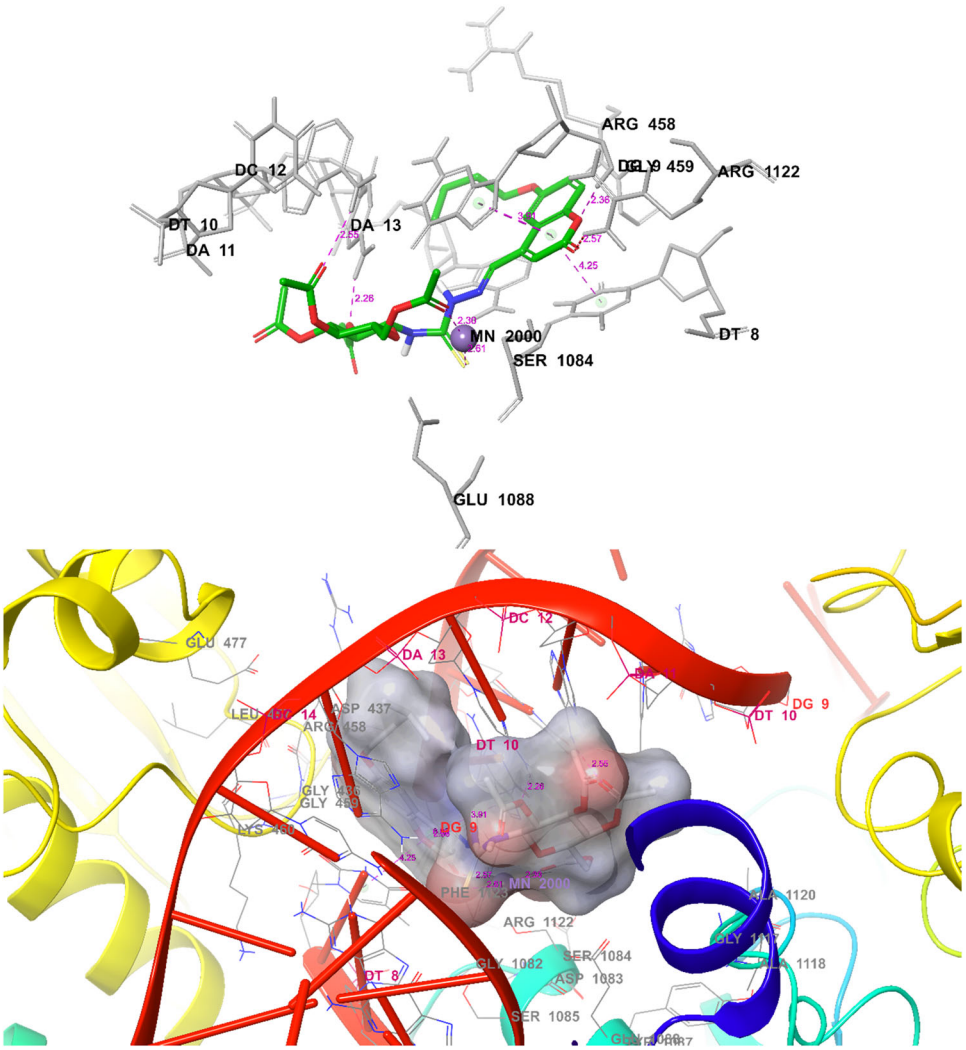


Fig. 3 Interacting mode of compound **6g** (colored elements) without and with helix form in the active region surrounded in DNA gyrase active site. The numbers in violet colors were distances for hydrogen bonding and π–π stacking interactions. DNA helices were in magenta colors. Residues that had active interactions were in thin-tube representations. Ligand **6g** was in thick tube in color by elements

Experimental

Chemistry

Instruments

Melting points were determined by open capillary method on STUART SMP3 instrument (BIBBY STERILIN, UK) and are uncorrected. IR 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 and 125 MHz, respectively, using DMSO-d_6 as solvent and TMS as an internal standard; ESI-EI-HRMS and ESI/HR mass spectra were recorded on EI-MS LTQ Orbitrap XL or Thermo Scientific Exactive Plus Orbitrap spectrometers (Thermo-Scientific, USA) in methanol using the ESI method. The analytical thin-layer chromatography was performed on silica gel 60WFS$_{254}$ aluminum sheets (Merck, Germany) and was visualized with UV light. Chemical reagents in high purity were purchased from the Merck Chemical Company (in Viet Nam). All materials were of reagent grade for organic synthesis. 4-Methyl-6-hydroxy-2*H*-chromen-2-one (**2**) and was prepared by reaction of hydroquinone (**1**) with ethyl acetoacetate in the presence of 70% sulfuric acid according to Russell's procedure (*see* Scheme 1) [44]. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazide (**5**) was prepared from D-glucose via corresponding bromide and isocyanate derivatives according to the literature procedure (see Scheme 2) [37, 45].

General procedure for the synthesis of 6-alkoxy-4-methyl-2*H*-chromen-2-ones (3a–g)

To the suspension of 4-methyl-6-hydroxy-2*H*-chromen-2-one (**2a**, 0.02 mol) in dry acetone (20 mL) was added anhydrous potassium carbonate (0.04 mol, 5.5 g). The mixture was heated under reflux for 10 min with stirring.

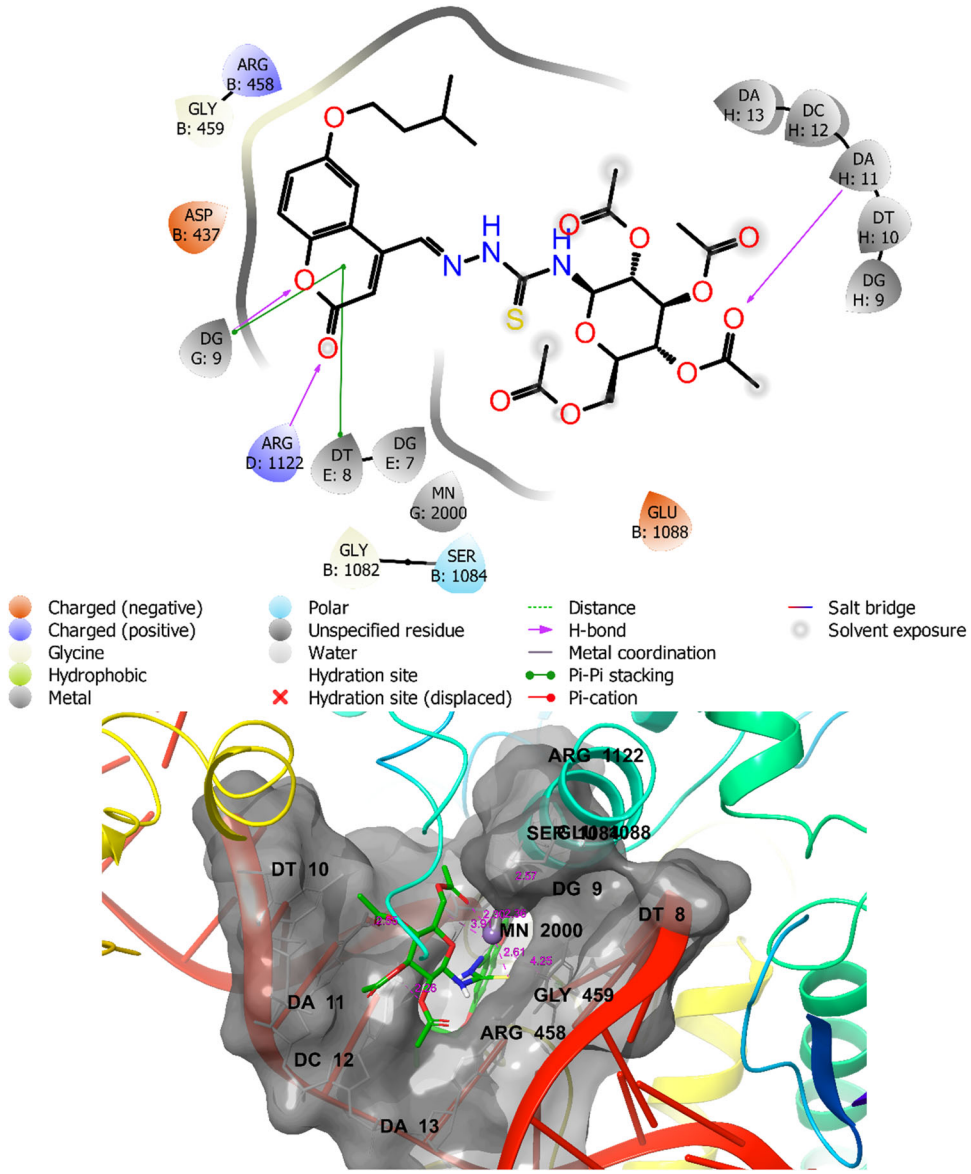


Fig. 4 Two-dimensional diagram (top) in ligand interaction of compound **6g** in DNA gyrase active site showed active ligand-receptor interactions. The pocket on this enzyme was shown by electrostatic potential surface (bottom). Ligand **6g** was in thick tube in color by elements (carbon was in green color, oxygen in red, nitrogen in blue, sulfur in yellow)

Appropriate alkyl halide (bromide or iodide) was added. In cases of bromide derivatives, KI (0.001 mol, 0.17 g) was supplemented. Reaction mixture continued to heat under reflux for 20 h. Solvent was removed completely under reduced pressure. Water (20 mL) was added to residue in order to dissolve inorganic salts. The precipitate was filtered, washed with water until pH 7, and crystallized from 96% ethanol to yield the title compounds **3a–g**.

4-Methyl-6-ethoxy-2H-chromen-2-one (3a) From **2a** (0.02 mol, 3.52 g), ethyl iodide (0.024 mol, 1.93 mL). Reaction time: 16 h. Yield: 3.47 g (85%) of **3a** as pale-yellow crystals. M.p.: 116–117 °C (from 96% ethanol), ref. [46], 116–118 °C (from 96% ethanol). IR (KBr), ν (cm^{-1}) 1715 ($\nu_{C=O}$ lactone), 1572, 1485 ($\nu_{C=C}$ arene), 1165, 1067 (ν_{COC} lactone); ^{1}H NMR (500 MHz, DMSO-d_6, ppm), δ (ppm): 7.27 (d, $J = 9.0$ Hz, 1H, H-8), 7.16 (dd, $J = 9.0$, 2.5 Hz, 1H, H-7), 7.11 (d, $J = 2.5$ Hz, 1H, H-5), 6.34 (s, 1H, H-3), 4.07 (q, $J = 7.0$ Hz, 2H, 6-O**CH$_2$**CH$_3$), 2.39 (s, 3H, 4-CH$_3$), 1.35 (t, $J = 7.0$ Hz, 3H, 6-OCH$_2$**CH$_3$**); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 160.3 (C=O lactone, C-2), 155.3 (C-6), 153.4 (C-8a), 147.6 (C-4), 120.5 (C-5), 119.7 (C-7), 117.8 (C-8), 115.1 (C-4a), 109.2 (C-3), 64.2 (6-O**CH$_2$**CH$_3$), 18.6 (4-CH$_3$), 15.0 (6-OCH$_2$**CH$_3$**); ESI-MS: $C_{12}H_{12}O_3$, calc. for $M =$ 204.1 Da, found: m/z 204.2 ($[M]^+$).

4-Methyl-6-propoxy-2H-chromen-2-one (3b) From **2a** (0.02 mol, 3.52 g), propyl iodide (0.022, 2.4 mL). Reaction time: 16 h. Yield: 3.27 g (75%) of **3b** as pale-yellow crystals. M.p.: 119 – 121.5 °C (from 96% ethanol). IR (KBr), ν cm^{-1}: 1713 ($\nu_{C=O}$ lactone), 1576, 1497 ($\nu_{C=C}$ arene),

1173, 1029 (ν_{COC} lactone); ^{1}H NMR (500 MHz, $CDCl_3$, ppm), δ (ppm): 7.32 (d, $J = 8.5$ Hz, 1H, H-8), 7.21 (d, $J = 8.5$ Hz, 1H, H-7), 7.18 (s, 1H, H-5), 6.38 (s, 1H, H-3), 4.02 (t, $J = 7.0$ Hz, 2H, 6-O**CH$_2$**CH$_2$CH$_3$), 2.43 (s, 3H, 4-CH$_3$), 1.76 (sextet, $J = 6.5$ Hz, 2H, 6-OCH$_2$**CH$_2$**CH$_3$), 1.00 (t, $J = 7.25.0$ Hz, 3H, 6-OCH$_2$CH$_2$**CH$_3$**); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 160.4 (C=O lactone, C-2), 155.5 (C-6), 153.5 (C-8a), 147.6 (C-4), 120.6 (C-5), 119.9 (C-7), 117.9 (C-8), 115.1 (C-4a), 109.4 (C-3), 70.2 (6-O**CH$_2$**CH$_2$CH$_3$), 22.5 (4-CH$_3$), 18.6 (6-OCH$_2$**CH$_2$**CH$_3$), 10.9 (6-OCH$_2$CH$_2$**CH$_3$**); ESI-MS: $C_{13}H_{14}O_3$, calc. for M + H = 219.10 Da, M + Na = 241.08 Da, found: *m/z* 219.16 ([M + H]$^+$), 241.09 ([M + Na]$^+$).

4-Methyl-6-isopropoxy-2H-chromen-2-one (3c) From **2a** (0.02 mol, 3.52 g), isopropyl iodide (0.022, 2.4 mL). Reaction time: 16 h. Yield: 2.62 g (60%) of **3d** as pale-yellow crystals. M.p.: 120–122 °C (from 96% ethanol). IR (KBr), ν (cm^{-1}): 1709 ($\nu_{C=O}$ lactone), 1573, 1490 ($\nu_{C=C}$ arene), 1170, 1020 (ν_{COC} lactone); ^{1}H NMR (500 MHz, DMSO-d_6, ppm), δ (ppm): 7.68 (d, $J = 3.0$ Hz, 1H, H-5), 6.93–6.88 (m, 2H, H-7, and H-8), 6.48 (s, 1H, H-3), 4.23–4.14 [m, 1H, 6-O**CH**(CH$_3$)$_2$], 2.43 (s, 3H, 4-CH$_3$), 1.02–0.98 [m, 6H, 6-OCH(**CH$_3$)$_2$**]; ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 162.2 (C=O lactone, C-2), 160.6 (C-6), 155.2 (C-4), 153.8 (C-8a), 126.8 (C-4a), 113.4 (C-8), 112.8 (C-7), 111.5 (C-3), 101.5 (C-5), 68.2 [6-O**CH**(CH$_3$)$_2$], 22.3 [6-OCH(**CH$_3$)$_2$**], 19.5 (4-CH$_3$); ESI-MS: $C_{13}H_{14}O_3$, calcd. for M + H = 219.10 Da, M + Na = 241.08 Da, found: *m/z* 219.15 ([M + H]$^+$), 241.15 ([M + Na]$^+$); Elemental anal. for $C_{13}H_{14}O_3$, calcd.: C, 71.54; H, 6.47%; found: C, 71.32; H, 6.24%.

4-Methyl-6-butoxy-2H-chromen-2-one (3d) From **2a** (0.02 mol, 3.52 g), butyl bromide (0.022 mol, 2.58 mL), and KI (0.001 mol, 0.17 g). Reaction time: 12 h. Yield: 4.65 g (85%) of **3c** as white crystals. M.p.: 107–109 °C (from 96% ethanol). IR (KBr), ν cm^{-1}: 1721 ($\nu_{C=O}$ lactone), 1569, 1428 ($\nu_{C=C}$ arene), 1242, 1166 (ν_{COC} lactone); ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 7.28 (d, $J = 9.0$ Hz, 1H, H-7), 7.18 (dd, $J = 9.0$, 3.0 Hz, 1H, H-8), 7.15 (d, $J = 2.3$ Hz, 1H, H-3), 6.35 (d, $J = 1.0$ Hz, 1H, H-5), 4.01 (t, $J = 6.5$ Hz, 2H, 6-O**CH$_2$**CH$_2$CH$_2$CH$_3$), 2.40 (s, 4-CH$_3$), 1.70 (quintet, $J = 6.5$ Hz, 2H, 6-OCH$_2$**CH$_2$**CH$_2$CH$_3$), 1.44 (sextet, $J = 7.0$ Hz, 2H, 6-OCH$_2$CH$_2$**CH$_2$**CH$_3$), 0.93 (t, $J = 7.0$ Hz, 3H, 6-OCH$_2$CH$_2$CH$_2$**CH$_3$**); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 159.8 (C=O lactone, C-2), 155.0 (C-6), 152.9 (C-8a), 147.0 (C-4), 120.0 (C-5), 119.3 (C-7), 117.3 (C-8), 114.6 (C-4a), 108.7 (C-3), 67.8 (6-O**CH$_2$**CH$_2$CH$_2$CH$_3$), 30.7 (6-OCH$_2$**CH$_2$**CH$_2$CH$_3$), 18.7 (4-CH$_3$), 18.1 (6-OCH$_2$CH$_2$**CH$_2$**CH$_3$), 13.6 (6-OCH$_2$CH$_2$CH$_2$**CH$_3$**); ESI-MS: $C_{14}H_{16}O_3$, calc. for (M + 2H) = 234.2 Da, found: *m/z* 234.1 ([M + 2H]$^+$).

4-Methyl-6-isobutoxy-2H-chromen-2-one (3e) From **2a** (0.02 mol, 3.52 g), isobutyl bromide (0.022 mol, 2.59 mL), and KI (0.1 mmol, 0.17 g). Reaction time: 12 h. Yield: 3.49 g (75%) of **3f** as white crystals. M.p.: 103–105 °C (from 96% ethanol). IR (KBr), ν (cm^{-1}): 1718 ($\nu_{C=O}$ lactone), 1520, 1500, 1480 ($\nu_{C=C}$ arene), 1248, 1167 (ν_{COC} lactone); ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 7.60 (d, $J = 8.75$ Hz, 1H, H-7), 6.92 – 6.87 (m, 2H, H-5, and H-8), 6.16 (s, 1H, H-3), 4.04 [d, $J = 8.0$ Hz, 2H, 6-O**CH$_2$**CH(CH$_3$)$_2$], 2.36 (s, 3H, 4-CH$_3$), 1.73–1.67 [m, 1H, 6-OCH$_2$**CH**(CH$_3$)$_2$], 0.93 [d, $J = 7.0$ Hz, 6H, 6-OCH$_2$CH(**CH$_3$)$_2$**]; ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 162.2 (C=O lactone, C-2), 160.6 (C-6), 155.2 (C-4), 153.8 (C-8a), 126.8 (C-4a), 113.4 (C-8), 112.8 (C-7), 111.5 (C-3), 101.5 (C-5), 68.4 [6-O**CH$_2$**CH(CH$_3$)$_2$], 31.0 [6-OCH$_2$**CH**(CH$_3$)$_2$], 19.1 [6-OCH$_2$CH(**CH$_3$)$_2$**], 18.5 (4-CH$_3$); ESI-MS: $C_{14}H_{16}O_3$, calcd. for M + H = 233.12 Da, M + Na = 255.10 Da, found: *m/z* 233.19 ([M + H]$^+$), 255.08 ([M + Na]$^+$); ESI-HRMS: calcd. for M + H = 233.1178 Da, found: 233.1185 ([M + H]$^+$, 100%); elemental anal. for $C_{14}H_{16}O_3$, calcd.: C, 72.39; H, 6.94%; found: C, 72.57; H, 6.61%.

4-Methyl-6-pentoxy-2H-chromen-2-one (3f) From **2a** (0.02 mol, 3.52 g), pentyl bromide (0.022 mol, 3.12 mL), and KI (0.001 mol, 0.17 g). Reaction time: 12 h. Yield: 4.88 g (84%) of **3d** as white crystals. M.p.: 90–92 °C (from 96% ethanol). IR (KBr), ν cm^{-1}: 1719 ($\nu_{C=O}$ lactone), 1568, 1492 ($\nu_{C=C}$ arene), 1248, 1170 (ν_{COC} lactone); ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 7.28 (d, $J = 9.0$ Hz, 1H, H-7), 7.18 (dd, $J = 8.75$, 2.3 Hz, 1H, H-8), 7.15 (d, $J = 3.0$ Hz, 1H, H-3), 6.45 (d, $J = 0.5$ Hz, 1H, H-3), 4.00 (t, $J = 6.5$ Hz, 2H, 6-O**CH$_2$**CH$_2$CH$_2$CH$_2$CH$_3$), 2.40 (s, 3H, 4-CH$_3$), 1.72 (quintet, $J = 6.5$ Hz, 2H, 6-OCH$_2$**CH$_2$**CH$_2$CH$_2$CH$_3$), 1.37–1.35 (m, 4H, 6-OCH$_2$CH$_2$**CH$_2$CH$_2$**CH$_3$), 0.88 (t, $J = 7.0$ Hz, 3H, 6-OCH$_2$CH$_2$CH$_2$CH$_2$**CH$_3$**); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 159.9 (C=O lactone, C-2), 155.0 (C-6), 153.0 (C-8a), 147.1 (C-4), 120.1 (C-5), 119.3 (C-7), 117.4 (C-8), 114.6 (C-4a), 108.8 (C-3), 68.1 (6-O**CH$_2$**CH$_2$CH$_2$CH$_2$CH$_3$], 28.3 (6-OCH$_2$**CH$_2$**CH$_2$CH$_2$CH$_3$), 27.7 (6-OCH$_2$CH$_2$**CH$_2$**CH$_2$CH$_3$), 21.8 (6-OCH$_2$CH$_2$CH$_2$**CH$_2$**CH$_3$), 18.1 (4-CH$_3$), 13.9 (6-OCH$_2$CH$_2$CH$_2$CH$_2$**CH$_3$**]; ESI-MS: $C_{15}H_{18}O_3$, calc. for (M + H) = 247.1 Da, found: *m/z* 247.2 ([M + H]$^+$).

4-Methyl-6-isopentoxy-2H-chromen-2-one (3g) From **2a** (0.02 mol, 3.52 g), isopentyl bromide (0.024 mol, 2.88 mL), and KI (0.1 mmol, 0.17 g). Reaction time: 12 h. Yield: 3.85 g (79%) of **3h** as white crystals. M.p.: 99–101 °C (from 96% ethanol). IR (KBr), ν (cm^{-1}): 1708 ($\nu_{C=O}$ lactone), 1571 ($\nu_{C=C}$ arene), 1243, 1169 (ν_{COC} lactone); ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 7.24 (t, $J = 6.0$ Hz, 1H, H-7), 7.14 (dd, $J = 8.75$, 3.0 Hz, 1H, H-8), 7.10 (s, 1H, H-3), 6.31 (d, $J = 1.0$ Hz, 1H, H-5), 4.00 [t, $J = 6.5$ Hz, 2H, 6-O**CH$_2$**CH$_2$CH(CH$_3$)$_2$], 2.37 (s, 3H, 4-CH$_3$), 1.76–1.74 [m, 1H, 6-OCH$_2$CH$_2$**CH**(CH$_3$)$_2$], 1.59 [q, $J = 13.5$ Hz, 2H,

6-$OCH_2\mathbf{CH_2}CH(CH_3)_2$], 0.91 [t, $J = 7.0$ Hz, 6H, 6-$OCH_2CH_2CH(\mathbf{CH_3})_2$]; ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 159.8 (C=O lactone, C-2), 154.9 (C-6), 152.9 (C-4), 147.0 (C-8a), 120.0 (C-4a), 119.3 (C-8), 117.3 (C-7), 114.6 (C-3), 108.8 (C-5), 66.5 [6-$O\mathbf{CH_2}CH_2CH(CH_3)_2$], 37.6 [6-$OCH_2\mathbf{CH_2}CH(CH_3)_2$], 24.5 [6-$OCH_2CH_2\mathbf{CH}(CH_3)_2$], 22.3 [6-$OCH_2CH_2CH(\mathbf{CH_3})_2$], 18.1 (4-$CH_3$); ESI-MS: $C_{15}H_{18}O_3$, calcd. for M + H = 247.1 Da, found: *m/z* 247.3 ($[M + H]^+$); ESI-HRMS: calcd. for M + H = 247.1334 Da, found: *m/z* 247.1341 ($[M + H]^+$, 100%); elemental anal. for $C_{15}H_{18}O_3$, calcd.: C, 73.15; H, 7.37%; found: 73.42; H, 7.57%.

General procedure for synthesis of 6-alkoxy-2-oxo-2*H*-chromene-4-carbaldehydes (4a–g)

To the solution of corresponding 6-alkoxy-4-methyl-2-oxo-2*H*-chromen-2-ones (**3a–g**, 0.02 mol) in xylene (50 mL) was added activated selenium dioxide (0.027 mol, 3.8 g). The reaction mixture was boiled under reflux for 24 h with gently stirring. The hot reaction mixture then was filtered, the filtrate was cooled in ice bath, and separated product was filtered, crystallized from toluene to afford the title aldehydes **4a–g**.

6-Ethoxy-2-oxo-2H-chromene-4-carbaldehyde (4a) From **3a** (0.2 mol, 4.08 g). Reaction time: 24 h. Yield: 2.83 g (65%) of **4a** as yellow crystals. M.p.: 163–168 °C (from toluene). IR (KBr), ν cm^{-1}: 1736 ($\nu_{C=O}$ lactone), 1708 ($\nu_{C=O}$ aldehyde), 1565, 1430 ($\nu_{C=C}$ arene), 1243, 1054 (ν_{COC} lactone); ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 10.15 (s, 4-CHO), 7.96 (d, $J = 3.0$ Hz, 1H, H-5), 7.40 (d, $J = 9.0$ Hz, 1H, H-8), 7.27 (dd, $J = 9.5$, 3.0 Hz, 1H, H-7), 7.16 (s, H, 1H, H-3), 4.09 (q, $J = 7.0$ Hz, 2H, 6-$O\mathbf{CH_2}CH_3$), 1.37 (t, $J = 7.0$ Hz, 3H, 6-$OCH_2\mathbf{CH_3}$); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 193.6 (4-CHO), 160.1 (C=O lactone, C-2), 155.0 (C-6), 148.2 (C-4), 143.0 (C-8a), 125.3 (C-4a), 119.9 (C-7), 117.3 (C-8), 109.1 (C-3), 63.7 (6-$O\mathbf{CH_2}CH_3$), 14.5 (6-$OCH_2\mathbf{CH_3}$); ESI-MS: $C_{12}H_{10}O_4$, calc. for M + H = 219.07 Da, M + Na = 241.05 Da, found: *m/z* 219.15 ($[M + H]^+$), 219.11 ($[M + Na]^+$).

6-Propoxy-2-oxo-2H-chromene-4-carbaldehyde (4b) From **3b** (0.2 mol, 4.4 g). Reaction time: 24 h. Yield: 3.1 g (67%) of **4b** as yellow crystals. M.p.: 143–145 °C (from toluene). IR (KBr), ν cm^{-1}: 1723 ($\nu_{C=O}$ lactone), 1703 ($\nu_{C=O}$ aldehyde), 1542, 1434 ($\nu_{C=C}$ arene), 1244, 1045 (ν_{COC} lactone); ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 10.16 (s, 4-CHO), 7.99 (d, $J = 3.0$ Hz, 1H, H-5), 7.43 (d, $J = 9.0$ Hz, 1H, H-8), 7.30 (dd, $J = 9.5$, 2.5 Hz, 1H, H-7), 7.19 (s, H, 1H, H-3), 3.98 (t, $J = 7.0$ Hz, 2H, 6-$O\mathbf{CH_2}CH_2CH_3$), 1.77 (sextet, $J = 7.0$ Hz, 2H, 6-$OCH_2\mathbf{CH_2}CH_3$), 1.01 (t, $J = 7.0$ Hz, 2H, 6-$OCH_2CH_2\mathbf{CH_3}$); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 194.1 (4-CHO), 160.6 (C=O lactone, C-2), 155.8 (C-6), 148.7 (C-4), 143.6 (C-8a), 125.9 (C-4a), 120.5 (C-7), 118.2 (C-8), 115.9 (C-3), 109.7 (C-5), 70.2 (6-$O\mathbf{CH_2}CH_2CH_3$), 22.4 (6-$OCH_2\mathbf{CH_2}CH_3$), 10.8 (6-$OCH_2CH_2\mathbf{CH_3}$); ESI-MS: $C_{13}H_{12}O_4$, calc. for M + H = 233.08 Da, M + Na = 255.06 Da, found: *m/z* 233.12 ($[M + H]^+$), 255.17 ($[M + Na]^+$).

6-Isopropoxy-2-oxo-2H-chromene-4-carbaldehyde (4c) From **3c** (0.2 mol, 4.4 g). Reaction time: 24 h. Yield: 2.32 g (50%) of **4c** as yellow crystals. M.p.: 134–135 °C (from toluene). IR (KBr), ν (cm^{-1}): 1734 ($\nu_{C=O}$ lactone), 1704 ($\nu_{C=O}$ aldehyde), 1525, 1432 ($\nu_{C=C}$ arene), 1246, 1054 (ν_{COC} lactone); ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 10.12 (s, 4-CHO), 8.54 (d, $J = 9.0$ Hz, 1H, H-5), 6.92 (m, 1H, H-3), 6.96–6.94 (m, 1H, H-8), 6.35–6.34 (d, $J = 8.0$ Hz, 1H, H-6), 4.05–4.03 [m, 1H, 6-$O\mathbf{CH}(CH_3)_2$], 0.99–0.96 [m, 6H, 6-$OCH(\mathbf{CH_3})_2$]; ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 194.2 (4-CHO), 161.5 (C-4), 161.3 (C-7), 156.4 (C=O lactone, C-2), 143.4 (C-8a), 121.5 (C-5), 112.6 (C-3), 110.9 (C-4a), 108.1 (C-6), 101.4 (C-8), 70.1 [6-$O\mathbf{CH}(CH_3)_2$], 10.6 [6-$OCH(\mathbf{CH_3})_2$]; ESI-MS: $C_{13}H_{12}O_4$, calcd. for M + H = 233.08 Da, M + Na = 255.06 Da, found: *m/z* 233.15 ($[M + H]^+$), 255.19 ($[M + Na]^+$); ESI-HRMS: calcd. for M + H = 233.0814 Da, found: *m/z* 233.0819 ($[M + H]^+$, 100%); elemental anal. for $C_{13}H_{12}O_4$, calcd.: C, 67.23; H, 5.21%; found: C, 67.41; H, 5.09%.

6-Butoxy-2-oxo-2H-chromene-4-carbaldehyde (4d) From **3c** (0.2 mol, 4.64 g). Reaction time: 24 h. Yield: 3.31 g (67%) of **4d** as yellow crystals. M.p.: 110–112 °C (from toluene). IR (KBr), ν cm^{-1}: 1735 ($\nu_{C=O}$ lactone), 1700 ($\nu_{C=O}$ aldehyde), 1565, 1435 ($\nu_{C=C}$ arene), 1244, 1150 (ν_{COC} lactone); ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 10.16 (s, 4-CHO), 7.99 (d, $J = 3.0$ Hz, 1H, H-5), 7.46 (d, $J = 9.0$ Hz, 1H, H-8), 7.32 (dd, $J = 9.5$, 2.5 Hz, 1H, H-7), 7.17 (s, H, 1H, H-3), 4.04 – 4.01 (m, 2H, 6-$O\mathbf{CH_2}CH_2CH_2CH_3$), 1.76–1.70 (m, 2H, 6-$OCH_2\mathbf{CH_2}CH_2CH_3$), 1.50–1.42 (m, 2H, 6-$OCH_2CH_2\mathbf{CH_2}CH_3$), 0.95 (t, $J = 7.0$ Hz, 3H, 6-$OCH_2CH_2CH_2\mathbf{CH_3}$); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 194.1 (4-CHO), 160.6 (C=O lactone, C-2), 155.8 (C-6), 148.7 (C-4), 143.5 (C-8a), 125.8 (C-4a), 120.5 (C-7), 119.5 (C-8), 118.2 (C-3), 112.0 (C-5), 68.4 (6-$O\mathbf{CH_2}CH_2CH_2CH_3$), 31.1 (6-$OCH_2\mathbf{CH_2}CH_2CH_3$), 19.2 (6-$OCH_2CH_2\mathbf{CH_2}CH_3$), 14.1 (6-$OCH_2CH_2CH_2\mathbf{CH_3}$); ESI-MS: $C_{14}H_{14}O_4$, calc. for M + H = 247.10 Da, M + Na = 269.08 Da, found: *m/z* 247.12 ($[M + H]^+$), 269.15 ($[M + Na]^+$).

6-Isobutoxy-2-oxo-2H-chromene-4-carbaldehyde (4e) From **3e** (0.2 mol, 4.64 g). Reaction time: 24 h. Yield: 3.38 g (65%) of **5e** as yellow crystals. M.p.: 123–125 °C (from toluene). IR (KBr), ν (cm^{-1}): 1733 ($\nu_{C=O}$ lactone), 1694 ($\nu_{C=O}$ aldehyde), 1515, 1425 ($\nu_{C=C}$ arene), 1250, 1154 (ν_{COC} lactone); ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm):

10.16 (s, 4-CHO), 7.99 (d, $J = 3.5$ Hz, 1H, H-5), 7.43 (d, $J = 9.0$ Hz, 1H, H-8), 7.29 (dd, $J = 9.0$, 3.0 Hz, 1H, H-7), 7.19 (s, H, 1H, H-3), 3.83 [d, $J = 6.5$ Hz, 2H, 6-O**CH**$_2$CH(CH_3)$_2$], 2.03 [septet, $J = 6.5$ Hz, 1H, 6-OCH$_2$**CH**(CH_3)$_2$], 0.98 [d, $J = 7.0$ Hz, 6H, 6-OCH$_2$CH(**CH$_3$)$_2$**]; ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 194.1 (4-CHO), 160.6 (C-4), 155.8 (C-6), 148.7 (C=O lactone, C-2), 143.6 (C-8a), 125.9 (C-4a), 120.5 (C-3), 118.2 (C-8), 115.9 (C-7), 109.7 (C-5), 74.3 [6-O**CH$_2$**CH(CH_3)$_2$], 27.5 [6-OCH$_2$**CH**(CH_3)$_2$], 18.9 [6-OCH$_2$CH(**CH$_3$)$_2$**]; ESI-MS: $C_{14}H_{14}O_4$, calcd. for M + H = 247.10 Da, M + Na = 269.08 Da, found: *m/z* 247.15 ([M + H]$^+$), 269.17 ([M + Na]$^+$); ESI-HRMS: calcd. for M + H = 247.0970 Da, found: *m/z* 247.0978 ([M + H]$^+$, 100%); Elemental anal. for $C_{14}H_{14}O_4$, calcd.: C, 68.28; H, 5.73%; found: C, 68.47; H, 5.51%.

6-Pentoxy-2-oxo-2H-chromene-4-carbaldehyde (4f) From **3f** (0.2 mol, 4.92 g). Reaction time: 24 h. Yield: 3.22 g (61%) of **4f** as yellow crystals. M.p.: 112–114 °C. IR (KBr), ν (cm^{-1}): 1738 ($\nu_{C=O}$ lactone), 1703 ($\nu_{C=O}$ aldehyde), 1567, 1437 ($\nu_{C=C}$ arene), 1243, 1156 (ν_{COC} lactone); ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 10.14 (s, 4-CHO), 7.94 (d, $J = 3.5$ Hz, 1H, H-5), 7.39 (d, $J = 9.0$ Hz, 1H, H-8), 7.32 (dd, $J = 9.0$, 3.5 Hz, 1H, H-7), 7.16 (s, H, 1H, H-3), 3.98 (t, 2H, $J = 7.0$ Hz, 6-O**CH$_2$**CH$_2$CH$_2$CH$_2$CH$_3$), 1.74 (quintet, 2H, $J = 7.0$ Hz, 6-OCH$_2$**CH$_2$**CH$_2$CH$_2$CH$_3$), 1.43–1.33 (m, 4H, 6-OCH$_2$CH$_2$**CH$_2$CH$_2$**CH$_3$), 0.91 (t, $J = 7.0$ Hz, 3H, 6-OCH$_2$CH$_2$CH$_2$CH$_2$**CH$_3$**); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 194.1 (4-CHO), 160.6 (C=O lactone, C-2), 155.7 (C-6), 148.7 (C-4), 143.5 (C-8a), 125.8 (C-4a), 120.4 (C-7), 118.2 (C-8), 115.8 (C-3), 109.6 (C-5), 68.6 (6-O**CH$_2$**CH$_2$CH$_2$CH$_2$CH$_3$), 28.7 (6-OCH$_2$**CH$_2$**CH$_2$CH$_2$CH$_3$), 28.1 (6-OCH$_2$CH$_2$**CH$_2$**CH$_2$CH$_3$), 22.4 (6-OCH$_2$CH$_2$CH$_2$**CH$_2$**CH$_3$), 14.4 (6-OCH$_2$CH$_2$CH$_2$CH$_2$**CH$_3$**); ESI-MS: $C_{15}H_{16}O_4$, calc. for $M = 260.1$ Da, found: *m/z* 260.8 ([M]$^+$).

6-Isopentoxy-2-oxo-2H-chromene-4-carbaldehyde (4g) From **3g** (0.2 mol, 4.92 g). Reaction time: 24 h. Yield: 2,60 g (50%) of **5g** as yellow crystals. M.p.: 114–116 °C (from toluene). IR (KBr), ν (cm^{-1}): 1738 ($\nu_{C=O}$ lactone), 1700 ($\nu_{C=O}$ aldehyde), 1562, 1438 ($\nu_{C=C}$ arene), 1273, 1156 (ν_{COC} lactone); ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 10.16 (s, 4-CHO), 7.97 (d, $J = 3.0$ Hz, 1H, H-5), 7.45–7.39 (m, 1H, H-8), 7.33–7.27 (m, 1H, H-7), 7.18 (s, 1H, H-3), 4.03 [t, $J = 6.75$ Hz, 2H, 6-O**CH$_2$**CH$_2$CH(CH_3)$_2$], 1.84–1.76 [m, 1H, 6-OCH$_2$CH$_2$**CH**(CH_3)$_2$], 1.66–1.61 [m, 2H, 6-OCH$_2$**CH$_2$**CH(CH_3)$_2$], 0.94 [d, 6H, $J = 6.5$ Hz, 6-OCH$_2$CH$_2$CH(**CH$_3$)$_2$**]; ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 193.6 (4-CHO), 160.1 (C-4), 154.5 (C-6), 148.2 (C=O lactone, C-2), 143.1 (C-8a), 125.2 (C-4a), 119.9 (C-3), 117.7 (C-8), 117.3 (C-7), 109.3 (C-5), 66.6 [6-O**CH$_2$**CH$_2$CH(CH_3)$_2$], 37.3 [6-OCH$_2$**CH$_2$**CH(CH_3)$_2$], 24.6 [6-OCH$_2$CH$_2$**CH**(CH_3)$_2$], 22.4 [6-OCH$_2$CH$_2$CH**(CH$_3$)$_2$**]; ESI-MS: C15H16O4, calcd. for M + H = 261.11 Da, M + Na = 283.09 Da, found: *m/z* 261.18 ([M + H]$^+$), 283.21 ([M + Na]$^+$); ESI-HRMS: calcd. for M + H = 261.1127 Da, found: 261.1121 ([M + H]$^+$, 100%); elemental anal. for $C_{15}H_{16}O_4$, calcd.: C, 69.22; H, 6.20%; found: C, 69.39; H, 6.04%.

General procedure for synthesis of 6-alkoxy-2-oxo-2*H*-chromene-4-carbaldehyde *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiosemicarbazones (6a–g)

To a slurry of *N*-(1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranosyl) thiosemicarbazide **5** (2 mmol) in absolute ethanol (10 mL) was added appropriate 6-alkoxy-2-oxo-2*H*-chromene-4-carbaldehydes (**4a–g**, 2 mmol). Glacial acetic acid (5 mol%) as catalyst was added dropwise with stirring. The obtained mixture was then irradiated in microwave oven at power of 600 W for 9–13 min, cooled to room temperature, and the separated precipitate was filtered and recrystallized from 96% ethanol to yield the compounds **6a–g**.

6-Ethoxy-2-oxo-2H-chromene-4-carbaldehyde *N*-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)thiosemicarbazone (6a) From **4a** (2 mmol, 218 mg) and **5** (2.2 mmol, 421 mg). Reaction time: 9 min. Yield: 397 mg (64%) of **6a** as yellow crystals. M.p.: 189–191 °C (from 96% ethanol), $[\alpha]_D^{25} + 76.1$ ($c = 0.21$, $CHCl_3$). IR (KBr), ν (cm^{-1}): 3330 and 3250 (ν_{NH}), 1748 ($\nu_{C=O\ ester}$), 1730 ($\nu_{C=O}$ lactone), 1518 (νC=C arene), 1235, 1042 ($\nu_{COC\ ester}$), 1090 ($\nu_{C=S}$); ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 12.06 (s, 1H, NH-2), 9.24 (d, $J = 8.5$ Hz, 1H, NH-4), 8.53 (s, 1H, CH=N), 7.40 (d, $J = 9.0$ Hz, 1H, H-8″), 7.37 (s br, 1H, H-3″), 7.30 (s br, 1H, H-5″), 7.26 (dd, $J =$ 9.25, 2.75 Hz, 1H, H-7″), 5.98 (t, $J = 9.0$ Hz, H-1′), 5.38 (dd, $J = 10.0$, 3.5 Hz, 1H, H-3′), 5.35 (d, $J = 9.0$ Hz, 1H, H-2′), 5.33 (d, $J = 3.5$ Hz, H-4′), 4.35 (t, $J = 6.5$ Hz, 1H, H-5′), 4.11 (q, 2H, $J = 7.0$ Hz, 6-O**CH$_2$**CH$_3$), 4.05 (d, 2H, $J = 6.5$ Hz, 1H, H-6′a, 1H, H-6′b), 2.14–1.94 (s, 4 × 3H, 4 × **CH$_3$**CO), 1.37 (t, 3H, $J = 7.0$ Hz, 6-OCH$_2$**CH$_3$**); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 178.9 (C=S), 170.0–169.4 (C=O ester, 4 × CH$_3$**CO**), 160.0 (C=O lactone, C-2″), 154.8 (C-6″), 147.8 (C-8″a), 144.0 (C-4″), 136.9 (CH=N), 120.0 (C-7″), 118.1 (C-8″), 117.5 (C-4″a), 112.4 (C-3″), 107.5 (C-5″), 82.1 (C-1′), 71.8 (C-5′), 70.7 (C-3′), 68.7 (C-2′), 67.5 (C-4′), 63.8 (6-O**CH$_2$**CH$_3$), 61.3 (C-6′), 20.5–20.3 (4 × **CH$_3$**CO), 14.5 (6-OCH$_2$**CH$_3$**); ESI-MS: $C_{27}H_{31}N_3O_{12}S$, calc. for M–H=620.2 Da, found: *m/z* 620.9 ([M–H]$^-$); ESI-HRMS(+): calc. for. M + H = 622.1707 Da, M + Na = 644.1526 Da, found: *m/z* 622.1713 [M + H]$^+$, 644.1531 [M + Na]$^+$.

6-Propoxy-2-oxo-2H-chromene-4-carbaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-thiosemicarbazone (6b) From **4b** (2 mmol, 232 mg) and **5** (2.2 mmol, 421 mg). Reaction time: 11 min. Yield: 470 mg (74%) of **6b** as yellow crystals. M.p.: 121–122 °C (from 96% ethanol), $[\alpha]_D^{25} + 83.5$

($c = 0.25$, $CHCl_3$). IR (KBr), ν (cm^{-1}): 3539 and 3277 (ν_{NH}), 1748 ($\nu_{C=O\ ester}$), 1737 ($\nu_{C=O}$ lactone), 1516 ($\nu C = C$ arene), 1252 and 1049 ($\nu_{COC\ ester}$), 1080 ($\nu_{C=S}$); ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 12.05 (s, 1H, NH-2), 9.26 (d, $J = 8.5$ Hz, 1H, NH-4), 8.51(s, 1H, CH=N), 7.34 (d, $J = 9.5$ Hz, 1H, H-8″), 7.33 (s, 1H, H-3″), 7.26 (dd, $J = 8.7$, 1.75 Hz, 1H, H-7″), 7.22 (s, 1H, H-5″), 5.98 (t, $J = 9.0$ Hz, H-1′), 5.39 (dd, $J =$ 10.2, 3.75 Hz, 1H, H-3′), 5.37 (d, $J = 9.0$ Hz, 1H, H-2′), 5.33 (d, $J = 2.3$ Hz, H-4′), 4.35 (t, $J = 6.5$ Hz, 1H, H-5′), 4.06 (d, 2H, $J = 6.5$ Hz, 1H, H-6′a, 1H, H-6′b), 3.96 (t, 2H, $J =$ 7.0 Hz, 6-O**CH$_2$**CH$_2$CH$_3$), 2.15–1.94 (s, 4 × 3H, 4 × **CH$_3$**CO), 1.76 (sextet, 2H, $J = 7.0$ Hz, 6-OCH$_2$**CH$_2$**CH$_3$), 0.99 (t, 3H, $J = 7.0$ Hz, 6-OCH$_2$CH$_2$**CH$_3$**); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 178.9 (C=S), 170.0–169.4 (C=O ester, 4 × CH$_3$**CO**), 160.1 (C=O lactone, C-2″), 154.8 (C-6″), 147.8 (C-8″a), 143.9 (C-4″), 136.7 (CH=N), 119.9 (C-7″), 118.1 (C-8″), 118.0 (C-4″a), 111.9 (C-3), 107.4 (C-5″), 82.2 (C-1′), 71.8 (C-5′), 70.8 (C-3′), 69.6 (O**CH$_2$**CH$_2$CH$_3$), 68.7 (C-2′), 67.5 (C-4′), 61.3 (C-6′), 21,9 (6-OCH$_2$**CH$_2$**CH$_3$), 20.5–20.3 (4 × **CH$_3$**CO), 10.3 (6-OCH$_2$CH$_2$**CH$_3$**); ESI-MS: $C_{28}H_{33}N_3O_{12}S$, calc. for $M =$ 635.2 Da, found: *m/z* 635.4 ($[M]^+$); ESI-HRMS(+): calc. for. M + H = 636.1863 Da, M + Na = 658.1683 Da, found: *m/z* 636.1868 $[M + H]^+$, 658.1689 $[M + Na]^+$.

6-Isopropoxy-2-oxo-2H-chromene-4-carbaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-thiosemicarbazone (6c) From **4c** (2 mmol, 232 mg) and **5** (2.2 mmol, 421 mg). Reaction time: 12 min. Yield: 432 g (68%) of **6c** as yellow crystals. M.p.: 191–193 °C (from 96% ethanol), $[\alpha]_D^{25} +$ 84.5 ($c = 0.25$, $CHCl_3$). IR (KBr), ν (cm^{-1}): 3540 and 3266 (ν_{NH}), 1751 ($\nu_{C=O}$ ester), 1715 ($\nu_{C=O}$ lactone), 1600, 1522, and 1490 ($\nu_{C=C}$ arene), 1242 and 1043 (ν_{COC} ester), 1090 ($\nu_{C=S}$); ^{1}H NMR (DMSO-d_6) δ (ppm): 12.09 (s, 1H, NH-2), 9.17 (d, 1H, $J = 9.0$ Hz, NH-4), 8.54 (s, 1H, CH=N), 8.45 (s, 1H, H-3″), 7.83–7.45 (m, 3H, H-5″, H-7″, H-8″), 6.03 (t, 1H, $J = 9.25$ Hz, H-1′), 5.43 (t, 1H, $J = 9.25$ Hz, H-3′), 5.29 (t, 1H, $J = 9.25$ Hz, H-2′), 4.97 (t, 1H, $J = 9.75$ Hz, H-4′), 4.22 (dd, 1H, $J = 12.25$, 4.75 Hz, H-6′a), 4.12 (ddd, 1H, $J = 9.88$, 5.13, 2.63 Hz, H-5′), 4.08 [sextet, 2H, $J = 6.5$ Hz, 6-O**CH**(CH$_3$)$_2$], 3.99 (dd, 1H, $J = 10.5$, 2.0 Hz, H-6′b), 2.00–1.92 (s, 4 × 3H, **CH$_3$**CO), 0.95 [d, 6H, $J = 6.5$ Hz, 6-OCH(**CH$_3$**)$_2$]; ^{13}C NMR (DMSO-d_6) δ (ppm): 179.0 (C=S), 170.0–169.4 (C=O ester, 4 × CH$_3$**CO**), 160.1 (C=O lactone, C-2″), 155.0 (C-8″), 147.0 (C-8″a), 144.0 (C-4″), 137.4 (CH=N), 120.0 (C-5″), 118.1 (C-7″. C-6″), 117.6 (C–4″a), 107.6 (C-3″), 81.7 (C-1′), 72.7 (C-5′), 72.4 (C-3′), 71.0 (C-2′), 67.8 (C-4′), 61.8 (C-6′), 66.6 [6-O**CH**(CH$_3$)$_2$], 22.4 [6-OCH(**CH$_3$**)$_2$], 20.5–20.3 (4 × **CH$_3$**CO); ESI-MS: $C_{28}H_{33}N_3O_{12}S$, calc. for $M = 635.2$ Da, found: *m/z* 635.5 ($[M]^+$); ESI-HRMS(+): calc. for. M + H = 636.1863 Da, M + Na = 658.1683 Da, found: *m/z* 636.1869 $[M + H]^+$, 658.1691 $[M + Na]^+$.

6-Butoxy-2-oxo-2H-chromene-4-carbaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-thiosemicarbazone (6d) From **4d** (2 mmol, 246 mg) and **5** (2.2 mmol, 421 mg). Reaction time: 13 min. Yield: 402 mg (62%) of **6d** as yellow crystals. M.p.: 119–121 °C (from 96% ethanol), $[\alpha]_D^{25} + 81.7$ ($c = 0.24$, $CHCl_3$). IR (KBr), ν (cm^{-1}): 3286 and 3251 (ν_{NH}), 1753 ($\nu_{C=O\ ester}$), 1718 ($\nu_{C=O}$ lactone), 1530, 1500 ($\nu C = C$ arene), 1241 and 1042 ($\nu_{COC\ ester}$), 1089 ($\nu_{C=S}$); ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 12.04 (s, 1H, NH-2), 9.27 (d, $J = 8.0$ Hz, 1H, NH-4), 8.52 (s, 1H, CH = N), 7.36 (d, $J = 9.5$ Hz, 1H, H-8″), 7.34 (s, 1H, H-3″), 7.23 (d, $J = 7.0$ Hz, 1H, H-7″), 7.22 (s, 1H, H-5″), 5.98 (t, $J = 9.25$ Hz, H-1′), 5.39 (dd, $J = 10.0$, 3.5 Hz, 1H, H-3′), 5.35 (d, $J = 10.0$ Hz, 1H, H-2′), 5.33 (d, $J = 2.3$ Hz, H-4′), 4.35 (t, $J = 6.25$ Hz, 1H, H-5′), 4.06 (d, 2H, $J = 6.5$ Hz, 1H, H-6′a, 1H, H-6′b), 4.01 (t, 2H, $J = 7.5$ Hz, 6-O**CH$_2$**CH$_2$CH$_2$CH$_3$), 2.15–1.94 (s, 4 × 3H, 4 × **CH$_3$**CO), 1.72 (quintet, 2H, $J = 7.5$ Hz, 6-OCH$_2$**CH$_2$**CH$_2$CH$_3$), 1.44 (sextet, 2H, $J = 7.5$ Hz, 6-OCH$_2$CH$_2$**CH$_2$**CH$_3$), 0.94 (t, 3H, $J = 7.5$ Hz, 6-OCH$_2$CH$_2$CH$_2$**CH$_3$**); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 178.9 (C=S), 170.0–169.3 (C=O ester, 4 × CH$_3$**CO**), 160.0 (C=O lactone, C-2″), 155.0 (C-6″), 147.8 (C-8″a), 143.9 (C-4″), 136.7 (CH=N), 119.9 (C-7″), 118.0 (C-8″), 117.5 (C-4″a), 111.9 (C-3″), 107.4 (C-5″), 82.1 (C-1′), 71.8 (C-5′), 70.7 (C-3′), 68.7 (C-2′), 67.5 (C-4′), 67.8 (6-O**CH$_2$**CH$_2$CH$_2$CH$_3$), 61.3 (C-6′), 30.7 (6-OCH$_2$**CH$_2$**CH$_2$CH$_3$), 20.5–20.3 (4 × **CH$_3$**CO), 18.7 (6-OCH$_2$CH$_2$**CH$_2$**CH$_3$), 13.6 (6-OCH$_2$CH$_2$CH$_2$**CH$_3$**); ESI-MS: $C_{29}H_{35}N_3O_{12}S$, calc. for M − H = 648.2 Da, found: *m/z* 648.8 ($[M - H]^-$); ESI-HRMS(+): calc. for. M + H = 650.2020 Da, M + Na = 672.1839 Da, found: *m/z* 650.2025 $[M + H]^+$, 672.1834 $[M + Na]^+$.

6-Isobutoxy-2-oxo-2H-chromene-4-carbaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-thiosemicarbazone (6e) From **4e** (2 mmol, 246 mg) and **5** (2.2 mmol, 421 mg). Reaction time: 13 min. Yield: 480 mg (74%) of **6e** as yellow crystals. M.p.: 120–122 °C (from 96% ethanol), $[\alpha]_D^{25} + 81.7$ ($c = 0.24$, $CHCl_3$). IR (KBr), ν (cm^{-1}): 3263 (ν_{NH}), 1752 ($\nu_{C=O}$ ester), 1715 ($\nu_{C=O}$ lactone), 1600, 1529 ($\nu_{C=C}$ arene), 1237 and 1035 (ν_{COC} ester), 1090 ($\nu_{C=S}$); ^{1}H NMR (DMSO-d_6) δ (ppm): 12.22 (s, 1H, NH-2), 9.11 (d, 1H, $J = 9.0$ Hz, NH-4), 8.45 (s, 1H, CH = N), 7.84 (d, 1H, $J = 8.5$ Hz, H-5″), 7.07–7.04 (m, 2H, H-6″, H-8″), 7.04 (s, 1H, H-3″), 5.95 (t, 1H, $J = 9.25$ Hz, H-1′), 5.43 (t, 1H, $J = 9.5$ Hz, H-3′), 5.36 (t, 1H, $J = 9.0$ Hz, H-2′), 4.95 (t, 1H, $J = 9.75$ Hz, H-4′), 4.26 (dd, 1H, $J = 12.3$, 5.0 Hz, H-6′a), 4.12 (ddd, 1H, $J = 10.0$, 4.5, 2.25 Hz, H-5′), 3.99 (dd, 1H, $J = 12.25$, 1.75 Hz, H-6′b), 3.85 [d, 2H, $J =$ 6.5 Hz, 6-O**CH$_2$**CH(CH$_3$)$_2$]; 2.05 [sextet, 1H, $J = 6.5$ Hz, 6-OCH$_2$**CH**(CH$_3$)$_2$], 2.00–1.93 (s, 12H, 4 × **CH$_3$**CO); 0.99 [d, 6H, $J = 6.5$ Hz, 6-OCH$_2$CH(**CH$_3$**)$_2$]; ^{13}C NMR (DMSO-d_6), δ (ppm): 179.0 (C=S), 170.1–169.3 (C=O ester, 4 × CH$_3$**CO**), 161.9 (C=O lactone, C-2″), 160.3 (C-7″), 155.5 (C-8″a), 144.5

(C-4″), 137.4 (CH=N), 125.7 (C-5″), 113.0 (C-8″), 110.3 (C-4″a), 109.2 (C-3″), 101.6 (C-6″), 81.6 (C-1′), 72.7 (C-5′), 72.3 (C-3′), 70.8 (C-2′), 67.8 (C-4′), 61.7 (C-6′), 67.8 (O**C**H_2), 27.6 (OCH_2**C**H(CH_3)$_2$), 20.5-20.3 (4 × **C**H_3CO), 18.9 (OCH_2CH(**C**H_3)$_2$); ESI-MS: $C_{29}H_{35}N_3O_{12}S$, calc. for M − H = 648 Da, found: *m/z* 648 ([M−H]$^-$); ESI-HRMS(+): calc. for. M + H = 650.2020 Da, M + Na = 672.1839 Da, found: *m/z* 650.2026 $[M+H]^+$, 672.1844 $[M+Na]^+$.

6-Pentoxy-2-oxo-2H-chromen-2-one-4-carbaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)thiosemicarbazone (6f) From **4f** (2 mmol, 260 mg) and **5** (2.2 mmol, 421 mg). Reaction time: 13 min. Yield: 464 mg (70%) of **6f** as yellow crystals. M.p.: 166–168 °C (from 96% ethanol), $[\alpha]_D^{25}$ + 80.3 ($c = 0.26$, $CHCl_3$). IR (KBr), ν (cm^{-1}): 3203 and 3263 (ν_{NH}), 1756 ($\nu_{C=O\ ester}$), 1715 ($\nu_{C=O}$ lactone), 1571, 1527 ($\nu_{C=C}$ arene), 1224 and 1038 ($\nu_{COC\ ester}$), 1089 ($\nu_{C=S}$); ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 12.04 (s, 1H, NH-2), 9.28 (d, $J = 8.0$ Hz, 1H, NH-4), 8.53 (s, 1H, CH=N), 7.37 (d, $J = 9.0$ Hz, 1H, H-8″), 7.36 (s, 1H, H-6″), 7.25 (s, 1H, H-5″), 7.24 (d, $J = 7.5$ Hz, 1H, H-7″), 5.98 (t, $J = 8.75$ Hz, H-1′), 5.39 (dd, $J = 10.0$, 4.5 Hz, 1H, H-3′), 5.35 (d, $J = 9.0$ Hz, 1H, H-2′), 5.33 (d, $J = 4.5$ Hz, H-4′), 4.35 (t, $J = 6.25$ Hz, 1H, H-5′), 4.05 (d, 2H, $J = 6.5$ Hz, 1H, H-6′a, 1H, H-6′b), 4.01 [t, 2H, $J = 7.0$ Hz, 6-O**C**H_2CH_2(CH_2)$_2$CH_3], 2.15–1.94 (s, 4 × 3H, **C**H_3CO), 1.74 (quintet, 2H, $J = 7.0$ Hz, 6-OCH_2**C**H_2CH_2CH_2CH_3), 1.42–1.32 (m, 4H, (6-OCH_2CH_2**C**H_2**C**H_2CH_3), 0.87 (t, 3H, 6-OCH_2CH_2CH_2CH_2**C**H_3); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 179.0 (C=S), 170.0–169.3 (C=O ester, 4 × CH_3**CO**), 160.1 (C=O lactone, C-2″), 155.0 (C-6″), 147.8 (C-8″a), 144.0 (C-4″), 136.6 (CH=N), 119.9 (C-7″), 118.0 (C-8″), 117.6 (C-4″a), 112.0 (C-3″), 107.4 (C-5″), 82.2 (C-1′), 71.8 (C-5′), 70.7 (C-3′), 68.7 (C-2′), 68.0 (6-O**C**H_2CH_2CH_2CH_3), 67.5 (C-4′), 61.3 (C-6′), 28.3 (6-OCH_2**C**H_2CH_2CH_2CH_3), 27.6 (6-OCH_2CH_2**C**H_2CH_2CH_3), 21.8 (6-OCH_2CH_2CH_2**C**H_2CH_3), 20.5–20.3 (4 × **C**H_3CO), 13.8 (6-OCH_2CH_2CH_2CH_2**C**H_3); ESI-MS: $C_{30}H_{37}N_3O_{12}S$, calc. for $M = 663.2$ Da, found: *m/z* 663.2 ([M]$^+$); ESI-HRMS(+): calc. for. M + H = 664.2176 Da, M + Na = 686.1996 Da, found: *m/z* 664.2181 $[M+H]^+$, 686.1991 $[M+Na]^+$.

6-Isopentoxy-2-oxo-2H-chromen-2-one-4-carbaldehyde N-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)thiosemicarbazone (6g) From **4g** (2 mmol, 260 mg) and **5** (2.2 mmol, 421 mg). Reaction time: 13 min. Yield: 464 mg (70%) of **6g** as yellow crystals. M.p.: 179–181 °C (from 96% ethanol), $[\alpha]_D^{25}$ + 80.5 ($c = 0.26$, $CHCl_3$). IR (KBr), ν (cm^{-1}): 3570 and 3270 (ν_{NH}), 1753 ($\nu_{C=O}$ ester), 1719 ($\nu_{C=O}$ lactone), 1600, 1530 ($\nu_{C=C}$ arene), 1241 and 1043 (ν_{C-O} ester), 1090 ($\nu_{C=S}$); ^{1}H NMR (DMSO-d_6) δ (ppm): 12.09 (s, 1H, NH-2), 9.17 (d, 1H, $J = 9.0$ Hz, NH-4), 8.54 (s, 1H, CH=N), 8.45 (s, 1H, H-3″), 7.83–7.45 (m, 3H, H-5″, H-7″, H-8″), 6.03 (t, 1H, $J = 9.25$ Hz, H-1′), 5.43 (t, 1H, $J = 9.25$ Hz, H-3′), 5.29 (t, 1H, $J = 9.25$ Hz, H-2′), 4.97 (t, 1H, $J = 9.75$ Hz, H-4′), 4.22 (dd, 1H, $J = 12.25$, 4.75 Hz, H-6′a), 4.12 (ddd, 1H, $J = 9.88$, 5.13, 2.63 Hz, H-5′), 4.08 [t, 2H, $J = 6.5$ Hz, 6-O**C**H_2CH_2CH(CH_3)$_2$], 3.99 (dd, 1H, $J = 10.5$, 2.0 Hz, H-6′b), 2.00–1.92 (s, 4 × 3H, **C**H_3CO); 1.81 [sextet, 1H, $J = 6.5$ Hz, 6-OCH_2CH_2**C**H(CH_3)$_2$], 1.65 [q, 2H, $J = 6.5$ Hz, 6-OCH_2**C**H_2CH(CH_3)$_2$], 0.95 [d, 6H, $J = 6.5$ Hz, 6-OCH_2CH_2CH(C**H**$_3$)$_2$]; ^{13}C NMR (DMSO-d_6) δ (ppm): 179.0 (C=S), 170.0–169.4 (C=O ester, 4 × CH_3**CO**), 160.1 (C=O lactone, C-2″), 155.0 (C-8″), 147.0 (C-8″a), 144.0 (C-4″), 137.4 (CH=N), 120.0 (C-5″), 118.1 (C-7″. C-6″), 117.6 (C-4″a), 107.6 (C-3″), 81.7 (C-1′), 72.7 (C-5′), 72.4 (C-3′), 71.0 (C-2′), 67.8 (C-4′), 61.8 (C-6′), 66.6 (OCH_2), 37.4 [6-OCH_2**C**H_2CH(CH_3)$_2$], 24.6 [6-OCH_2CH_2**C**H(CH_3)$_2$], 22.4 [6-OCH_2CH_2CH(**C**H_3)$_2$], 20.5–20.3 (4 × **C**H_3CO); ESI-MS: $C_{30}H_{37}N_3O_{12}S$, calc. for $[M+H]^+ = 664$ Da, found: *m/z* 664; ESI-HRMS(+): calc. for. M + H = 664.2176 Da, M + Na = 686.1996 Da, found: *m/z* 664.2182 $[M+H]^+$, 686.1990 $[M+Na]^+$.

Biological assays

In vitro antimicrobial activity

All the synthesized thiosemicarbazone **6a–g** were evaluated for in vitro antibacterial and antifungal activities against Gram-positive and Gram-negative bacteria organisms. Gram-positive were chosen *Bacillus subtilis* (ATCC 11774), *Staphylococcus aureus* (ATCC 11632), *Staphylococcus epidermidis* (ATCC 12228); Gram-negative bacteria were *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 4352), *Pseudomonas aeruginosa* (ATCC 25923), and *Salmonella typhimurium* (ATCC 1402). Three methicillin-resistant *S. aureus* (MRSA198-1, MRSA198-2, and MRSA198-3 were also chosen. The evaluations were performed by using MIC, as described in our previous article [42]. The microbroth dilutions technique was applied using Mueller-Hinton broth [47]. The drug references were ciprofloxacin, methicillin, and vancomycin (Table 1). The solutions that had the concentrations of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 µg/mL were prepared by further diluting the test compounds and standard drugs prepared above. The inoculum was prepared using a 4–6-h broth adjusted to a turbidity equivalent of a 0.5 McFarland standard, diluted in broth media to give a final concentration of 5×10^5 CFU/mL in the test tray. 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. All the experiments were performed three times. The MIC values for all tested compounds and reference drug are listed in Tables 1 and 2.

In vitro antifungal activity

The compounds **6a–g** were evaluated for their in vitro antifungal activity against three fungi, including *Aspergillus niger* (439), *Aspergillus flavus* (ATCC 204304), *Saccharomyces cerevisiae* (SH 20), and *Candida albicans* (ATCC 7754), using the agar dilution method with Saburoud's dextrose agar (Hi-Media), as described in our previous article [42]. Miconazole and fluconazole were used as drug references for antifungal activity. The solutions, which had the concentrations of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 µg/mL of each tested compound and standard drugs, were prepared. Suspensions of each microorganisms were prepared to contain 10 CFU/mL and applied to agar plates, which had been serially diluted with compounds to be tested. The plates were incubated at 35 °C. After 72 h, the MICs were determined [47]. Minimal inhibitory concentrations for each compound were investigated against standard fungal strains. All the experiments were performed three times. The MIC values for all tested compounds and reference drug are shown in Table 4.

In vitro kinase assessment

The determinations of in vitro enzyme inhibitory activity of compound **6g** determination against *S. aureus* DNA gyrase, Topoisomerase IV, and Dihydrofolate Reductase enzymes were performed using ciprofloxacin and methotrexate as reference drugs according to the previously literature reported methods [48, 49].

Molecular docking

The two-dimensional structures (.mae) of six compounds (ligands) were drawn, and the structure was analyzed by using 2D sketcher and 3D builder of Maestro 11.5 (Schrödinger, LLC, New York, NY, USA) [50]. The three-dimensional structures of these compounds (ligands) were generated from two-dimensional structures using LigPrep Tool (a Schrödinger suite tool). The tautomeric isomers (including 32 isomers) for the ligands were searched and energy minimizations were carried out by applying the OPLS 2005 force fields, at pH 7.0 ± 2.0. The Epik v.4.3 methodology was used when preparing the ligands. Crystal structure of two enzymes, including *S. aureus* Gyrase complexed with Ciprofloxacin and DNA was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/structure/2XCT, PDB ID: 2XCT). These structures were solved by X-ray crystallography at 3.35 Å resolution. Coordinates of the protein-ligand complexes were fixed for errors in atomic representations and optimized using Protein Preparation Wizard Maestro v. 11.5 (Maestro, v. 11.5: Schrödinger, LLC, New York, NY, USA). The molecular docking was accomplished and analyzed via the Glide v. 7.8 docking tool. The receptor grid was located in the center based on the active site of the protein, using the receptor grid generation tool. The ligands were flexibly docked in grid box using the Monte Carlo-based simulation algorithm and a high-throughput virtual screening (HTVS) method without any constraints was employed that generated binding poses based on energy.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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