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Short communication

Synthesis of substituted-phenyl-1,2,4-triazol-3-thione analogues with modified D-glucopyranosyl residues and their antiproliferative activities

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ABSTRACT

A series of D-glucopyranosyl-1,2,4-triazole-3-thione derivatives **1a**–**1d** were synthesized by the reaction of 1,2,4-triazole-3-thione Schiff bases **5a**–**5d** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide. We demonstrate the conversion of **2** to **5**, without the necessity of purification of both oxadiazole and triazole intermediates to afford the compounds **5**. Their structures were confirmed by standard studies of ^{1}H NMR, IR, MS and elemental analysis. Analogues **5** and **1** have shown cytotoxic activity against human MCF-7 and Bel-7402 malignant cell lines.

1. Introduction

1,2,4-Triazoles nucleus and their derivatives emerge rapidly with the advance of modern heterocyclic chemistry, promising a variety of medical applications such as antibacterial, antifungal, anticancer, antitumor, anticonvulsant, anti-inflammatory, and analgesic properties [1–6]. 1,2,4-Triazole nucleus has been incorporated into a wide variety of therapeutically interesting molecules to transform them into better drugs [7–9].

Recently, for the rapid development of drug resistance, new antiproliferative agents should be designed and synthesized with chemical characteristics clearly differ from those of existing agents. 4-Amino-3-mercapto-1,2,4-triazol derivative is an ideal heterocyclic by virtue of its vicinal nucleophiles amino and mercapto groups constitutes a ready-made building block for construction of various organic heterocycles [10]. It has been established that introduction of 4-methyl mercapto phenyl to different heterocycles has yielded many biologically active compounds endowed with wide spectrum of pharmacological and antimicrobial activities [11]. Nitrogen-containing heterocyclic molecules constitute the largest portion of chemical entities, which are part of many natural products, fine chemicals, and biologically active pharmaceuticals vital for enhancing the quality of life [12].

Schiff bases of 1,2,4-triazoles find diverse applications and extensive biological activity. Schiff bases derived from 3-substituted-4-amino-5-mercapto-1,2,4-triazoles show analgesic, antimicrobial, anti-inflammatory, and antidepressant activities [13]. The incorporation of the 1,2,4-triazole unit into Schiff base macrocycles is of considerable current interest as complexes of 1,2,4-triazoles are being developed for potential use in applications such as magnetic materials and photochemically driven molecular devices [14].

It was known that carbohydrates and their derivatives have a significant biological role and occur widely in all living matter and function even appear to be essential to the process of infection by certain pathogenic species [15]. High levels of glycosylation are one of the many molecular changes that accompany malignant transformations. These changes are characteristic for cancer cells and can protect them from immune surveillance and chemotherapeutic agents, and enhance their metastatic capacity [16]. The carbohydrate moiety can be expected to play the role of drug carrier and improve the selectivity of compounds for cancerous cell lines. For example, vaccination using synthetic tumor-associated antigens such as carbohydrate antigens, holds promise for generating a specific antitumor response by targeting the immune system to cancer cells [17]. The galectin family of carbohydrate receptors represents a promising target for drug delivery. Galectin-3 is selected as a model receptor for targeting of glycoside-bearing drug

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carriers and some reports describe the upregulation of galectin-3 in the progression of colorectal cancer [18]. The incorporation of a carbohydrate moiety on a 1,2,4-triazoles scaffold unleashes the potential to access a new dimension of structural diversity to complement the vast structural diversity already inherent to 1,2,4-triazoles.

The aim of the present study was to modify the bioactivities of 1,2,4-triazole Schiff bases and gain the relative derivatives with better curing effect, optimization of hydrophilic/lipophilic character, and improve the bioavailability by inducing glycosyl groups. During the course of this work, we prepared some 1,2,4-triazole-3-thione Schiff bases and the related D-glucopyranosyl derivatives. The newly synthesized compounds were evaluated for their cytotoxic activity against MCF-7 and Bel-7402 cell lines.

2. Chemistry

The synthesis of the 1,2,4-triazoles Schiff base and 2,3,4,6-tetra-*O*-acetyl-σ-D-glucopyranosyl-1,2,4-triazole-3-thione derivatives is illustrated and outlined in Scheme 1.

We prepared 5-phenyl-1,3,4-oxadiazole-2(3*H*)-thione (**3**) from benzohydrazide (**2**) and carbon disulfide in boiling absolute ethanol with a yield of 90% [19]. Thione derivative (**3**) readily reacted with hydrazine in absolute ethanol to produce the sulphhydryl derivative of 1,2,4-triazole (**4**) in 68% yield [20].

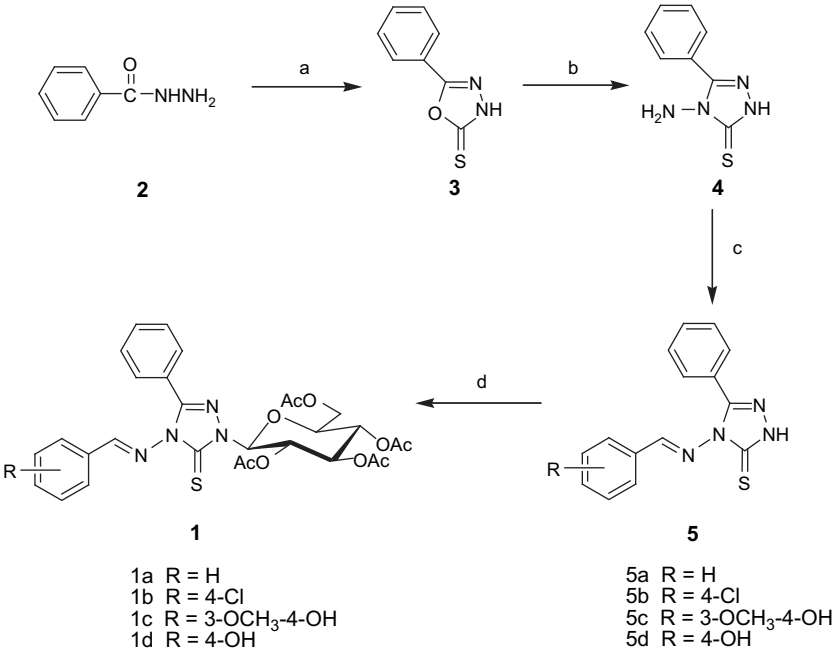
1,2,4-Triazole-3-thione Schiff bases (**5a**–**5d**) were synthesized by the reaction of 4-amino-5-phenyl-2*H*-1,2,4-triazole-3(4*H*)-thione (**4**) with aromatic aldehydes in glacial acetic acid medium. In literature, synthesis of 1,2,4-triazole Schiff bases was catalyzed with acid or base in ethanol medium [21]. The reaction requires several hours. And, the product must be purified by crystallization or column chromatography. Here we brought up a better approach: we used glacial acetic acid as catalyst and solvent; and the reaction took only about 10 min. Furthermore, the products **5** can be purified easily by washing with ethanol. The conversion of **2** to **5a**–**5d** can be performed to achieve an overall yield of 50% in three step, without the necessity of purification of either oxadiazole or triazole intermediates. Sugar esters were conveniently prepared using Koenigs–Knorr reaction, phase-transfer catalysis, etc. [22–24]. Recently, phase-transfer catalysis has proven to be a very useful method for various synthetic transformations. This methodology allows synthetic modifications under mild conditions that were heretofore reserved to very drastic reagents and reaction media [25]. Then, the compounds **5** were treated with 2,3,4,6-tetra-*O*-acetyl-σ-D-glucopyranosyl bromide and Bu_4NBr in ethanol, producing the desired series of 1,2,4-triazole-3-thione derivatives (**1a**–**1d**).

The chemical structures of the compounds were confirmed by ^1H NMR, IR, MS spectra and elemental analysis and the results are presented in Section 5.

3. Biological evaluation

The newly synthesized 1,2,4-triazole-3-thione derivatives **5** and **1** were evaluated for their antiproliferative activity against estrogen receptor positive breast adenocarcinoma MCF-7 and human liver cancer cell lines Bel-7402. The MTT assay was widely applied to examine in vitro cytotoxicity of the drug molecule after 24 h cell treatment [26]. The results, including the IC_{50} values of compounds **5**, **1** and the reference compound 5-fluorouracil [27], are summarized in Table 1.

Table 1 shows that all the compounds were active in μM range against the MCF-7 and Bel-7402 malignant cell lines. The glycosyl esters **1a**–**1d** showed similar activity against MCF-7 and Bel-7402 cells when compared to 5-fluorouracil. Meanwhile, **1a**–**1d** showed higher antiproliferative activity than Schiff bases **5a**–**5d**, demonstrating that our strategy of modifying these molecules was successful. Perhaps, both bioactive component group and structure were shown to improve the bioactivity of 1,2,4-triazole-3-thiones. Interestingly, **1c** showed more potent cytotoxic activity against MCF-7 cells being 2.7-fold more potent than the reference compound **5c**. This analogue was also active against Bel-7402 cells, but it was almost 2.3-fold less potent with respect to the parent molecule **5c**. Compound **1b** was approximately 2-fold and 1.4-fold more active



Scheme 1. Synthesis of the 1,2,4-triazoles Schiff base and D-glucopyranosyl-1,2,4-triazole-3-thione derivatives. Reagents and conditions: (a) CS_2, KOH, EtOH, 80 °C, 8 h, 90% of **3**; (b) NH_2NH_2, EtOH, 90 °C, 8 h, 68% of **4**; (c) Aromatic aldehydes, CH_3COOH, 120 °C, 10 min, 82% of **5a**, 86% of **5b**, 78% of **5c**, 79% of **5d**; (d) 2,3,4,6-tetra-*O*-acetyl-σ-D-glucopyranosyl bromide, $NaHCO_3$, Bu_4NBr, rt, 24 h, 61% of **1a**, 67% of **1b**, 63% of **1c**, 62% of **1d**.

Table 1
Cytotoxicity of the target compounds against MCF-7 and Bel-7402 cells in vitro.

Compound	IC_{50}[a,b] (μM)	
	MCF-7[c]	Bel-7402
5a	31.8 ± 2.2	22.5 ± 1.8
5b	30.5 ± 2.1	24.0 ± 1.5
5c	26.3 ± 2.3	19.6 ± 1.3
5d	29.9 ± 2.0	25.2 ± 1.7
1a	18.0 ± 1.2	10.7 ± 0.8
1b	14.1 ± 1.1	17.4 ± 1.0
1c	9.7 ± 0.6	8.6 ± 0.6
1d	12.8 ± 1.0	11.3 ± 0.9
5-Fluorouracil	4.3 ± 0.7	4.7 ± 0.4

[a] IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control.
[b] Values are expressed as mean ± SD ($n = 3$).
[c] MCF-7, human breast cancer cells; Bel-7402, human hepatocellular cancer cell.

than compound **5b** against MCF-7 and Bel-7402 cells, respectively. As shown in Table 1, we have demonstrated that the modified compound series **1a**–**1d** performed more effectively than the reference compounds **5a**–**5d** to kill the cancer cells (MCF-7 and Bel-7402). We are interested in testing these compounds on a broader range of cancer cell lines.

Due to such cytotoxicity against certain malignant cell lines, and their nontoxicity towards the normal MCF-7 and Bel-7402 cells, the analogues **1a**–**1d** may serve as important leads in the synthesis of more potent and selective anticancer agents. In order to search the relationships between biological activity and structure, it should be performed the synthesis and a more detailed biological evaluation of a series of 1,2,4-triazole-3-thione analogues bearing different sugar groups in their amide moiety. Further investigations of the structure–toxicity and structure–activity relationship of an expanded library of 1,2,4-triazole-3-thione derivatives are currently underway in our group.

4. Conclusion

In summary, we have accomplished the synthesis of various 1,2,4-triazole-3-thione derivatives bearing Schiff bases **5a**–**5d** and 2,3,4,6-tetra-*O*-acetyl-σ-D-glucopyranosyl-1,2,4-triazole-3-thione derivatives (**1a**–**1d**). The conversion of **2** to **5a**–**5d** was carried out without purification of both oxadiazole and triazole intermediates to afford the compounds **5** about 50% overall yield (three steps), respectively.

Analogues **5** and **1** have shown a potent cytotoxic activity against human malignant cell lines (MCF-7 and Bel-7402). The preliminary results obtained in this structure–activity relationship study have shown that in both series, the introduction of 2,3,4,6-tetra-*O*-acetyl-σ-D-glucopyranosyl bromide into 1,2,4-triazole-3-thione system is potentially of interest to improve the cytotoxic activity of this series.

All these findings support the need for further investigations to clarify the features underlying the antiproliferative potential of these new 1,2,4-triazole derivatives. It may be concluded that the fusion of 1,2,4-triazole nuclei in the case of the examined carbohydrates might result in bioactive molecules of potency, particularly if the substituents are designed with optimum toxophoric requirements.

5. Experimental protocols

5.1. Chemistry

Melting points were measured on an XRC-1 melting point apparatus, and are uncorrected. FT Infrared (IR) spectra were recorded in KBr disks using FD-5DX spectrometer. ^{1}H NMR spectra were obtained on a Varian INOVA-400 Spectrometer, using $CDCl_3$ as a solvent; TMS (δ 0.00 ppm) and trifluoroacetic acid were used as internal standard. All NMR chemical shifts are reported as δ values in parts per million (ppm) and coupling constants (J) are given in hertz (Hz). The splitting pattern abbreviations are as follows: s, singlet; d, doublet; br, broad peak; and m, multiplet. Mass spectra were carried out on a ZAB-HS mass spectrometer. Elemental analyses were recorded with Vario-EL-III Spectrometer.

2,3,4,6-Tetra-*O*-acetyl-σ-D-glucopyranosyl bromide was synthesized by our laboratory [16]. 4-Dimethylamino pyridine (DMAP) was purchased from ACROS. Absolute ethanol (CH_3CH_2OH) and dichloromethane (CH_2Cl_2) were dried and purified using standard technique. Other reagents were of the highest commercially available quality.

5.1.1. General procedure for compounds **3** and **4**

Compound **2** (0.27 g, 2.0 mmol) and KOH (0.17 g, 3.0 mmol) were dissolved in 50 mL absolute ethanol. CS_2 (0.18 mL, 3.0 mmol) was added dropwise. The mixture was refluxed for 8 h, and then distilled under reduced pressure. The residue was added into distilled water and the solid formed was filtered. The filtrate was acidified and set to pH = 1 with 2 M HCl solution and turned to slurry. The product was obtained by filtration and dissolved in ethyl acetate. The solution dried with $MgSO_4$ and evaporated by vacuum. The compound **3** was obtained.

The mixture of compound **3** (0.36 g, 2.0 mmol), 80% hydrate hydrazine aqueous solution (0.4 mL, 2.5 mmol) and absolute ethanol (20 mL) was refluxed at 90 °C for 8 h. Then the mixture was cooled and evaporated by vacuum. Crystallization of the product from ethanol gave pink solid of compound **4**.

*5.1.1.1. 5-Phenyl-1,3,4-oxadiazole-2(3H)-thione(**3**).* White solid, yield: 90%, m.p. 199–201 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.50–7.54 (m, 2H, C_6H_5); 7.57–7.61 (m, 1H, C_6H_5); 7.93–7.96 (m, 2H, C_6H_5); 10.69 (s, 1H, NH). Anal. calcd for $C_8H_6N_2OS$: C 53.92, H 3.39, N 15.72; found C 53.84, H 3.35, N 15.79.

*5.1.1.2. 4-Amino-5-phenyl-2H-1,2,4-triazole-3(4H)-thione (**4**).* Pink solid, yield: 68%, m.p.192–193 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.86 (s, 2H, NH_2); 7.51–7.53 (m, 2H, C_6H_5); 7.78 (d, 1H, $J = 7.6$ Hz, C_6H_5); 8.08–8.10 (m, 2H, C_6H_5); 11.17 (s, 1H, NH). Anal. calcd for $C_8H_8N_4S$: C 49.98, H 4.19, N 29.14; found C 49.92, H 4.15, N 29.18.

5.1.2. General procedure for the synthesis of 4-(arylmethylideneamino)-5-phenyl-2H-1,2,4-triazole-3(4H)-thiones (**5a**–**5d**)

Aromatic aldehydes (2.0 mmol) were dissolved in glacial acetic acid (4.0 mL) and compound **4** (0.38 g, 2.0 mmol) was added. The reaction mixture was refluxed for 10 min. Then the mixture was cooled and the solid separated was filtered and washed with ethanol. After drying, compounds **5** were obtained.

*5.1.2.1. (E)-4-(Benzylideneamino)-5-phenyl-2H-1,2,4-triazole-3(4H)-thione (**5a**).* White solid, yield: 82%, m.p. 180–182 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.47–7.52 (m, 5H, C_6H_5); 7.52–7.57 (m, 1H, C_6H_5); 7.88–7.90 (m, 2H, C_6H_5); 7.95–7.97 (m, 2H, C_6H_5); 10.07 (s, 1H, CH=N); 11.21 (s, 1H, NH). IR (KBr), ν/cm^{-1}: 3028, 1603, 1543, 1502, 1363, 1277. MS: $m/z = 280.7$ ($[M+1]^+$); Anal. calcd for $C_{15}H_{12}N_4S$: C 64.26, H 4.31, N 19.98; found C 64.23, H 4.38, N 19.92.

*5.1.2.2. (E)-4-(4-Chlorobenzylideneamino)-5-phenyl-2H-1,2,4-triazole-3(4H)-thione (**5b**).* Yellow solid, yield: 78%, m.p. 218–220 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.46–7.54 (m, 5H, C_6H_5), 7.82 (d, 2H, $J = 7.6$ Hz, C_6H_4), 7.92 (d, 2H, $J = 7.2$ Hz, C_6H_4), 10.16 (s, 1H, CH=N), 10.94 (s, 1H, NH). IR (KBr), ν/cm^{-1}: 3030, 1599, 1539, 1500, 1356,

1281. MS: $m/z = 314.9$ ($[M+1]^+$); Anal. calcd for $C_{15}H_{11}ClN_4S$: C 57.23, H 3.52, N 17.80; found C 57.15, H 3.58, N 17.83.

*5.1.2.3. (E)-4-(4-Hydroxy-3-methoxybenzylideneamino)-5-phenyl-2H-1,2,4-triazole-3(4H)-thione (**5c**).* Yellow solid, yield: 80%, m.p. 171–173 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.95 (s, 3H, OCH_3), 7.03 (d, 1H, $J = 8.0$ Hz, C_6H_5), 7.20 (dd, 1H, $J = 1.6$ Hz, $J = 8.4$ Hz, C_6H_3), 7.45 (d, 2H, $J = 7.2$ Hz, C_6H_3), 7.50 (d, 2H, $J = 1.6$ Hz, C_6H_5), 7.88–7.90 (m, 2H, C_6H_5), 9.70 (s, 1H, CH═N), 11.17 (s, 1H, NH). IR (KBr), ν/cm^{-1}: 3070, 1593, 1514, 1383, 1292. MS: $m/z = 327.4$ ($[M+1]^+$); Anal. calcd for $C_{16}H_{14}N_4O_2S$: C 58.88, H 4.32, N 17.17; found C 58.94, H 4.28, N 17.15.

*5.1.2.4. (E)-4-(4-Hydroxybenzylideneamino)-5-phenyl-2H-1,2,4-triazole-3(4H)-thione (**5d**).* White solid, yield: 84%, m.p. 227–229 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.94 (d, 2H, $J = 8.8$ Hz, C_6H_4), 7.52–7.54 (m, 3H, C_6H_5), 7.75 (d, 2H, $J = 9.2$ Hz, C_6H_4), 7.86–7.89 (m, 2H, C_6H_5), 9.37 (s, 1H, CH═N), 10.42 (s, 1H, OH), 14.16 (s, 1H, NH). IR (KBr), ν/cm^{-1}: 3030, 1606, 1568, 1512, 1356, 1284. MS: $m/z = 297.3$ ($[M+1]^+$); Anal. calcd for $C_{15}H_{12}N_4OS$: C 60.79, H 4.08, N 18.91; found C 60.85, H 4.02, N 18.87.

*5.1.3. General procedure for the synthesis of N-2-2,3,4,6-tetra-O-acetyl-σ-D-glucopyranosyl-4-(arylmethylideneamino)-5-phenyl-2H-1,2,4-triazole-3(4H)-thiones (**1a**–**1d**)*

Compounds **5** (**5a**–**5d**, 2.2 mmol) and 4-dimethylamino pyridine (DMAP, 0.13 g, 1.0 mmol) were suspended in a mixture of 10 mL dichloromethane and 4% aqueous KOH (3.0 mL, 2.4 mmol). The suspension was slowly heated to completely dissolve with vigorously stirring. A solution of 2,3,4,6-tetra-*O*-acetyl-σ-D-glucopyranosyl bromide (0.82 g, 2.0 mmol) in dichloromethane (10 mL) was added dropwise carefully. The reaction solution was heated to reflux and stirred for 5 h under nitrogen atmosphere. The solution was washed with 4% aqueous NaOH (15 mL) and distilled water (20 mL × 3). The organic layer was dried over with anhydrous Na_2SO_4, and the filtrate was distilled under reduced pressure to remove the solvent. The residue was purified by column chromatography (silica gel 60, mesh size 200–300, ethyl acetate/petroleum ether, v/v) to giveproducts **1** (**1a**–**1d**).

*5.1.3.1. N-2-2,3,4,6-tetra-O-acetyl-σ-D-glucopyranosyl-(E)-4-(benzylideneamino)-5-phenyl-2H-1,2,4-triazole-3(4H)-thione (**1a**).* White solid, yield: 61%, m.p. 209–211 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.95 (s, 3H, $COCH_3$); 2.05 (s, 3H, $COCH_3$); 2.07 (s, 3H, $COCH_3$); 2.09 (s, 3H, $COCH_3$); 4.03 (dd, 1H, $J = 4.8$ Hz, $J = 2.0$ Hz); 4.19 (dd, 1H, $J = 12.4$ Hz, $J = 2.0$ Hz); 4.32 (dd, 1H, $J = 12.8$ Hz, $J = 4.8$ Hz); 5.29 (t, 1H, $J = 9.6$ Hz); 5.45 (t, 1H, $J = 9.6$ Hz); 5.96 (t, 1H, $J = 9.6$ Hz); 6.31 (d, 1H, $J = 9.2$ Hz); 7.45–7.52 (m, 5H, C_6H_5); 7.55–7.57 (m, 1H, C_6H_5); 7.86–7.89 (m, 2H, C_6H_5); 7.93–7.96 (m, 2H, C_6H_5); 10.03 (s, 1H, CH═N). IR (KBr), ν/cm^{-1}: 3477, 3062, 2958, 1751, 1064, 1493, 1429, 1369, 1223, 1072, 1036, 924, 847, 758, 692, 607. MS: $m/z = 611.0$ ($[M+H]^+$), 633.0 ($[M+Na]^+$); Anal. calcd for $C_{29}H_{30}N_4O_9S$: C 57.04, H 4.95, N 9.18; found C 57.12, H 4.90, N 9.13.

*5.1.3.2. N-2-2,3,4,6-tetra-O-acetyl-σ-D-glucopyranosyl-(E)-4-(4-chlorobenzylidene amino)-5-phenyl-2H-1,2,4-triazole-3(4H)-thione (**1b**).* White solid, yield: 67%, m.p. 185–187 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.95 (s, 3H, $COCH_3$); 2.05 (s, 3H, $COCH_3$); 2.07 (s, 3H, $COCH_3$); 2.08 (s, 3H, $COCH_3$); 4.03 (dd, 1H, $J = 10.0$ Hz, $J = 2.4$ Hz); 4.19 (dd, 1H, $J = 12.4$ Hz, $J = 2.0$ Hz); 4.32 (dd, 1H, $J = 12.4$ Hz, $J = 5.2$ Hz); 5.28 (t, 1H, $J = 9.6$ Hz); 5.45 (t, 1H, $J = 9.6$ Hz); 5.95 (t, 1H, $J = 9.6$ Hz); 6.30 (d, 1H, $J = 9.6$ Hz); 7.46–7.52 (m, 5H, C_6H_5); 7.80 (d, 2H, $J = 8.4$ Hz, C_6H_4); 7.91 (d, 2H, $J = 8.4$ Hz, C_6H_4); 10.12 (s, 1H, CH═N). IR (KBr), ν/cm^{-1}: 3458, 2970, 1747, 1597, 1491, 1423, 1371, 1227, 1072, 1036, 931, 850, 775, 694, 607. MS: $m/z = 645.0$ ($[M+H]^+$), 667.0 ($[M+Na]^+$); Anal. calcd for $C_{29}H_{29}ClN_4O_9S$: C 53.99, H 4.53, N 8.69; found C 53.92, H 4.52, N 8.72.

*5.1.3.3. N-2-2,3,4,6-tetra-O-acetyl-σ-D-glucopyranosyl-(E)-4-(4-hydroxy-3-methoxy benzylideneamino)-5-phenyl-2H-1,2,4-triazole-3(4H)-thione (**1c**).* White solid, yield: 63%, m.p. 201–203 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.95 (s, 3H, $COCH_3$); 2.05 (s, 3H, $COCH_3$); 2.08 (s, 3H, $COCH_3$); 2.09 (s, 3H, $COCH_3$); 3.98 (s, 3H, OCH_3); 4.04 (dd, 1H, $J = 4.8$ Hz, $J = 2.4$ Hz); 4.20 (dd, 1H, $J = 12.4$ Hz, $J = 2.0$ Hz); 4.33 (dd, 1H, $J = 12.4$ Hz, $J = 4.8$ Hz); 5.29 (t, 1H, $J = 9.6$ Hz); 5.45 (t, 1H, $J = 9.6$ Hz); 5.96 (t, 1H, $J = 9.6$ Hz); 6.31 (d, 1H, $J = 9.6$ Hz); 7.01 (d, 1H, $J = 8.0$ Hz, C_6H_5); 7.19 (dd, 1H, $J = 1.6$ Hz, $J = 8.4$ Hz, C_6H_3); 7.43 (d, 2H, $J = 7.2$ Hz, C_6H_3); 7.51 (d, 2H, $J = 1.6$ Hz, C_6H_5); 7.88–7.90 (m, 2H, C_6H_5); 9.89 (s, 1H, CH═N). IR (KBr), ν/cm^{-1}: 3428, 3066, 2927, 1750, 1603, 1515, 1442, 1372, 1227, 1070, 1038, 959, 871, 769, 695, 653, 577. MS: $m/z = 656.9$ ($[M+H]^+$), 679.0 ($[M+Na]^+$); Anal. calcd for $C_{30}H_{32}N_4O_{11}S$: C 54.87, H 4.91, N 8.53; found C 54.92, H 4.86, N 8.55.

*5.1.3.4. N-2-2,3,4,6-tetra-O-acetyl-σ-D-glucopyranosyl-(E)-4-(4-hydroxybenzylidene amino)-5-phenyl-2H-1,2,4-triazole-3(4H)-thione (**1d**).* White solid, yield: 62%, m.p. 213–215 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.95 (s, 3H, $COCH_3$); 2.05 (s, 3H, $COCH_3$); 2.08 (s, 3H, $COCH_3$); 2.09 (s, 3H, $COCH_3$); 4.04 (dd, 1H, $J = 4.8$ Hz, $J = 2.4$ Hz); 4.20 (dd, 1H, $J = 12.4$ Hz, $J = 2.0$ Hz); 4.33 (dd, 1H, $J = 12.4$ Hz, $J = 4.8$ Hz); 5.30 (t, 1H, $J = 9.6$ Hz); 5.45 (t, 1H, $J = 9.6$ Hz); 5.97 (t, 1H, $J = 9.6$ Hz); 6.32 (d, 1H, $J = 9.2$ Hz); 6.91 (d, 2H, $J = 8.8$ Hz, C_6H_4); 7.43–7.49 (m, 3H, C_6H_5); 7.73–7.75 (m, 2H, C_6H_5); 7.93 (d, 2H, $J = 8.8$ Hz, C_6H_4); 9.65 (s, 1H, CH═N); 10.50 (s, 1H, OH). IR (KBr), ν/cm^{-1}: 3421, 3290, 3064, 2927, 1751, 1604, 1516, 1439, 1371, 1223, 1063, 1038, 924, 850, 771, 692, 604. MS: $m/z = 626.9$ ($[M+H]^+$), 649.0 ($[M+Na]^+$); Anal. calcd for $C_{29}H_{30}N_4O_{10}S$: C 55.58, H 4.83, N 8.94; found C 55.52, H 4.87, N 8.96.

5.2. In vitro cytotoxicity assay

The antiproliferative activities of **5a**–**5d** and **1a**–**1d** were assessed by use of the MTT assay. The MTT assay is a simple nonradioactive colorimetric assay to measure cell cytotoxicity, proliferation, or viability. MTT is a yellow, water-soluble, tetrazolium salt. Metabolically active cells are able to convert this dye into a water-insoluble dark blue formazan by reductive cleavage of the tetrazolium ring. Formazan crystals then can be dissolved and quantified by measuring the absorbance of the solution at 570 nm, and the resultant value is related to the number of living cells.

The effect of all compounds on the cells' proliferation efficiency was determined after 24 h incubation with cells. To determine cell proliferation, the MCF-7 cell lines and Bel-7402 cell lines were individually plated at a density of 1×10^4 cells/well in 96-well plates at 37 °C in 5% CO_2 atmosphere. After 24 h of culture, the medium in the wells was replaced with the fresh medium containing compounds of varying concentrations respectively. In the microplate, five wells were tested in parallel for each concentration of the synthesized compounds. After 24 h, 10 μL of MTT dye solution (5 mg/mL in phosphate buffer pH 7.4) was added to each well and incubated for 4 h at 37 °C and 5% CO_2 for exponentially growing cells and 10 min for steady-state confluent cells. The formazan crystals were solubilized with 100 μL of DMSO and the solution was vigorously mixed to dissolve the reacted dye. The absorbance of each well was read on a microplate reader (DYNATECH MR7000 instruments) at 570 nm. The spectrophotometer was calibrated to zero using culture medium without cells. We selected inhibitory effect to evaluate side effects of the silica-coated fluorescent compounds to cells proliferation. The inhibitory effect of all compounds was calculated as percentage inhibition in comparison

to the value obtained in untreated well to which no compounds were added.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2009.05.030.

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