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Efficient synthesis and antimicrobial activity of some novel S-β-D-glucosides of 5-aryl-1,2,4-triazole-3-thiones derivatives

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ABSTRACT

A series of 3-S-β-D-glucosides-4-arylideneamino-5-aryl-1,2,4-triazoles were rationally designed and synthesized according to the principle of superposition of bioactive substructures by the combination of 1,2,4-triazole, Schiff base and glucosides. The structures of the target compounds have been characterized by ^{1}H NMR, ^{13}C NMR, IR, MS and HRMS. All the newly synthesized compounds have been evaluated for their antimicrobial activities in vitro against *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8099) as well as *Monilia albican* (ATCC 10231). The bioactive assay showed that most of the tested compounds displayed variable inhibitory effects on the growth of the Gram-positive bacterial strain (*Staphylococcus aureus*), Gram-negative bacterial strains (*Escherichia coli*) and fungal strains (*Monilia albican*). All the target compounds exhibited better antifungal activity than antibacterial activity. Especially, compounds **6b**, **6c**, **6f**, **6j**, **6k** and **6l** showed excellent activity against fungus *Monilia albican* with MIC values of 16 μg/mL.

1,2,4-Triazoles nucleus and their derivatives have emerged 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] Several compounds possessing 1,2,4-triazole nucleus are clinically used drugs, for example, fluconazole, itraconazole and terconazole.[7,8] These drugs act by displacing lanosterol from cytochrome $P450_{14\alpha DM}$ and, in this manner, block the biosynthesis of ergosterol, an essential component of the fungal cell membrane. Cytochrome $P450_{14\alpha DM}$ oxidatively removes the 14-α-methyl group of lanosterol by oxygenization and NADPH.[9]

On the other hand, glycosylsulfanyl heterocycles have attracted much attention because of their biological activity and in particular because of their inhibition of the activity of enzymes.[10,11] They have excellent chemoselectivity in glycosylation processes as both donors and acceptors, particularly via reaction processes that involve active and latent glycosylation protocols.[10] The synthesis and investigation of biological activity of 1,2,4-triazole glycosides have been stimulated by the finding that Ribavirin, β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, is remarkable in its broad spectral activity against DNA and RNA viruses.[12]

Schiff bases have also been reported a broad range of biological activities, including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral and antipyretic properties.[13] Schiff base hydrazone ligands containing 1,2,4-triazole nucleus have aroused great interest in chemistry and biology for many years due to their facile synthesis and wide applications.[14]

Prompted by these observations and in continuation of search for bioactive molecules, we designed and synthesized a series of novel 3-S-β-D-glucosides-4-arylideneamino-5-aryl-1,2,4-triazoles. In our design we emphasized the strategy of combining three molecules, 1,2,4-triazole nucleus, Schiff base and glycosyl, in one frame. The newly synthesized compounds **6a–6l** was screened for their in vitro antibacterial activities against *Escherichia coli*, *Staphylococcus aureus* and *Monilia albican*. The bioactive assay showed that most of the tested compounds displayed variable inhibitory effects on the growth of the Gram-positive bacterial strain, Gram-negative bacterial strains and fungal strains.

The route employed for synthesis of the target compounds is shown in Scheme 1. Hydrazides (**1a** and **1b**) and potassium aroyl dithiocarbazate (**2a** and **2b**) were obtained by the reported method.[15,16] The salt **2** underwent ring closure with an excess of hydrazine hydrate to give the key intermediate 4-amino-1,2,4-triazole-3-thione **3**. The ring closure reaction may have a nucleophilic substitution–elimination mechanism (Scheme 2). The ring closure reaction mechanism is discussed as Scheme 2. The Schiff base **4a–4l** were produced by reaction of 4-amino-1,2,4-triazole-3-thi-

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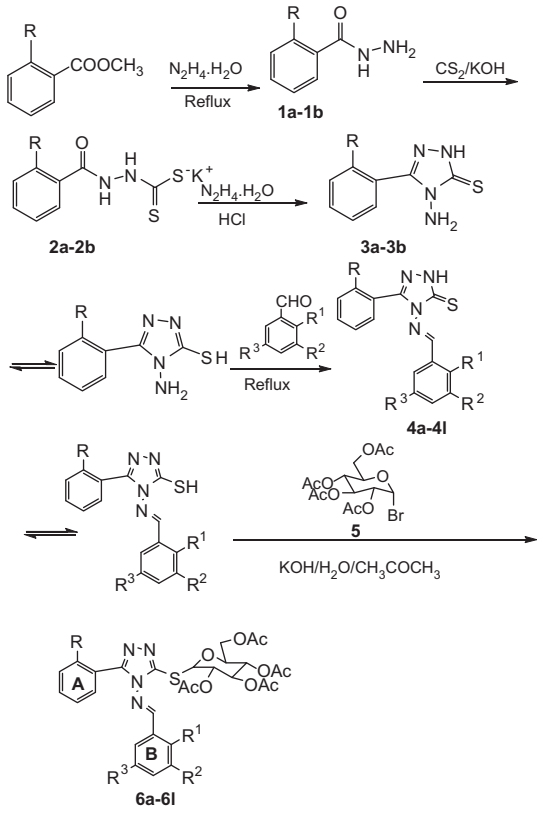
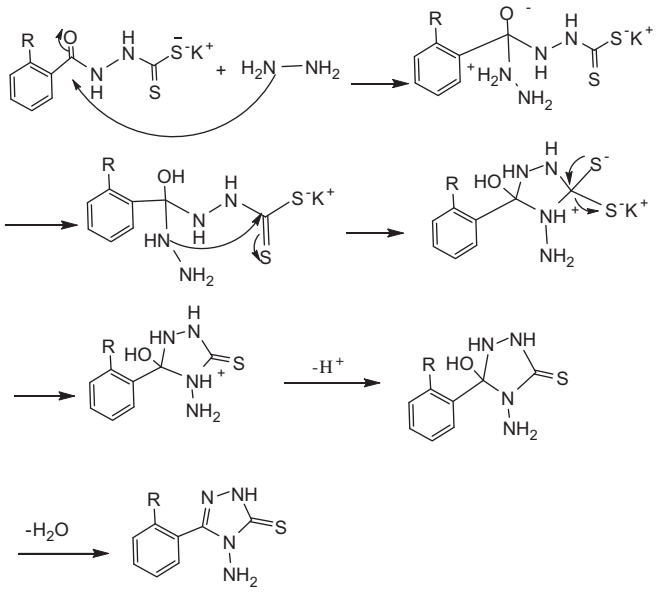


a:R=OH,R^1=Cl,R^2=H,R^3=H; b:R=OH,R^1=OH,R^2=Br,R^3=Br;
c:R=OH,R^1=OH,R^2=H,R^3=Br d:R=OH,R^1=H,R^2=H,R^3=H;
e:R=OH,R^1=OH,R^2=H,R^3=H; f:R=OH,R^1=OH,R^2=H,R^3=Cl;
g:R=H,R^1=OH,R^2=H,R^3=H; h:R=H,R^1=H,R^2=H,R^3=H;
i:R=H,R^1=Cl,R^2=H,R^3=H; j:R=H,R^1=OH,R^2=Br,R^3=Br;
k:R=H,R^1=OH,R^2=H,R^3=Cl; l:R=H,R^1=OH,R^2=H,R^3=Br;

Scheme 1. Synthetic pathway of compounds **6a–6l**.



Scheme 2. Representative postulated mechanism of ring closure reaction for synthesis of compound **3**.

one derivative **3** with appropriate aryl aldehydes heated under reflux in ethanol containing a few drops of hydrochloric acid as a catalyst. 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide compound **5** was obtained by the reported method.[17] Compounds **4a–4l** and **5** were used as starting materials to prepare new functionalized 1,2,4-triazoleS-β-D-glucosides. Thus, glucosidation of compounds **4a–4l** with 1.2 M equiv of 2,3,4,6-tetra-*O*-acetyla-α-D-glucopyranosyl bromide **5** afforded a chromatographically separable product, namely, 3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-1-thio)-4-arylideneamino-5-aryl-1,2,4-triazoles **6a–6l**. To optimize the synthetic reaction condition for compounds **6a–6l**, we tested a mild inorganic base (KOH) and two kinds of organic base (pyridine and triethylamine), potassium hydroxide proved to be the most effective one, giving the desired compounds in better yield and as the single product.

The structures of all newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS and HRMS studies.

Compounds 4-amino-1,2,4-triazole-3-thiones **3** may exist in tautomeric equilibrium in solution, which enables two possible configurations of the sulfide group as thione and thiol (Fig. 1), in the present study, the thione structure was dominated in the solid state. These constitutional isomers were distinguished by usual spectroscopic methods of IR and ^{1}H NMR. The appearance of a C=S absorption peak in the region 1248–1260 cm^{-1} indicated that the triazoles are in their thione form. The ^{1}H NMR spectra of compounds **3** (measured in DMSO-d_6) exhibited the NH signals (NH function of the triazoline ring) as a singlet at 13.86 ppm which also supports the proposed thione structure[18]. A signal in the region 5.62 ppm, integrating to two protons, was assigned to NH_2 protons.

The structures of compounds **4a–4l** were confirmed on the basis of both microanalysis and spectral data. These compounds exist in the tautomeric forms in the solid state as indicated by their IR spectra similarly as compounds **3**. The ^{1}H NMR spectra of these compounds (measured in DMSO-d_6) support the predominant 2,4-dihydrothione structure (NH signals of these compounds appeared as a singlet at δ 14.14–14.20, consistent with related reported thione structures.[18–20]) Furthermore, their proton NMR spectra lacked singlets characteristic of NH_2 protons, and showed new singlet characteristic for –N=CH– proton recorded in the range of 9.14–9.96 ppm (integration for one proton) confirmed the conversion of compounds **3** into 4-arylideneamino-5-aryl-1,2,4-triazoles-3-thhone derivatives **4a–4l**.

The structures identification for compounds **6a–6l** was based on spectroscopic and chemical evidences. The *S*-β-D-configuration of compounds **6a–6l** is supported by their ^{1}H NMR data, which revealed the anomeric proton signal around δ 5.4 with a coupling constant value of 9.9–10.5 Hz consistent with the reported data for S-β-D glycosides.[21] In the IR spectra, the disappearance of a C=S absorption peak in the region 1252–1278 cm^{-1} also supports the proposed structure. Meanwhile, four singlets in the region of δ 1.91–2.13 were attributed to four acetyl groups.

The in vitro minimum inhibitory and bactericidal concentrations (MICs and MBCs) of compounds against Gram-positive, Gram-negative bacteria as well as fungi were determined by the method of National Committee for Clinical Laoratory (NCCLS).[22] The in vitro minimal inhibitory concentration and bactericidal concentration values are summarized in Table 1. Fluconazole triclosan and gentamicin were used as standard drugs.

MICs and MBCs of tested compounds showed that most of the compounds displayed better antifungal activity than antibacterial activity. However, it was revealed that all newly synthesized compounds exhibited poor antibacterial activity compared to that of the control drugs triclosan and gentamicin.

The results obtained on Gram-negative bacteria showed that compounds **6b** (MIC = 16 μg/mL, MBC = 16 μg/mL) and **6j** (MIC = 16 μg/mL, MBC = 16 μg/mL) exhibited much higher activities than the other compounds,while compound **6g** showed weak activity against *Escherichia coli*.

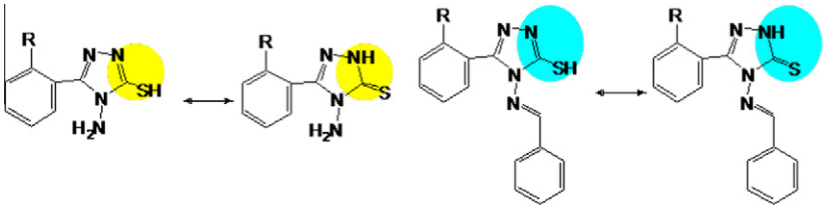


Figure 1. Schematic representation of thione-thiol tautomerism of triazoles.

Table 1
In vitro antimicrobial activities for some synthetic compounds **6a–6l** expressed as MIC and MBC (μg/mL)

Compd.	Gram-negative bacteria *Escherichia coli*		Gram-positive bacteria *Staphylococcus aureus*		Fungus *Monilia albican*	
	MIC	MBC	MIC	MBC	MIC	MBC
6a	128	128	128	128	32	64
6b	16	16	32	32	16	32
6c	128	128	64	128	16	16
6d	128	256	128	256	32	32
6e	64	64	128	128	64	64
6f	128	256	128	128	16	32
6g	—	—	64	128	32	64
6h	128	128	64	64	32	64
6i	128	128	128	128	64	64
6j	16	16	16	32	16	16
6k	128	128	64	128	16	32
6l	128	128	32	32	16	16
Fluconazole	128	128	16	32	2	4
Triclosan	2	4	4	4	32	64
Gentamicin	2	4	2	2	4	4

MIC: minimum inhibitory concentration (the lowest concentration that inhibited the bacterial growth). MBC: minimum bactericidal concentration (the lowest concentration at which no bacterial growth was observed).
—: Indicates the compound has no activity.

The results mentioned in Table 1 indicated that compounds **6b**, **6j** and **6i** have shown a promising antibacterial activity against *Staphylococcus aureus* with MIC values of 32, 16 and 32 μg/mL, respectively.

The MICs of antifungal results revealed that compounds **6b**, **6c**, **6f**, **6j**, **6k** and **6l** showed excellent activity with MIC values of 16 μg/mL than rest of the compounds.

From the in vitro antimicrobial activity data, preliminary structure–activity relationship of the synthesized compounds **6a–6l** was studied. The compounds **6a–6l** exhibited good antibacterial activity against bacteria *Staphylococcus aureus*, *Escherichia coli* and antifungal activity against fungi *Monilia albican*. All the compounds exhibited better antifungal activity than antibacterial activity.

Meanwhile, the halogen substituents on the phenyl ring **A** as well as **B** in compounds **6a–6l** affected the antibacterial activity to a certain extent. The presence of an electron-withdrawing substituent($R^2 = R^3 = Br$) on the phenyl ring **B** is beneficial for antibacterial activity, such as compound **6j**, which showed better activity against *Escherichia coli*, and also registered higher inhibitory effect against *Staphylococcus aureus* and *Monilia albican* than most of the other compounds. To compare the antimicrobial activity of the bromine and chlorine moiety on the phenyl ring, we find compound **6c** ($R^3 = Br$, MIC = 64 μg/mL) showed higher inhibitory effect against *Staphylococcus aureus* than compound **6f** ($R^3 = Cl$, MIC = 128 μg/mL). While, the antifungi activity of **6e** ($R^1 = OH$, MIC = 64 μg/mL) and **6d** ($R^1 = H$, MIC = 32 μg/mL) indicate the introduction of –OH group at R^1 decrease the activity compared to non –OH group at R^1.

In conclusion, in this study we described an efficient method for the preparation of 3-S-β-D-glucosides-4-arylideneamino-5-aryl-1,2,4-triazoles. All the compounds **6a–6l** were tested for their in vitro antibacterial activity against *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8099) and for their antifungal activity against fungus *Monilia albican* (ATCC 10231). Most of the synthesized compounds exhibited moderate to good activity towards Gram positive and Gram negative bacteria as well as fungi species. Compounds **6b** (MIC = 16 μg/mL, MBC = 16 μg/mL) and **6j** (MIC = 16 μg/mL, MBC = 16 μg/mL) appeared more effective against *Escherichia coli* than the other compounds (MIC = 64–128 μg/mL). From structure–activity relationship, we can conclude that glycosylation improved the biological activity of these compounds against Gram negative bacteria. The introduction of halogen atoms (–Br) into the pharmacophore structure increased the antimicrobial activity. Introduction of –OH group at R^1 on **B** ring decreased in activity against *Monilia albican*.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.038.

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