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Synthesis of Schiff and Mannich bases of new *s*-triazole derivatives and their potential applications for removal of heavy metals from aqueous solution and as antimicrobial agents†

Zeinab A. Hozien,[a] Ahmed F. M. EL-Mahdy,*[ab] Ahmad Abo Markeb,[a] Laila S. A. Ali[a] and Hassan A. H. El-Sherief*[a]

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An efficient, simple, and one-pot double Mannich reaction was performed for the synthesis of cyclized 2-methyl-6-substituted-6,7-dihydro-5*H*-*s*-triazolo[5,1-*b*]-1,3,5-thiadiazines *via* a reaction of 5-methyl-1*H*-*s*-triazole-3-thiol (**1**) with formaldehyde and primary aliphatic amines in ethanol at room temperature, while with primary aromatic amines, uncyclized 3-methyl-1-((substituted-amino)methyl)-1*H*-*s*-triazole-5-thiols were produced. Under Mannich reaction conditions, 4-amino-3-methyl-*s*-triazole-5-thiol (**8**) reacted with formaldehyde only in boiling ethanol or at room temperature to afford 3-methyl-5,6-dihydro-*s*-triazolo[3,4-*b*]-1,3,4-thiadiazole without incorporation of secondary amine. Furthermore, after reaction of compound **8** with aromatic aldehydes under different reaction conditions, uncyclized Schiff's bases were produced. Therefore, reaction of these Schiff's bases with primary or secondary amines with formaldehyde in ethanol at room temperature afforded the corresponding Mannich bases **13–14**. The structures of all new compounds were confirmed using spectral analysis. Furthermore, most of the synthesized derivatives showed high efficiency for removal of Pb^{2+}, Cd^{2+}, Ca^{2+}, and Mg^{2+} from aqueous solutions, as well as antimicrobial activity.

1. Introduction

The therapeutic importance of *s*-triazole derivatives has been documented in the numerous roles they play as antimicrobial,[1–4] antiviral,[5] antifungal,[6] anti-inflammatory,[7] antitumoral[8,9] anticonvulsant,[10,11] antidepressant,[12] hypoglycemic,[13] antihypertensive,[14] analgesic,[15] plant growth regulating,[16] and anticoagulant agents.[17] For instance, commonly prescribed drugs that possess an *s*-triazole nucleus are ribavirin, rizatriptan, alprazolam, which are used as antiviral, antimigraine, and anxiolytic agents, respectively, as well as fluconazole and itraconazole, which are used as antifungal agents.[18–22] In addition, derivatives of *s*-triazole have been used for environmental,[23] industrial,[24] and agricultural applications.[25–27]

Due to the strong biological and medical activities of *s*-triazole moieties and their widespread use, it is necessary to synthesise new *s*-triazole derivatives in a shorter amount of time with minimum steps under mild reaction conditions. Therefore, we selected the attractive Mannich reaction to quickly synthesise various derivatives of *s*-triazole under mild conditions with high yield. In the last decade, the Mannich reaction has been highly utilized as one of the potential multi component reactions (MCRs) due to its performance in the building of carbon–nitrogen (C–N) and carbon–carbon (C–C) single bonds.[28] It is well-known that compounds with one active hydrogen atom undergo a single Mannich reaction, while compounds with two neighboring active hydrogen atoms undergo a double Mannich reaction.[29–31] Recently, we reported the triple Mannich reaction of 6-amino-2-(ethylthio)pyrimidin-4(3*H*)-one, which contains three active hydrogen atoms, and the quadruple Mannich reaction of 6-amino-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one, which contains four active hydrogen atoms.[32,33]

Biologically, the efficient route for the modification of biologically active compounds is the amino alkylation of aromatic substrates by Mannich reaction.[34] Therefore, several applications of Mannich bases have been reported as potential biological agents. For example, Mannich bases can be used as anticancer, antimalarial, antitubercular, vasorelaxant, and analgesic drugs.[35–40] In addition, a literature survey reveals that the incorporation of *N*-methylpiperazine, piperidine, or morpholine rings into Mannich bases results in antimicrobial activity.[41,42] Furthermore, we reported the application of a double Mannich reaction as an efficient route for the synthesis

[a]*Chemistry Department, Faculty of Science, Assiut University, Egypt. E-mail: dr_hassanahmed@yahoo.com*

[b]*Department of Materials and Optoelectronic Science, National Sun Yat-sen University, Kaohsiung, 80424, Taiwan. E-mail: ahmedelmahdy@mail.nsysu.edu.tw*

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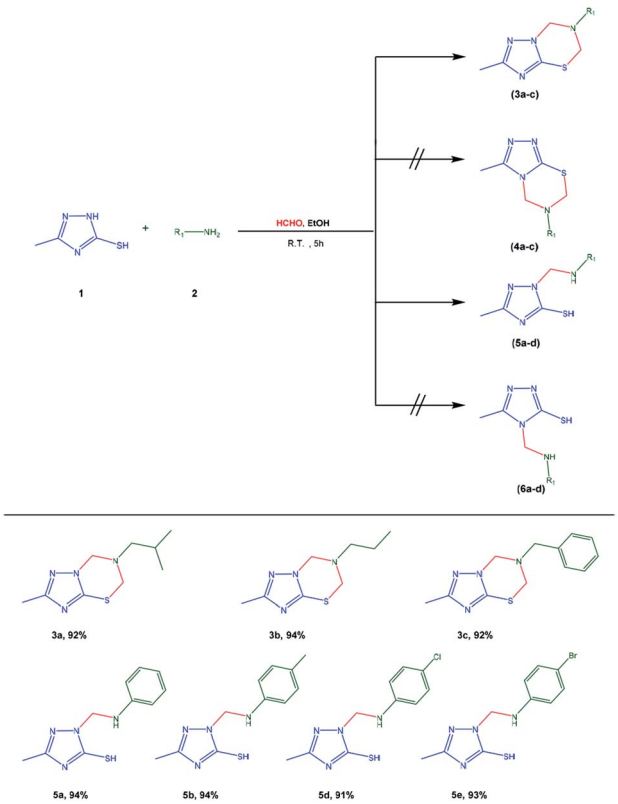
of biologically active triazolothiadiazine,[30] pyrimidothiadiazine,[43] pyrazolopyrimidine,[44] and thiadiazinobenzimidazole.[45] Moreover, one of the most concise and effective approaches to the preparation of the biologically active *s*-triazolo-1,3,5-thiadiazines is based on the double Mannich-type condensation of 2-mercaptoazole or -azine derivatives with primary amines and an excess of formaldehyde.[46]

Although several pollutants have been registered as contaminants of emerging concern in water, no studies have been reported for the applications of triazole Mannich base compounds in water treatment processes. When metal and heavy metal ions exist in water above the maximum contaminated levels, environmental problems can result, according to the World Health Organization (WHO) and the U.S. Environmental Protection Agency (EPA).[47,48] As a result of these facts, this work presents the synthesis of some new 2-methyl-6-substituted-6,7-dihydro-5*H*-*s*-triazolo[5,1-*b*]-1,3,5-thiadiazines, 3-methyl-1-((substituted-amino)methyl)-1*H*-*s*-triazole-5-thiol, 3-methyl-5,6-dihydro-*s*-triazolo[3,4-*b*]-1,3,4-thiadiazole, and Mannich bases possessing both the *s*-triazole nucleus as well as morpholine, *N*-methylpiperazine, or piperidine moieties and the investigation of their biological activities as well as their efficiency for the removal of metal and heavy metal ions from water.

2. Results and discussion

2.1. Chemistry

In the context of our progressive research on the preparation of creative heterocyclic frameworks through minimum steps and under mild reaction conditions,[49–51] including the Mannich reaction, with this study, we endeavored to investigate the behavior of 5-methyl-1*H*-*s*-triazole-3-thiol (**1**) and 4-amino-5-methyl-4*H*-*s*-triazole-3-thiol (**8**) as nucleophiles with both primary aliphatic, aromatic, and secondary amines. Interestingly, when compound **1** was allowed to react with one equivalent of a primary aliphatic amine such as an isobutyl amine, propyl amine, or benzyl amine **2** and excess of aqueous formaldehyde (37%) either in boiling ethanol or at room temperature for 5 hours, single products with unproven structures were produced (Scheme 1). In addition to mass spectroscopy, Fourier transform infrared (FTIR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy confirmed that the products might be cyclized 2-methyl-6-substituted-6,7-dihydro-5*H*-*s*-triazolo[5,1-*b*]-1,3,5-thiadiazines (**3a–c**) or other isomeric products such as 3-methyl-6-substituted-6,7-dihydro-5*H*-*s*-triazolo[3,4-*b*]-1,3,5-thiadiazines (**4a–c**). The FTIR spectra of the isolated products revealed the appearance of specific absorption bands at wavenumbers of 2960–2870, 1618–1580, and 1495–1427 cm^{-1} for C–H aliphatic, C=N, and C=C vibration bonds, respectively. The isolated product from the Mannich reaction using benzylamine as a nucleophile exhibited an absorption band at 3030 cm^{-1} that was attributed to C–H aromatic vibration bonds. In addition, all isolated products lacked the characteristic NH absorption band. The ^{1}H-NMR spectra of the isolated products showed singlet signals at 4.60–4.69 and 5.05–5.09 ppm that corresponded to two methylene groups, confirming the reaction



Scheme 1 Mannich reaction of 3-methyl-1*H*-*s*-triazole-5-thiol.

of compound **1** with two equivalent molecules of formaldehyde and the occurrence of a double-Mannich reaction. The other protons of CH_3 triazole appeared at the expected chemical shift of $\delta = 2.35$–2.38 ppm. The isolated product from isobutyl amine exhibited signals at $\delta = 0.94$–0.96, 1.67–1.98, and 2.54 ppm for $CH(C\underline{H}_3)_2$, $C\underline{H}(CH_3)_2$, and $C\underline{H}_2$–CH protons, while the isolated products from propyl and benzyl amines exhibited signals at $\delta = 0.96$ ppm for $CH_2C\underline{H}_3$, 1.60 ppm for $C\underline{H}_2CH_3$, 2.73 ppm for $N–C\underline{H}_2CH_2$, 3.93 ppm for $C\underline{H}_2$-Ph, and 7.29–7.37 ppm for aromatic protons. The ^{13}C-NMR spectra further confirmed the performance of the double Mannich reaction by the appearance of two signals at $\delta = 68.03$ and 58.33 ppm for the methylene carbons. The mass spectra of the products from isobutyl, propyl, and benzyl amines revealed a molecular ion peak of 212.08 [M^+], 198.02 [M^+], and 246.09 [M^+], respectively. All these findings confirmed the performance of the double Mannich reaction, while expecting that the isolated products were **3a–c** or **4a–c**.

Recently, Haijian *et al.* reported the synthesis of *s*-triazolo[3,4-*b*]-1,3,5-thiadiazines through the double Mannich reaction of 5-aryl-2*H*-*s*-triazole-3(4*H*)-thiones with polyformaldehyde and *S*-(−)-α-phenylethylamine in the presence of hydrogen chloride as an acidic catalyst.[52] The formation of *s*-triazolo[3,4-*b*]-1,3,5-thiadiazines rather than *s*-triazolo[5,1-*b*]-1,3,5-thiadiazines due to the protonation of highly basic N-2 directed the Mannich cyclization step to occur on N-4. Consequently, this reported result supports the formation of products **3a–c** due to the higher nucleophilicity of N-2 as compared to N-4 in the neutral

condition, and therefore, precluded the formation of products **4a–c** as previously reported.[30,43]

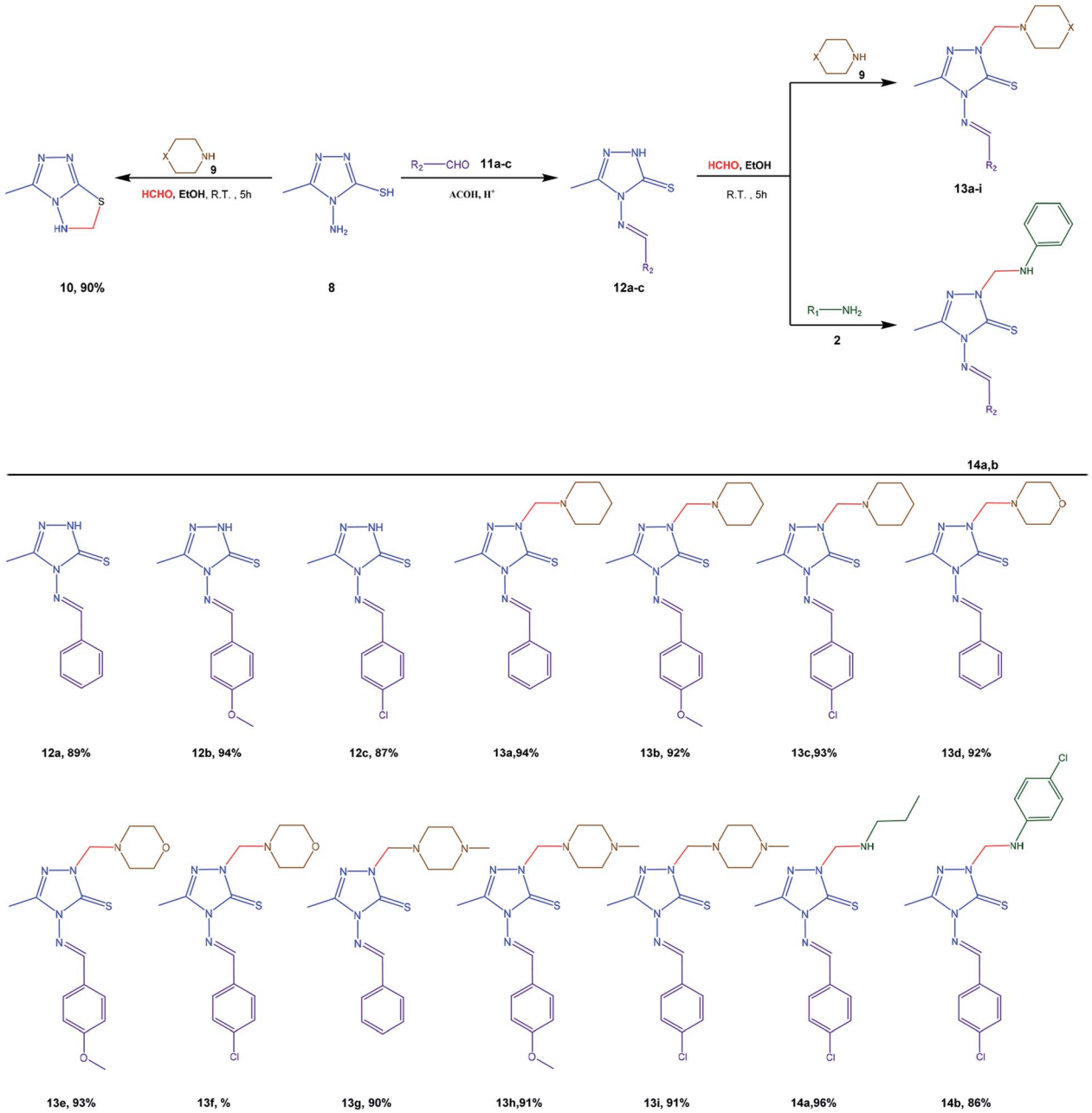
In comparison, after treatment of compound **1** with primary aromatic amines such as aniline, *p*-toluidine, *p*-chloroaniline, and *p*-bromoaniline **2** under the same conditions, single products of the uncyclized 3-methyl-1-((substituted-amino)methyl)-1*H*-*s*-triazole-5-thiols (**5a–d**) rather than 3-methyl-4-((substituted-amino)methyl)-4*H*-*s*-triazole-3-thiol (**6a–d**) were produced due to the higher nucleophilicity of N-2 as compared to N-4 in the neutral condition (Scheme 1). The FTIR spectra of compounds **5a–d** were characterized by the appearance of absorption bands at wavenumbers of 3369–3340, 3100–3034, 2987–2914, and 1594–1570 cm^{-1} assigned to NH, aromatic C–H, aliphatic C–H, and C=N vibration bonds. In addition, the ^{1}H-NMR spectra of compounds **5a–d** showed two characteristic signals: a doublet at δ = 5.37–5.52 ppm for the methylene (CH_2–NH) group and a triplet signal at δ = 6.79–7.13 ppm for the secondary amine (CH_2–NH) group, confirming the performance of the singlet Mannich reaction and the reaction of compound **1** with one equivalent molecule of formaldehyde. The other protons for SH and methyl groups appeared at δ = 12.60–13.25 ppm (exchange with D_2O) and 2.18–2.40 ppm, respectively. As an example, the mass spectra of the obtained compounds **5a**, **5c**, and **5d** revealed a molecular ion peak at the value of 220 [M^+], 254 [Cl^{35}, M^+], 256 [Cl^{37}, M^+ + 2] and 298 [Br^{79}, M^+], 300 [Br^{81}, M^+ + 2], respectively. Similarly, the treatment of compound **1** with secondary amine such as morpholine under Mannich reaction conditions resulted in the production of 3-methyl-1-(morpholinomethyl)-1*H*-*s*-triazole-5-thiol (**7**), as shown in Scheme S1.† The FTIR spectrum of compound **7** exhibited several absorbance bands at 3100, 2983, and 1569 cm^{-1} for the SH, aliphatic C–H, and C=N vibration bonds. The ^{1}H-NMR spectrum of compound **7** showed a singlet signal at 4.99 ppm corresponding to the methylene group, in addition to the other methyl, (N–$(CH_2)_2$, O–$(CH_2)_2$), and thiol protons at δ = 2.35, 2.76, 3.70, and 12.50 ppm, respectively. The mass spectrum of the obtained compound **7** exhibited a molecular ion peak at the value of 214.03 [M^+].

We examined the behavior of 4-amino-3-methyl-*s*-triazole-5-thiol (**8**) towards the Mannich reaction through the treatment of compound **8** with secondary amine such as morpholine or piperidine (**9**) and excess of aqueous formaldehyde (37%) either in boiling ethanol or at room temperature for 5 hours. The reaction proceeded to yield a single product, and FTIR and ^{1}H-NMR spectroscopy indicated that it was 3-methyl-5,6-dihydro-*s*-triazolo[3,4-*b*]-1,3,4-thiadiazole (**10**), which highlighted the performance of the Mannich reaction between the amino (NH_2) and thiol (SH) groups of the *s*-triazole moiety without the incorporation of secondary amine molecules (Scheme 2). This result was further confirmed by the reaction of compound **8** with formaldehyde refluxing in ethanol or in acetic acid in the presence of sulfuric acid as catalyst to afford the same product **10**. The FTIR spectrum of compound **10** exhibited adsorption bands at 3236 (NH), 2989 (aliphatic C–H), and 1586 cm^{-1} (C=N), while its ^{1}H-NMR spectrum exhibited a doublet signal at δ = 5.34 ppm that was attributed to the methylene group, in addition to the other NH and methyl protons at δ = 7.38 and 2.31 ppm, respectively. The mass spectrum of the obtained compound **10** showed a molecular ion peak at the value of 140 [M^+].

In the condensation reaction of compound **8** with aromatic aldehydes such as benzaldehyde, *p*-methoxybenzaldehyde, and *p*-chlorobenzaldehyde (**11a–c**) either in boiling acetic acid or at room temperature, uncyclized Schiff's base 4-arylidenamino-3-methyl-*s*-triazole-5-thiones (**12a–c**) were produced, as shown in Scheme 2. Several trials were attempted to cyclize the Schiff's bases to *s*-triazolo[3,4-*b*]thiadiazoles, using different reaction conditions as previously reported, but they failed.[53] As a result of this condensation reaction of compound **8**, the product **12** contains one active hydrogen atom (NH) that might undergo a single Mannich reaction. Therefore, when treated with formaldehyde and secondary amine such as morpholine, piperidine, or *N*-methylpiperazine (**9**) in ethanol at room temperature, compounds **12a–c** afforded 4-arylideneamino-3-methyl-1-(*N*-morpholinomethyl)-*s*-triazole-5-thiones (**13d–f**) and 4-arylid eneamino-3-methyl-1-(*N*-methylpiperazinomethyl)-*s*-triazole-5-thiones (**13g–i**), respectively (Scheme 2). In addition, in the reaction of 4-((4-chlorobenzylidene)amino)-3-methyl-2,4-dihydro-3*H*-*s*-triazole-5-thione (**12c**) with primary amine such as propylamine or *p*-chloroaniline (**2**) under the same conditions, 4(4-chlorobenzylideneamino)-3-methyl-1-((propyl-amino) methyl)-1*H*-*s*-triazole-3-thione and 4(4-chlorobenzy-lideneamino)-3-methyl-1-((4-chlorophenylamino)methyl)-1*H*-*s*-triazole-3-thione (**14a,b**) were afforded, respectively (Scheme 2).

The IR spectra of compounds **12a–c** lacked NH_2 absorption bands and exhibited absorption bands at 3110–3102, 3080–3056, 2960–2942, and 1656–1586 cm^{-1} for the NH, aromatic C–H, aliphatic C–H, and C=N vibration bonds, respectively, while compounds **13a–i** lacked NH absorption bands and showed absorption bands at 3090–3015, 2968–2809, and 1610–1525 cm^{-1} that were attributed to aromatic C–H, aliphatic C–H, and C=N vibration bonds, respectively. In addition, compounds **14a,b** exhibited the NH absorption band at 3389–3235 cm^{-1}. The ^{1}H-NMR spectrum of compounds **12a–c** showed singlet signals at δ = 2.33–2.37, 9.70–10.00, and 13.57–13.77 (exchange with D_2O) ppm for the methyl (CH_3), imine (N=CH), and amine (NH) groups, respectively, as well as the other aromatic protons that appeared at δ = 7.14–7.92 ppm, while compound **12b** exhibited an additional singlet signal at δ = 3.68 ppm that was attributed to the methoxy (OCH_3) group. The ^{1}H-NMR spectra of compounds **13a–i** showed a singlet signal at δ = 5.00–5.13 ppm corresponding to the methylene (N–CH_2–N) group, in addition to the other protons at δ = 2.40–2.48 ppm for the CH_3 group, 7.01–7.88 ppm for the aromatic protons, and 9.70–10.60 ppm for the imine (–N=CH) group.

Compounds **13a–c** showed signals at δ = 1.27–1.69 ppm for three methylene ($3CH_2$ piperidine) groups and at δ = 2.77–2.80 ppm for two methylene (N($C\underline{H}_2$)$_2$ piperidine) groups. Compounds **13d–f** showed signals at δ = 2.70–2.85 and 3.60–3.71 ppm that were attributed to the methylene N$(CH_2)_2$ and O$(CH_2)_2$ protons of the morpholine moiety, while compounds **13g–i** showed signals at δ = 2.44–2.89 and 2.27–2.28 ppm for four methylene (N$(CH_2)_4$N) and (N–CH_3) protons of the piperazine moiety. In addition, compounds **13b**, **13e**, and **13h**



Scheme 2 Mannich reaction of 4-amino-5-methyl-4*H*-*s*-triazole-3-thiol (**8**) and its benzylidene derivatives (**12a–c**).

showed a singlet signal at $\delta = 3.88$–4.00 ppm that was attributed to the methoxy group. Furthermore, the ^{1}H-NMR spectra of compounds **14a,b** were characterized by the appearance of signals at $\delta = 2.38$–2.41 ppm for the methyl (CH_3), $\delta = 5.30$–5.40 ppm for the NH group, $\delta = 5.52$–5.53 ppm for the methylene (N–CH_2–N), and $\delta = 10.46$–10.52 ppm for the imine (N=CH) group, in addition to the other protons for propyl and phenyl groups.

The ^{13}C-NMR spectrum of the isolated product **12a**, as an example, exhibited signals at 163.78, 161.68, 148.86, and 11.22 ppm that were attributed to the C=S, C=N, N=CH, and methyl carbons and other expected aromatic carbons. The ^{13}C-NMR spectra of isolated products **13a**, **13d**, and **13h** showed signals at $\delta = 162.86$, 147.08, and 69.80 ppm corresponding to C=S, N=CH, and methylene (N–CH_2–N) carbons, in addition to the piperidine, morpholine, and *N*-methylpiperazine signals at $\delta = 52.79$ ($N(CH_2)_2$), 25.94 ($3CH_2$), 66.50 ($O(CH_2)_2$), 50.87 ($N(CH_2)_2$) and 54.90($2CH_2$), and 50.40 ($2CH_2$), respectively. Moreover, compound **13h** exhibited two additional signals at $\delta = 55.50$ and 46.01 ppm that were attributed to the methoxy (–O–CH_3) and methyl (N–CH_3) carbons, respectively. Finally, the mass spectra of compounds **13a–i** and **14a,b** showed the

expected molecular ion peaks at m/z = 315 [M^+], 345.30 [M^+], 372 [M + Na], 317 [M^+], 347 [M^+] 351.00 [M^+], 330 [M^+], 360.08 [M^+], 364.93 [M^+], 323 [M^+] and 392 [Cl^{35}, M^+], respectively.

2.2. Proposed mechanisms

The proposed mechanism of the obtained products **3a–c** and **5a–d** is illustrated in Scheme S2.† In the first step, a typical condensation reaction between the primary aliphatic amines with formaldehyde forms imines **15**, which are attached by (NH-1) of the starting compound **1** to provide the uncyclized intermediates **16a–c**. These intermediates **16a–c** reacted with another molecule of formaldehyde followed by dehydration and intramolecular cyclization to generate the cyclized compounds **3a–c**. The stability of the uncyclized intermediates **5a–d** with primary aromatic amines may be due to the resonance with the aryl moiety. Compound **10** can be formed through the nucleophilic attack of the amino (NH_2) group of the starting compound **8** with formaldehyde followed by intramolecular cyclization, as shown in Scheme S3.†

2.3. Screening of heavy metals and metal ion removal from aqueous solution

In this work, the compounds produced from the interaction of 5-methyl-1*H*-*s*-triazole-3-thiol (**1**) with primary aliphatic and aromatic amines were investigated for their ability to remove Pb^{2+}, Cd^{2+}, Ca^{2+}, and Mg^{2+} from aqueous solution. Fig. 1 illustrates that the removal percentage of metal and heavy metal ions was increased when the size of the side chain increased (**3a–c**). For example, 74.30%, 73.61%, and 72.29% of the Pb^{2+} in solution was removed using **3c**, **3a**, and **3b**, respectively. The removal efficiencies for Cd^{2+} were found to be 70.95%, 72.00%, and 72.24% for **3b**, **3a**, and **3c**, respectively. In addition, 62.45%, 61.26%, and 53.92% of Ca^{2+}, and 71.42%, 70.48%, and 70.27% of Mg^{2+} were removed using **3c**, **3a**, and **3b**, respectively.

The efficiencies were enhanced when compound **1** interacted with primary aromatic amines. Fig. 1 illustrates that the presence of a donating group such as methyl in the *para* position of the aromatic amine **5b** resulted in higher removal in comparison with the absence of one in **5a**, with 85.26%, 92.00%, 81.00%, and 91.00% removal using **5b** and 83.76%, 82.36%, 75.58%, and 88.81% removal using **5a** for Pb^{2+}, Cd^{2+}, Ca^{2+}, and Mg^{2+}, respectively. However, a decrease in the removal efficiency was observed when a withdrawing group such as a chloro or bromo group was in the *para* position. Also, a bromo group diminished the capacities of metal and heavy metal ion removal in comparison with the chloro group, as presented in Fig. 1. For instance, the efficacy of **5c** was found to be 83.55%, 82.20%, 70.28%, and 87.73%, while **5d** shows 82.74%, 81.81%, 66.87%, and 81.50% removal of Pb^{2+}, Cd^{2+}, Ca^{2+}, and Mg^{2+}, respectively.

Likewise, high capacity of removal was observed using compound **7**, where 84.24%, 82.34%, 72.17%, and 80.77% removal of Pb^{2+}, Cd^{2+}, Ca^{2+}, and Mg^{2+}, respectively, was demonstrated. Furthermore, to evaluate the efficiency of Mannich base derivatives produced by the reaction of 4-arylidenamino-3-methyl-*s*-triazole-5-thiones with secondary amines such as morpholine, piperidine, and *N*-methylpiperazine, or with primary amines such as propylamine, and *p*-chloroaniline, the removal of Pb^{2+}, Cd^{2+}, Ca^{2+}, and Mg^{2+} was tested. Fig. 2 shows the heavy metals and metal ions removed from aqueous solution using Mannich base derivative molecules and batch mode experiments. High efficiencies were obtained with lead, cadmium, and magnesium, while medium efficiency was found with calcium.

The efficiency of removal of Pb^{2+} by these Mannich base derivatives varied between 84.18–92.40%. The highest removal values were observed when a methoxy group was located in the *para* position of the benzaldehyde; then 92.40%, 86.24%, and 85.39% removal was obtained by using **13h**, **13b**, and **13e**, respectively. The Mannich base derivatives bearing a chloro group in the *para* position of benzaldehyde showed medium efficiency, where 85.99%, 85.31%, and 84.59% removal was achieved using **13i**, **13f**, and **13c**, respectively. Low removal in the case of unsubstituted benzaldehyde was found, with 84.81%, 84.56%, and 84.18% removal using **13g**, **13d**, and **13a**,

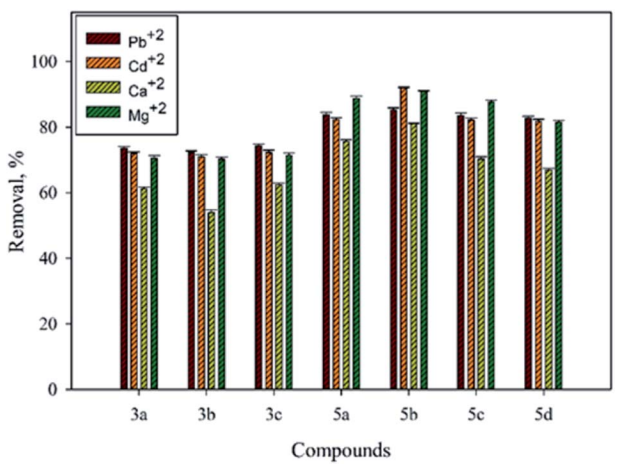


Fig. 1 Effect of the aliphatic and aromatic primary amine on the removal efficiencies of Pb^{2+}, Cd^{2+}, Ca^{2+}, and Mg^{2+}.

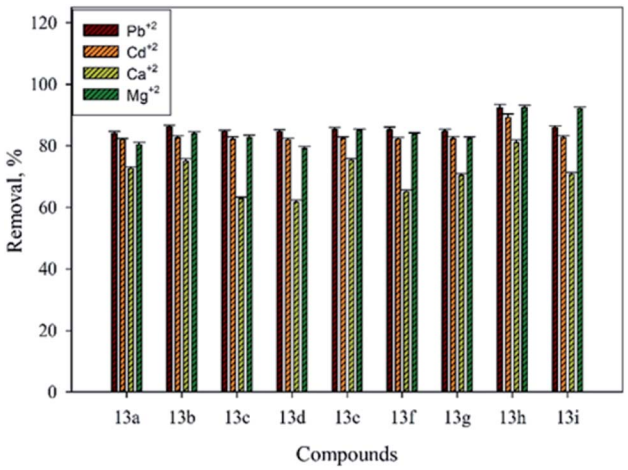


Fig. 2 Removal efficiencies of Pb^{2+}, Cd^{2+}, Ca^{2+}, and Mg^{2+} using Mannich base compounds.

respectively. Also, the compounds of Mannich bases under investigation demonstrated high efficiency for the removal of Cd^{2+}. The substitution of the methoxy group in the *para* position of benzaldehyde increased the removal efficiency of cadmium from 81.91–82.39% to 82.42–89.21%. For instance, the removal efficiency of Cd^{2+} when the methoxy group was present was found to be 82.66%, 82.42%, and 89.21% by **13b**, **13e**, and **13h**, respectively.

The same efficiency for **13a** and **13d** (81.91%) was observed without any withdrawing group in the *para* position of benzaldehyde. The presence of a chloro group in the *para* position of benzaldehyde decreased the efficiency of Cd^{2+} removal when compared with the methoxy group, and was 82.13%, 82.17%, and 82.77% by **13c**, **13f**, and **13i**, respectively. Interestingly, high and medium efficiencies were demonstrated for the removal of the metal ions responsible for water hardness (Ca^{2+} and Mg^{2+}). For the removal of Mg^{2+}, the tested Mannich bases exhibited high efficiency with values ranging between 79.16% and 92.49%, while for Ca^{2+}, the removal varied from 61.75% to 81.01%. However, the highest efficiencies were obtained for both cases of metal ion removal when the methoxy group was in the *para* position. For example, 81.01%, 75.33%, and 74.97% removal for Ca^{2+} and 92.49%, 85.02%, and 83.98% removal for Mg^{2+} were obtained when Mannich bases based on **13h**, **13e**, and **13b**, respectively were used. In the case of the chloro substituent group in the *para* position, the capacities to remove Ca^{2+} and Mg^{2+} ions were reduced. Then, 70.71%, 65.04%, and 62.89% removal of calcium and 92.14%, 83.71%, and 82.76% removal of magnesium from aqueous solution was attained utilizing **13i**, **13f**, and **13c**, respectively. In general, without substitution in the *para* position of benzaldehyde, the removal efficiencies were lower than those of substituent ones with methoxy and chloro groups. This could be attributed to the presence of the donating group, as the methoxy group enhances the electron density of the molecule.

Moreover, the removal efficiency of heavy metals and metal ions varied by the type of amino group, *e.g.*, primary or secondary. As shown in Fig. 3, high efficiency was found when propyl amine (**14a**) was used, where 93.92%, 87.74%, 85.43%, and 75.89% removal was obtained for Mg^{2+}, Pb^{2+}, Cd^{2+}, and Ca^{2+}, respectively. This could be attributed to the presence of the propyl amino ($CH_3CH_2CH_2NH$–) group, which facilitates the coordination between the metal and heavy metal ions and Mannich base molecules. Comparatively, when secondary amine, such as *N*-methylpiperazine, was used (**13i**), reduction of the removal efficiency was observed. Furthermore, **14b** shows that the lowest efficiency was due to the presence of withdrawal groups (chloro) in the *para* position. For example, 92.14%, 86.00%, 82.77%, and 70.81% removal occurred when **13i** was used, and 83.24%, 84.43%, 82.40%, and 70.65% removal was measured when **14a** was used.

Compound **10** exhibited the lowest values, with 21.06%, 7.41%, 9.09%, and 30.00% removal for Pb^{2+}, Cd^{2+}, Ca^{2+}, and Mg^{2+}, respectively. This could be attributed to the cyclization between compound **8** and formaldehyde. In accordance with the results obtained for these Mannich base compounds, it was observed that some molecules had a tendency to coordinate

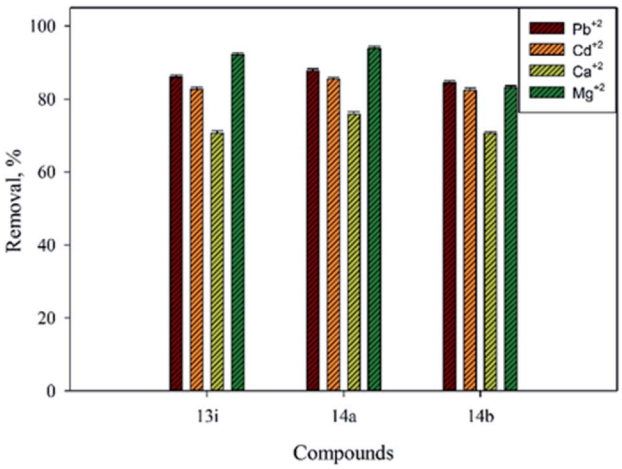


Fig. 3 Comparison of the removal efficiencies for Pb^{2+}, Cd^{2+}, Ca^{2+}, and Mg^{2+} using primary and secondary amines of Mannich base compounds.

with heavy metals; Pb^{2+} and Cd^{2+} tended to coordinate with metal ions, and Mg^{2+} and Ca^{2+} tended to coordinate with both metals and heavy metals. This could be attributed to the nature of heavy metals and metal ions, and the capacity of the Mannich base compounds to complex several metals and heavy metals. The different nature of these molecules may also be an important factor.

The pH is a critical parameter for metal and heavy metal ion adsorption from aqueous solution.[54] Therefore, in this study, the adsorption experiments were carried out at pH 6.0. At acidic pH, lower than 6, the compounds under investigation were protonated, which decreased the interaction between the heavy metals and metal ions and the adsorbent molecules. In addition, when the pH was higher than 6.0, the metals and heavy metals could be precipitated in hydroxide form $[M(OH)_2]$. Hence, the selected pH was 6.0, where surface groups such as the imino group became deprotonated and able to chelate the heavy metals and metal ions, as shown in eqn (1). This ensured that only ionic species existed in solution (Pb^{2+}, Cd^{2+}, Ca^{2+}, and Mg^{2+}) and eliminated the interference of hydroxide precipitation at pH above 6.0. Additionally, the highest removal efficiency could be obtained by avoiding the strong electrostatic repulsion that normally occurred at lower pH values.

$$M^{2+}_{(aq)} + x[L]_{(org)} \leftrightarrow [ML_x]_{(org)} \quad (1)$$

Therefore, a possible mechanism is the formation of coordination sites to receive the heavy metals and metal ions between an imine group and the thione of the *s*-triazole moiety, as shown in Fig. 4. The ability to form a complex is strongly affected by the nature of the substituent group and its position. Each substituent group has a specific effect on the coordination site that may increase or decrease the coordination capacity of the molecules. Thus, the efficiency in removing heavy metals and metal ions from aqueous solution is influenced by the electronic effect of substituents.

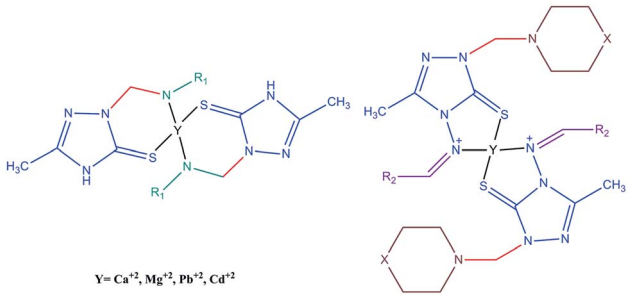


Fig. 4 Possible mechanism of metal and heavy metal ion removal.

Further study is required to confirm the mechanism of metal and heavy metal ion removal from water. For instance, X-ray structural analysis and phase purity of the formed complex is required, and FTIR spectroscopy is required to confirm the binding sites through the functional group of the synthesized compounds that attaches to the metal/heavy metal ions. Additionally, thermogravimetric analysis/differential thermal analysis (TGA/DTA) is useful for predicting the loss of water molecules, as well as the UV/Vis technique for identifying the stoichiometry of the formed complex.

2.4. Antimicrobial activity

Derivatives of *s*-triazoles are considered as potent antimicrobial agents.[55] Antimicrobial activity was evaluated *in vitro* using three strains of bacteria (*Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*) and three strains of fungi (*Aspergillus niger*, *A. flavus*, and *A. fumigatus*). The inhibition zones (mm) of the screened compounds were compared with those of chloramphenicol and clotrimazole, which were used as a reference for the antibacterial and antifungal tests, respectively. The well diffusion method was used because the inhibition zone diameters were clear with good definition. Also, an advantage of this method is that when the inoculum is added to the culture medium that is then poured into the plate, homogenous distribution and growth of the strain is obtained, which facilitates measurement of the inhibition zone diameters after 24 h.[56]

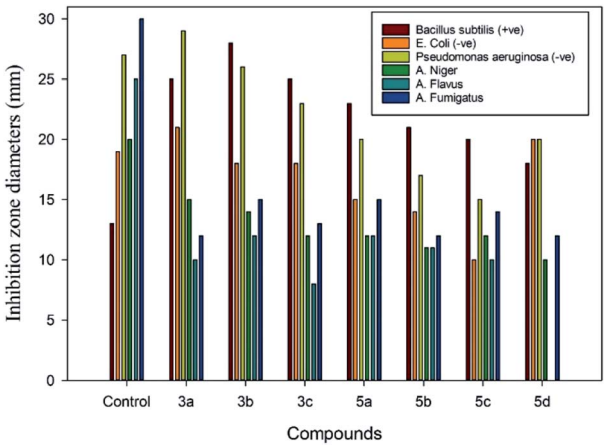


Fig. 5 Comparison of inhibition zone diameters for the antimicrobial activity of the synthesized compounds **3a–c** and **5a–d** at a concentration of 2.5 μg/0.1 mL.

Table 1 presents the antibacterial and antifungal activities of the synthesized compounds. In determining the efficiency of

Table 1 Screening results for antimicrobial activity, showing the inhibition zone (mm) after treatment with compounds **3a–c**, **5a–d**, **7**, **10**, **13a–i**, and **14a,b** at a concentration of 2.5 μg/0.1 mL

Compounds	*Escherichia coli* (−ve)	*Pseudomonas aeruginosa* (−ve)	*Bacillus subtilis* (+ve)	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus fumigatus*
3a	19	27	25	15	10	12
3b	21	29	28	14	12	15
3c	18	26	25	12	8	13
5a	18	23	23	12	12	15
5b	15	20	21	11	11	12
5c	14	17	20	12	10	14
5d	10	15	18	10	—	12
7	20	20	27	13	8	11
10	13	20	13	—	—	—
13a	—	—	—	12	—	14
13b	—	—	—	12	8	10
13c	24	22	—	2	4	—
13d	6	8	—	8	—	10
13e	22	18	2	6	—	10
13f	0	2	6	8	2	10
13g	14	10	—	20	8	16
13h	6	14	—	16	—	10
13i	10	—	—	22	—	16
14a	2	—	—	30	—	26
14b	4	2	—	14	12	18
Chloramphenicol	16	14	13	—	—	—
Clotrimazole	—	—	—	20	25	30

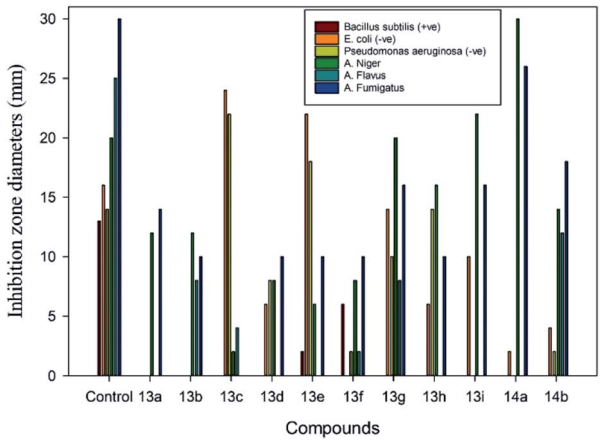


Fig. 6 Comparison of inhibition zone diameters for the antimicrobial activity of the synthesized compounds **13a–i** and **14a,b** at a concentration of 2.5 μg/0.1 mL.

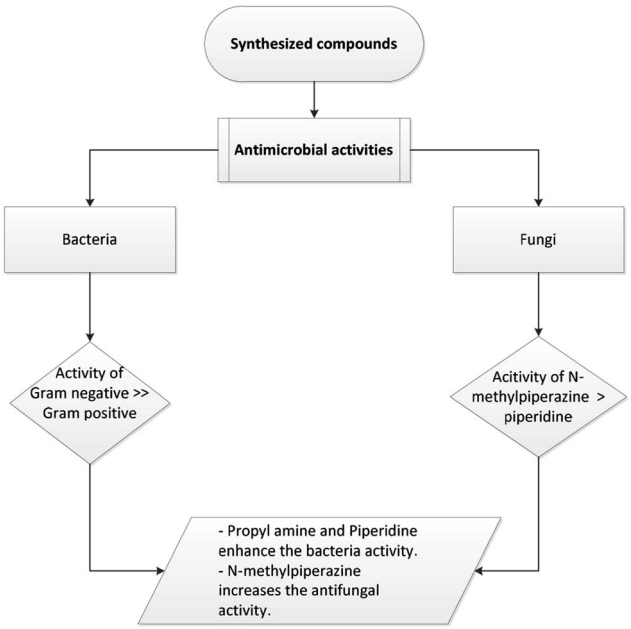


Fig. 7 Antimicrobial activity of *s*-triazole derivatives.

the compounds against the bacteria, the activity was higher with Gram-negative bacteria in comparison to Gram-positive bacteria. For instance, the inhibition zones ranged from 29 to 2 mm, and 28 to 2 mm for Gram (−ve) and Gram (+ve) bacteria, respectively. Compound **3b** showed the highest activity, with inhibition zones of 29 mm, 28 mm, and 21 mm against *Pseudomonas aeruginosa* (−ve), *Bacillus subtilis* (+ve), and *Escherichia coli* (−ve), respectively. Compounds **7**, **3a**, **3c**, and **5a** exhibited great activity with inhibition zones of 20, 19, and 18 mm towards *Escherichia coli* (−ve), while compounds **5b** and **5c** and showed low activity. In the case of *Pseudomonas aeruginosa* (−ve) and *Bacillus subtilis* (+ve), compounds **3a**, **3c**, **5a**, **5b**, **7**, **5c**, and **5d** were found to have strong activity compared to chloramphenicol, with inhibition zones of 14 mm and 13 mm, respectively (Fig. 5).

In measuring the efficiencies of the Mannich base compounds against the bacteria, the highest activity was observed with Gram-negative bacteria in comparison to Gram-positive bacteria. For instance, the inhibition zones ranged from 24 to 2 mm, and 13 to 2 mm for Gram (−ve) and Gram (+ve) bacteria, respectively. Compounds **13c**, **13e**, and **10** exhibited excellent activity towards both *Escherichia coli* (−ve), and *Pseudomonas aeruginosa* (−ve), as shown in Table 1 and Fig. 6. Moreover, moderate activity was shown using compounds **13d**, **13g**, **13h**, and **14b**, while low activity was observed for **13f**, **13i**, and **14a**. Furthermore, compounds **3a** and **13b** did not exhibit any activity against *Escherichia coli* (−ve) or *Pseudomonas aeruginosa* (−ve). Only compounds **10**, **13e**, and **13f** exhibited activity against *Bacillus subtilis* (+ve) compared to clotrimazole, with an inhibition zone of 13 mm.

In the same manner, all the synthesized compounds showed noteworthy antifungal activity against different strains of fungi, as shown in Table 1 and Fig. 5. Compounds **3a–c**, **5a–d**, and **7** were active against *A. niger*, *A. flavus*, and *A. fumigatus.* However, compound **5d** did not show any activity toward *A. flavus.* Interestingly, almost all Mannich base compounds exhibited antifungal activity against *A. niger*, *A. flavus*, and *A. fumigatus*, as shown in Table 1 and Fig. 6. Compounds **13g**, **13h**, and **14a** exhibited high activity, with inhibition zones of 20, 22, and 30 mm against *A. niger*, respectively. Additionally, compounds **14a** and **14b** exhibited significant activity against *A. fumigatus*, while moderate activity was found using compounds **13a**, **13b**, **13d**, **13f**, and **13h**. Compounds **13c** and **13e** presented the lowest activity against all species of fungi under investigation. Fig. 7 illustrates a simple explanation of the antimicrobial mechanism of action for the synthesized compounds.

3 Conclusions

We achieved an efficient one pot synthesis of the cyclized 2-methyl-6-substituted-6,7-dihydro-5*H*-*s*-triazolo[5,1-*b*]-1,3,5-thiadiazines *via* a double Mannich reaction of readily available 5-methyl-1*H*-*s*-triazole-3-thiol, formaldehyde, and primary aliphatic amines in ethanol at room temperature rather than other isomeric products such as 3-methyl-6-substituted-6,7-dihydro-5*H*-*s*-triazolo[3,4-*b*]-1,3,5-thiadiazines. By using primary aromatic amines, uncyclized 3-methyl-1-((substituted-amino)methyl)-1*H*-*s*-triazole-5-thiols rather than 3-methyl-4-((substituted-amino)methyl)-4*H*-*s*-triazole-3-thiol were obtained. Under the same reaction conditions, we investigated the behavior of 4-amino-3-methyl-*s*-triazole-5-thiol while undergoing the Mannich reaction through its treatment with secondary amine in boiling ethanol or at room temperature for 5 hours, and only 3-methyl-5,6-dihydro-*s*-triazolo[3,4-*b*]-1,3,4-thiadiazole was obtained without incorporation of secondary amine.

Additionally, after treatment of 4-amino-3-methyl-*s*-triazole-5-thiol with aromatic aldehydes either in boiling acetic acid or at room temperature, uncyclized Schiff's bases were produced. Mannich bases were then obtained by reaction of uncyclized

Schiff's bases with both primary and secondary amines under Mannich reaction conditions. The reaction products were easily isolated by filtration in very good to excellent yield and then purified by recrystallization with the proper solvent. All the synthesized products were used to remove Pb^{2+}, Cd^{2+}, Ca^{2+}, and Mg^{2+} from aqueous solution, with remarkable results. For example, 70.27–93.92%, 72.29–92.40%, 70.95–92.00%, and 53.92–89.00% removal values were obtained for the removal of Mg^{2+}, Pb^{2+}, Cd^{2+}, and Ca^{2+}, respectively. Compound **10** exhibited the lowest values of removal at 21.06%, 7.41%, 9.09%, and 30.00% for Pb^{2+}, Cd^{2+}, Ca^{2+}, and Mg^{2+}, respectively.

Our synthesized compounds exhibited biological activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus niger*, *A. flavus*, *and A. fumigatus*. Therefore, these compounds have dual functions and can be used in water remediation, and also for medical and pharmaceutical applications. In comparison with other materials reported in the literature, these adsorbents are highly competitive, as they have high removal efficiencies and promising features for potential applications. Further studies will focus on the adsorption mechanism and the effect of other parameters on our synthesized Mannich base compounds.

Conflicts of interest

There are no conflicts to declare.

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