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New bis- and tetrakis-1,2,3-triazole derivatives: Synthesis, DNA cleavage, molecular docking, antimicrobial, antioxidant activity and acid dissociation constants

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ABSTRACT

In this study, a series of bis– and tetrakis–1,2,3–triazole derivatives were synthesized using copper-catalyzed azide-alkyne cycloaddition (CuAAC) click chemistry in 73–95% yield. The bis– and tetrakis–1,2,3–triazoles exhibited significant DNA cleavage activity while the tetrakis–1,2,3–triazole analog **6g** completely degraded the plasmid DNA. Molecular docking simulations suggest that compound **6g** acts as minor groove binder of DNA by binding through several noncovalent interactions with base pairs. All bis- and tetrakis–1,2,3-triazole derivatives were screened for antibacterial activity against *E. coli*, *B. cereus*, *S. aureus*, *P. aeruginosa*, *E. hirae*, *L. pneumophila* subsp. *pneumophila* strains and antifungal activity against microfungus *C. albicans* and *C. tropicalis* strains. Compound **4d** exhibited the best antibacterial activity among bis–1,2,3–triazoles against *E. coli* and *E. hirae*, while **6c** exhibited the best antibacterial activity among tetrakis–1,2,3–triazoles against *E. hirae*. Furthermore, the best antifungal activity against *C. albicans* and *C. tropicalis* was reported for the compound **5**, while **6d** displayed the best antifungal activity against *C. tropicalis* and *C. albicans*. Reasonable iron chelating activities and DPPH radical scavenging abilities were found for some of the compounds. Finally, the acid dissociation constants (pK_a) of the bis–1,2,3–triazoles were also determined with the help of HYPERQUAD program using the data obtained from potentiometric titrations. The reported data here concludes that the bis- and tetrakis-1,2,3-triazoles are important cores that should be considered for further development of especially new anticancer agents acting through the DNA cleavage activity.

Introduction

In recent years, antimicrobial resistance has become one of the biggest struggles in the treatment of microbial related health problems and therefore, the need for new drugs have been increasing day by day. Microbial resistance to antimicrobial agents used over time has led to intensive work on the research and development of new antimicrobial agents, so there has been an ever-increasing interest in this field.[1]

The natural occurrence of DNA cleavage in various events such as DNA replication and transcription in the living system has made it an important target for treatment of cancer diseases. The development of effective anticancer agents such as bleomycin that act through DNA cleavage, and the critical and important applications of DNA cleavage in bioengineering, have led many researchers to focus on developing new DNA cleavage agents in recent years.[2] Oxygen play a vital role in biological processes and its concentration is controlled with great precision in living systems. On the other hand, some of the oxygen is partially reduced to reactive oxygen species (ROS) which contain radicals and

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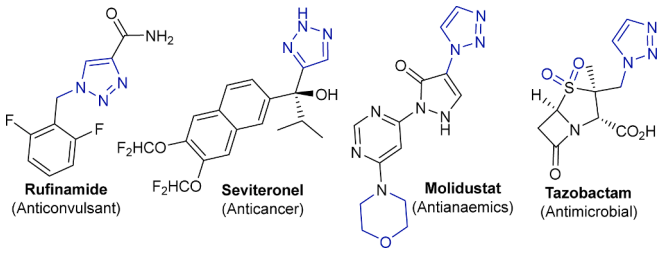


Figure 1. Some biologically active 1,2,3-triazole derivatives.

non-radical species. Large numbers of ROS are produced which lead to an imbalance between free radicals and the antioxidant defense under excessive stress conditions. If oxidative stress is too high for cells to maintain their own homeostasis, it may trigger the activation of various events such as proapoptotic pathways and necrosis, causing cell death and as a result, various disorders may emerge such as neurodegenerative, cardiovascular, cancer[3] and also Alzheimer's disease.[4]

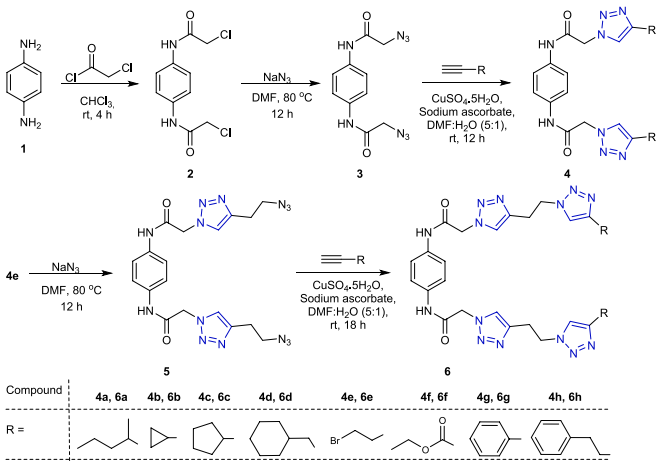
Triazole moieties 1,2,3-triazole and 1,2,4-triazole are found in the molecular structure of many drugs and are considered among the preferred cores in medicinal chemistry.[5]

The integration of 1,2,3-triazole to the molecular framework of biologically active molecules to increase their efficacy has received recent focus and is currently considered as a good strategy. Although 1,2,3-triazole cannot be obtained naturally, it has many desirable properties such as non-toxicity, having acidic and basic character, stability under physiological conditions, ability to make H-bonds and π-π stacking interactions. Click chemistry is widely used in the synthesis of 1,2,3-triazole derivatives, as it has many advantages over traditional methods such as mild reaction conditions, high yield, and also it allows obtaining stereo and region-specific products. In the last two decades, as a result of intensive studies on the synthesis and determination of bioactive properties of 1,2,3-triazole derivatives, a large number of drugs (Fig. 1) and drug candidates have been synthesized.[6]

Studies carried out in the last two decades showed that compounds bearing a 1,2,3-triazole ring exhibited anti(myco)bacterial,[7] antifungal,[8] DNA–cleavage/binding,[8,9] antioxidant[10] and anticancer[11] properties, and also useful agents for the treatment of neurodegenerative diseases.[12] It is also known that a large number of 1,2,3-triazole derivatives containing more than one 1,2,3-triazole moiety have been synthesized and exhibit a wide range of bioactivity such as antimicrobial,[13] antitumor,[14] DNA cleavage,[14,15] antioxidant[15,16] and various enzyme inhibitor activities.[17] Although, bis-triazole derivatives have been intensively studied in the literature, there are very limited studies on tris- and tetrakis-triazole derivatives.[18] Inspired by those facts, in our previously study,[15] we synthesized *N,N'*-(1,3-phenylene) bis(2-(4-R-1*H*-1,2,3-triazol-1-yl)acetamide) derivatives as bis-1,2,3-triazoles, and obtained high DNA cleavage activities.

Studies to determine physicochemical properties of pharmaceutics represent an important stage of drug research studies. pK_a values, which provide critical information about acidity, hydrogen bonding capacity, solubility, absorption, distribution, metabolism, elimination and transport of drugs to cells and other membranes, are one of the key parameters for drug development. Knowing pK_a values in advance gives the opportunity to see some problems that may arise in the later stages of drug research.[19] Today, there are many computer programs, such as HYPERQUAD which is amongst the most useful computer programs that yield results with high precision and accuracy when determining pK_a values from potentiometric data [19c–h] used to calculate pK_a values.[19a,b]

Computational methods are value added tools to help us investigate many chemical or biological events. Apart from our recent studies in which we used different *in silico* methods including quantum chemical[20] and molecular mechanic calculations,[21] molecular docking helped us to explain DNA cleavage activity of our recently synthesized ligands.[15] Therefore, in this study, we also aimed to run molecular docking



Scheme 1. Synthesis of the bis–1,2,3–triazole (**4a–h**, **5**) and tetrakis–1,2,3–-triazole derivatives (**6a–h**).

simulations to see possible DNA cleavage mechanism of promising ligands.

Due to all of the aforementioned points and in continuation of our previous work,[15] in this paper we present the synthesis of novel *N,N'*-(1,4-phenylene)bis(2-(4-R-1*H*-1,2,3-triazol-1-yl)acetamide) as bis–1,2,3–triazole and *N,N'*-(1,4-phenylene)bis(2-(4-(2-(4-R-1*H*-1,2,3-triazol-1-yl)ethyl)-1*H*-1,2,3-triazol-1-yl)acetamide) as tetrakis–1,2,3–triazole compounds and also their DNA–cleavage, antibacterial, antifungal and antioxidant properties. Moreover, molecular docking study to obtain binding details with the DNA and the pK_a values of the products are also reported.

The synthesis of bis–1,2,3–triazole **4a–h, 5** and tetrakis–1,2,3–-triazole **6a–h** derivatives was performed using CuAAC click chemistry in 73–95% yield. The benzene-1,4-diamine was used as starting compound and reacted with chloroacetyl chloride to obtain *N,N'*-(1,4-phenylene) bis(2-chloroacetamide) **2** as described in literature.[22] Subsequent reaction of compound **2** with sodium azide gave intermediate *N,N'*-(1,4-phenylene)bis(2-azidoacetamide) **3**. The prepared compound **3** was reacted with various alkyne compounds in the presence of sodium ascorbate (1.2 equivalent) and $CuSO_4{\cdot}5H_2O$ (0.6 equivalent) in DMF/water (5:1 v/v) mixture to obtain final products bis–1,2,3–triazoles **4a–h** in 80–95% yields (Scheme 1). The tetrakis–1,2,3–triazole **6a–h** derivatives (73–92% yields) were synthesized by reaction of *N,N'*-(1,4-phenylene)bis(2-(4-(2-azidoethyl)-1*H*-1,2,3-triazol-1-yl)acetamide) (**5**), which was obtained by reaction of **4e** with sodium azide and alkyne compounds using the protocol used to obtain compounds **4a–h** (Scheme 1). After the reaction of **4a–h, 5** and **6a–h** was completed, the crude mixture was precipitated with excess water and filtered. The leached mixture was washed with water, and then sequentially with dichloromethane and diethyl ether. The molecular structure of the obtained pure bis–1,2,3–triazole **4a–h, 5** and tetrakis–1,2,3–triazole **6a–h** compounds were characterized using NMR, FT-IR and HRMS techniques (Fig. S7-S74.).

The DNA cleavage activities of the bis–1,2,3–triazole **4a–h**, **5** and tetrakis–1,2,3–triazole **6a–h** compounds were performed by agarose gel electrophoresis and their chemical nuclease activities were evaluated using pBR322 plasmid DNA in Tris-HCl and NaCl buffer at 37 °C. DNA cleavage was screened by relaxation of supercoiled circular form into the nicked circular form and linear form. When plasmid DNA is run by agarose gel electrophoresis, it will be observed that the uncleaved supercoiled circular form (Form I) migrates relatively faster than the notched circular form (Form II) and the linear form (Form III). DNA cleavage activity was evaluated on the basis of observing one strand cleavage or double strand cleavage. When one strand cleavage occurs, the supercoiled form relaxes, creating a slower moving open circular

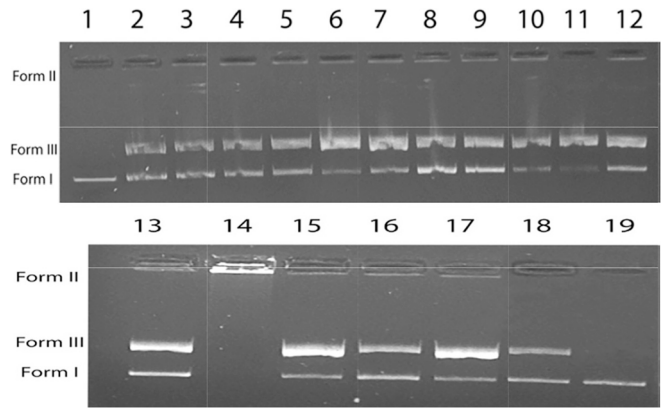


Figure 2. DNA cleavage activities of **4a–h**, **5**, **6a–h**. Lane 1: pBR 322 DNA; Lane 2, pBR 322 DNA + 250 μg/mL of **4a**; Lane 3, pBR 322 DNA + 250 μg/mL of **4b**; Lane 4, pBR 322 DNA + 250 μg/mL of **4c**; Lane 5, pBR 322 DNA + 250 μg/mL of **4g**; Lane 6, pBR 322 DNA + 250 μg/mL of **4e**; Lane 7 pBR 322 DNA + 250 μg/mL of **5**; Lane 8, pBR 322 DNA + 250 μg/mL of **6b**; Lane 9, pBR 322 DNA + 250 μg/mL of **4f**; Lane 10, pBR 322 DNA + 250 μg/mL of **4d**; Lane 11, pBR 322 DNA + 250 μg/mL of **4h**; Lane 12, pBR 322 DNA + 250 μg/mL of **6a**; Lane 13, pBR 322 DNA + 250 μg/mL of **6c**; Lane 14, pBR 322 DNA + 250 μg/mL of **6g**; Lane 15, pBR 322 DNA + 250 μg/mL of **6e**; Lane 16 pBR 322 DNA + 250 μg/mL of **6f**; Lane 17, pBR 322 DNA + 250 μg/mL of **6d**; Lane 18, pBR 322 DNA + 250 μg/mL of **6h**; Lane 19, pBR 322 DNA + DMSO.

form. However, when double strand cleavage occurs, linear form produces and moves between supercoiled and open circular forms.

The DNA cleavage activity results obtained for **4a–h**, **5**, **6a–h** are presented in Fig. 2. It is apparent from Lane 2–18 that all tested compounds **4a–h**, **5**, **6a–h** exhibited nuclease activity, while neither DNA nor DNA + DMSO showed cleavage activity (Lane 1 and Lane 19, respectively). All of the bis–1,2,3–triazoles **4a–h**, **5** exhibited nuclease activity by causing double chain breakage. In addition, the tetrakis–1,2,3–triazoles (**6a–f** and **6h)** also showed nuclease activity by double-strand break (Lane 8, 12, 13, 15–18, respectively). Excitingly, as shown in Lane 14, excellent nuclease activity was observed for the compound **6g** which completely cleaved the plasmid DNA. The results obtained emphasize the importance of bis– and tatrakis-1,2,3–triazoles to be considered as potential cores for the development of new anticancer agents acting through DNA cleavage activity. The striking nuclease activity of **6g** prompted us to run molecular modeling simulations to understand potential interactions with the DNA. Our previously generated DNA model[15] was used to dock compounds into the binding site of DNA that was assigned across the all of the minor and major grooves of DNA dodecamer D(CGCAAATTTGCG) in complexed with distamycin (PDB ID: 2DND; 2.20 Å). LigPrep[23] module of Schrödinger suite was used to generate possible ligand conformations, tautomers, protonation states at physiologic conditions. Glide XP (extra precision)[24] module was used to docked the compounds into the binding site of DNA. Molecular docking simulations were performed to find stable and favorable orientation of compounds that interact with the DNA. Docking results show that compound **6g** that showed excellent DNA cleavage activity makes favorable interactions with the DNA base pairs in the shallow and narrower minor groove rather than major groove (Fig. 3). Besides occupying A-T rich region, the terminal ends extend along the G-C regions too.

The phenyl ring makes a *T*-shaped π–π interaction with the adenine 4 of the DNA while the same moiety interacts with the adenine 5, cytosine 23 and glycine 22 by aromatic hydrogen bonds. Similar aromatic hydrogen bonds are observed between the other terminal phenyl ring with the thymine 9 and in the para substituted benzene ring, located at the center of **6g**, with the thymine 7. This thymine 7 is also involved in a strong hydrogen bond with the amide hydrogen of **6g**. Those overall strong noncovalent interactions provide **6g** shape complementarity that orients it to be in a parallel manner with respect to the DNA backbone (Fig. 3, left panel). Single bonds connecting aromatic rings in **6g** allow torsional rotation which makes it possible to adopt this specific shape. In an attempt to see the potential of 1,2,3-triazole core over 1,2,4-triazole in our compounds, 1,2,3-triazole core was replaced with 1,2,4-trizole in **6g** and molecular docking simulation was carried out. The neutral form of 1,2,4-trizole analog of **6g** was predicted to be very ineffective (unfavorable binding mode with positive binding energy).

The antibacterial properties of the bis–1,2,3–triazole **4a–h**, **5** and tetrakis–1,2,3–triazole **6a–h** compounds were screened against *E. hirae*, *B. cereus*, *S. aureus* as Gram-positive bacteria, *P. aeruginosa*, *L. pneumophila* subsp. *pneumophiia*, *E. coli* as Gram-negative bacteria. The antibacterial activities were measured to be in the range of 4–128 μg/mL (Table 1). Amongst the bis–1,2,3–triazole series **4a–h**, **5, 4d** bearing cyclohexylmethyl substituent exhibited the best antibacterial activity against *E. coli* and *E. hirae* strains (MIC values: 4 μg/mL and 8 μg/mL, respectively), while MIC values of 32 μg/mL or higher were

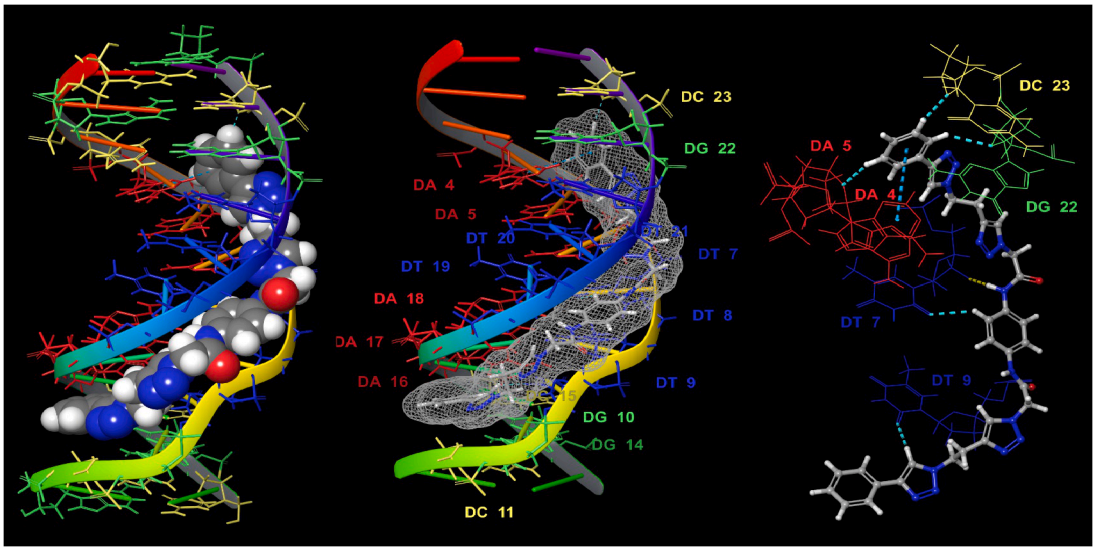


Figure 3. Binding mode of **6g** into the minor groove region of DNA. Left panel: space-filling model of **6g**, middle panel: surface representation of **6g**, right panel: noncovalent interactions of **6g** with the interacting base pairs. The nucleotides adenine, thymine, guanine, and cytosine are colored as red, blue, green and yellow, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
The MIC values (μg/mL) of **4a–h**, **5** and **6a–h** against the studied microbial strains.

Compound	EC[a]	BC[b]	SA[c]	PA[d]	EH[e]	LP[f]	CA[g]	CT[h]
4a	128	64	64	64	32	32	64	32
4b	64	64	128	128	64	128	128	64
4c	128	64	64	64	32	32	64	32
4d	4	64	64	64	8	16	16	32
4e	128	64	64	64	32	32	64	32
4f	128	64	32	64	64	64	64	32
4g	64	64	64	64	128	16	64	32
4h	128	32	32	16	64	64	64	64
5	32	64	64	64	32	32	8	16
6a	128	128	32	32	16	16	64	32
6b	128	64	64	64	64	32	128	64
6c	128	64	16	64	4	128	32	4
6d	128	64	64	64	16	32	8	4
6e	128	64	64	64	32	8	8	32
6f	128	32	32	64	64	32	128	64
6g	128	128	64	64	16	32	64	64
6h	128	128	64	64	64	64	64	16
Ampicillin	0.5	1	1	0.5	0.5	0.5	–	–
Fluconazole	–	–	–	–	–	–	0.5	0.5

[a] *E. coli.* [b] *B. cereus.* [c] *S. aureus.* [d] *P. aeruginosa.* [e] *E. hirae.* [f] *L. pneumophila* subsp. *pneumophila.* [g] *C. albicans.* [h] *C. tropicalis*

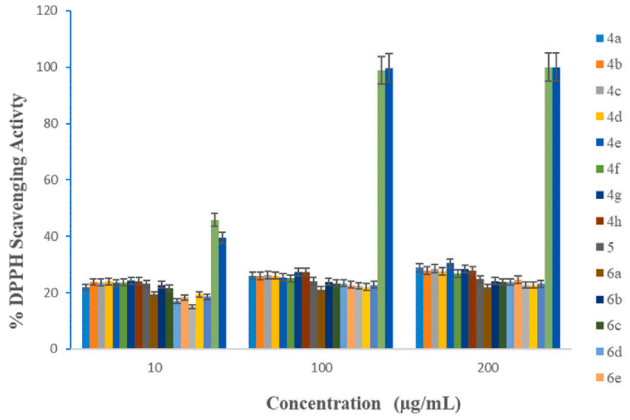


Figure 4. % Radical scavenging activity of the bis–1,2,3–triazoles **4a–h**, **5** and tetrakis–1,2,3–triazoles **6a–h.**

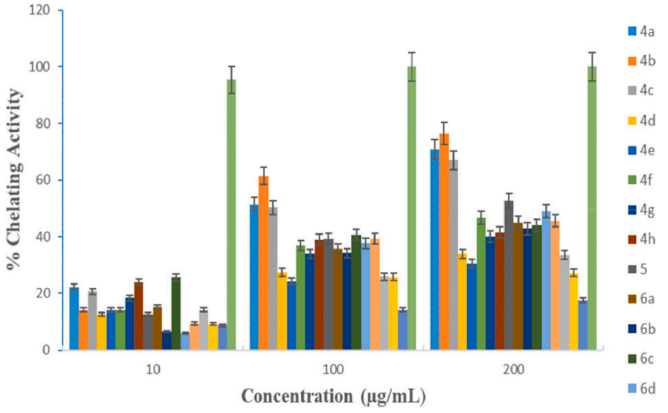


Figure 5. Chelating activity of **4a–h**, **5**, **6a–h.**

noted for the rest of the compounds. Although **4d** exhibited significant antibacterial activity against *E. coli* and *E. hirae* strains, the antibacterial activity is lower than that of ampicillin (MIC = 0.5 μg/mL). The bis–1,2,3–triazoles **4a–h**, **5** displayed moderate antibacterial activity against *B. cereus, S. aureus, P. aeruginosa* and *L. pneumophila* subsp. *pneumophiia* strains with an MIC of 16 μg/mL or higher, while ampicillin exhibited antibacterial activity against the same strains with a MIC of 0.5 μg/mL or 1 μg/mL. When the antibacterial activities of the tetrakis–1,2,3–triazoles **6a–h** were evaluated and compared with the ampicillin, all compounds had moderate activities (MIC: 16 μg/mL or higher) against *E. coli*, *B. cereus*, *S. aureus, P. aeruginosa* strains. However, **6c** bearing cyclopentyl substituent exhibited promising antibacterial activity against *E. hirae* strains (MIC: 4 μg/mL). Moreover, **6e** (R: cyclopentyl) showed reasonable antibacterial activity against *L. pneumophila* subsp. *pneumophiia* strains with a MIC of 8 μg/mL.

The antifungal activities of all compounds were also investigated using *C. albicans* and *C. tropicalis* strains. The activity values were found to be in the range of 4–128 μg/mL (Table 1). Compound **5** had the best antifungal activity against *C. albicans* and *C. tropicalis* strains (MIC: 8 μg/mL and 16 μg/mL, respectively) in the bis–1,2,3–triazole series. In addition, amongst the compounds that belong to the tetrakis–1,2,3–-triazole series, compounds **6d** and **6e** exhibited fair antifungal activities against *C. albicans* strains with a MIC of 8 μg/mL. Although fluconazole displayed antifungal activity against *C. tropicalis* strains with a MIC of 0.5 μg/mL, the antifungal activities of **6c** and **6d** against *C. tropicalis* strains were found promising (MIC: 4 μg/mL). We believe that both bis– and tetrakis–1,2,3–triazole structures should be considered encouraging for the development of new antibacterial and antifungal agents.

The antioxidant activities of the bis–1,2,3–triazole **4a–h**, **5** and tetrakis–1,2,3–triazole **6a–h** compounds were determined according to their DPPH scavenging activity measurement which is a fast, easy, economic and suitable method. DPPH radical scavenging activity study was performed following the method described by Blois.[25] Trolox and Ascorbic acids were used as reference compounds. Compared to reference compounds, all products tested exhibited moderate DPPH scavenging radical ability (Fig. 4 and Fig. S75). No significant change in DPPH radical scavenging activity was observed as the concentration of the compounds **4a–h**, **5** and **6a–h** was increased from 10 μg/mL to 200 μg/mL (Fig. 4 and Fig. S75). The lowest DPPH radical neutralization capacity was measured for compound **6a** (21.9%) at a concentration of 200 μg/mL, while the highest scavenging ability was noted for the compound **4e** (30.5%).

Within the framework of this study, the products **4a–h**, **5**, **6a–h** were tested for their ferrous chelating activities, and the obtained activities are given in Fig. 5 and Fig. S76. The ferrous chelating activities (concentration dependent) of the products **4a–h**, **5**, **6a–h** were compared to EDTA. The activities of the bis–1,2,3–triazoles **4a**, **4b**, **4c**, **4d**, **4e**, **4f**, **4g**, **4h** and **5** at 200 μg/mL were obtained as 70.8%, 76.5%, 67.1%, 33.8%, 30.4%, 46.5%, 40.1%, 41.4% and 52.8%, respectively. However, the measured activities for the tetrakis–1,2,3–triazoles **6a**, **6b**, **6c**, **6d**, **6e**,

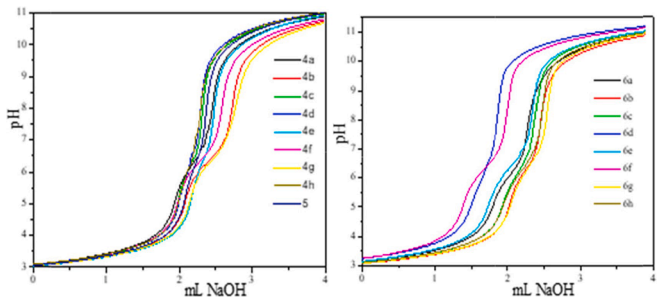


Figure 6. (a) Titration curves of **4a–h**, **5** (b) Titration curves of **6a–h** (25 ± 0.1 °C, I = 0.1 M NaCl, 20% (*v*/*v*) DMSO-water).

Table 2
Acid dissociation constants of the ligands (25 ± 0.1 °C, *I* = 0.1 M NaCl, 20% (*v*/*v*) DMSO-water).

Ligands	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}
4a	3.17 ± 0.01	6.37 ± 0.01	10.13 ± 0.02	11.66 ± 0.02
4b	2.77 ± 0.02	6.35 ± 0.02	10.25 ± 0.03	12.31 ± 0.03
4c	3.42 ± 0.01	6.25 ± 0.03	9.94 ± 0.02	11.13 ± 0.02
4d	3.53 ± 0.03	6.45 ± 0.01	10.35 ± 0.01	11.51 ± 0.01
4e	3.36 ± 0.01	6.33 ± 0.01	9.78 ± 0.02	11.31 ± 0.03
4f	3.05 ± 0.02	6.35 ± 0.01	10.19 ± 0.02	11.72 ± 0.01
4g	3.33 ± 0.02	6.40 ± 0.02	9.62 ± 0.01	11.24 ± 0.01
4h	3.50 ± 0.02	6.33 ± 0.02	9.89 ± 0.01	11.69 ± 0.01
5	2.33 ± 0.01	5.77 ± 0.03	10.40 ± 0.02	11.23 ± 0.02
6a	3.34 ± 0.03	6.14 ± 0.03	9.97 ± 0.01	11.22 ± 0.04
6b	3.11 ± 0.02	6.30 ± 0.03	10.23 ± 0.02	11.03 ± 0.03
6c	3.36 ± 0.01	6.26 ± 0.03	10.49 ± 0.03	11.49 ± 0.02
6d	3.62 ± 0.02	6.17 ± 0.02	10.51 ± 0.03	11.26 ± 0.04
6e	3.15 ± 0.03	6.26 ± 0.04	10.64 ± 0.03	11.23 ± 0.01
6f	3.07 ± 0.01	6.35 ± 0.02	10.72 ± 0.01	11.22 ± 0.04
6g	3.38 ± 0.01	6.39 ± 0.01	10.56 ± 0.02	11.16 ± 0.01
6h	3.33 ± 0.01	6.27 ± 0.01	10.55 ± 0.03	11.53 0.02

6f, **6g** and **6h** at the same concentration were 44.9%, 42.8%, 43.9%, 48.9%, 45.6%, 33.5%, 27.1%, and 17.3%, respectively. Amongst all, **4a**, **4b** and **4c** displayed higher chelating capacity. When the concentrations of **4a** and **4c** were increased from 10 μg/mL to 200 μg/mL, their activities increased from 22.0% to 70.8% and from 20.6% to 67.1%, respectively. The highest chelating activity was obtained with **4b** was 76.5% at 200 μg/mL. Although the iron chelating activities were lower than that of standard, the comparable activities obtained points out that **4a, 4b** and **4c** might be considered for further studies.

Due to the low water solubility and compatibility with the standard glass electrode and a large acidity range for determination of the pK_a values,[19d–h] DMSO-water mixture was preferred. Potentiometric measurements were carried out using a TitroLine® 7000 model automatic titrator with a combined glass electrode. The calibration of the glass electrode used was performed using a procedure available in literature.[19d–h] The pK_a values of the bis–1,2,3–triazoles **4a–h**, **5** and tetrakis–1,2,3–triazoles **6a–h** were determined potentiometrically in 20% (*v*/*v*) DMSO-water hydro-organic mixture at 25.0 ± 0.1 °C, at an ionic strength of 0.1 M NaCl. Four pK_a values for each **4a–h**, **5** and **6a–h** compounds were calculated using the HYPERQUAD computer program. Titration curves of **4a–h**, **5** and **6a–h** are given in Fig. 6 and distribution curves of **4a–h**, **5** and **6a–h** ligands are given in Fig. S77. Moreover, the calculated pK_a values are given in Table 2.

Since potentiometric titrations are carried out in an acidic medium, the probable fully protonated forms of the ligands can be as given in Fig. 7. The pK_a values of the protonated groups are expected to be very close to each other since the structure is symmetrical and there are two or four triazole rings in the molecular structure of the ligands. Although it is theoretically possible to identify the pK_a values of many protonated groups with thousands of potentiometric data, in practice this seems unlikely. According to the results obtained using the HYPERQUAD, four protonated species were obtained as LH, LH_2, LH_3 and LH_4. The calculated pK_{a1}, pK_{a2}, pK_{a3} and pK_{a4} values were obtained in a range of 2.33–3.62, 5.77–6.45, 9.62–10.72, and 11.03–12.31, respectively (Table 2).

The **4a–h**, **5** and **6a–h** contain two or four 1,2,3–triazole rings. The 1,2,3-triazol ring is a five-membered heterocyclic structure consisting of three consecutive nitrogen atoms followed by two carbon atoms. While one of the nitrogen atoms in the diazenyl group (-N═N-) in the molecular structure of the 1,2,3-triazole ring has strong acidic character, the other nitrogen and the tertiary imine nitrogen in the 1,2,3-triazole ring have strong basic character due to the electronic effects.[8,11] In addition, **4a–h**, **5** and **6a–h** contain substituted 1,2,3-triazole rings attached to the phenyl ring via two amide linkers at the para position. Because of delocalization between amide carbonyl groups and unpaired electrons on the nitrogen atoms, and also bonding to the phenyl ring, the amide carbonyl group is expected to have a weak acidic character.

Supported with the data obtained, the pK_{a1} values (2.33 – 3.62) can be attributed to the acidic nitrogen of the diazenyl group. The pK_{a2} values (5.77 – 6.45) indicating a weakly acidic character can be attributed to the amide carbonyl group. The pK_{a3} (9.62 – 10.72) and pK_{a4} (11.03 – 12.31) values are two values representing the strongly basic character. Although the nitrogen atom, which has a basic character, in the diazenyl group (-N═N-) and the tertiary imine nitrogen in the molecular structure of the 1,2,3-triazole ring are very basic, the tertiary imine nitrogen is expected to be more basic than the basic nitrogen atom in the diazenyl group in accordance with the literature.[8,11] In this context, the pK_{a3} (9.62 – 10.72), and pK_{a4} (11.03 – 12.31) values can be attributed to the basic nitrogen of the diazenyl group and the tertiary imine nitrogen, respectively.

In summary, the synthesis of the bis–1,2,3–triazole **4a–h**, **5** and

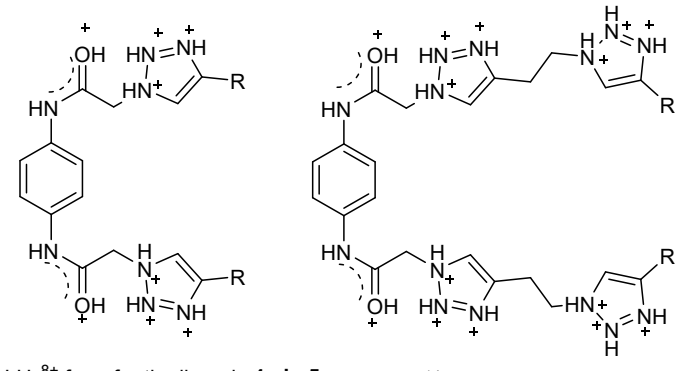


Figure 7. The proposed fully protonated form of the ligands **4a–h**, **5** (LH_8^{8+}) and **6a–h** (LH_{14}^{14+}).

tetrakis–1,2,3–triazole **6a–h** derivatives was described, and their DNA cleavage, antibacterial, antifungal and antioxidant activities were investigated in the present study. Intriguingly, compound **6g** exhibited excellent nuclease activity by cleaving plasmid DNA completely, while all other bis-, and tetrakis-1,2,3-triazole products exhibited remarkable nuclease activity by causing double chain breakage. Therefore, we report that bis- and tetrakis-1,2,3-triazole containing compounds can be considered for further improvement as new potential anticancer agents acting through DNA cleavage activity. Molecular docking studies reveal that compound **6g** binds to the minor groove region of DNA through strong noncovalent interactions and its flexibility allows it to adopt a unique orientation. Compound **4d** exhibited a significant antibacterial activity against *E. coli* and *E. hirae* with a MIC value of 4 μg/mL and 8 μg/mL, respectively, while **6c** and **6e** exhibited also remarkable antibacterial activity against *E. hirae* and *L. pneumophila* subsp. *pneumophiia* with a MIC value of 4 μg/mL and 8 μg/mL, respectively. In addition, **6c** and **6d** demonstrated a significant antifungal activity against *C. tropicalis* with a MIC value of 4 μg/mL, while **5**, **6d** and **6e** exhibited also a good antifungal activity against *C. albicans* with a MIC value of 8 μg/mL. However, the use of these compounds as antimicrobial agents still needs additional improvement. Although, all of the products exhibited a moderated DPPH scavenging activity, **4a**, **4b** and **4c** exhibited fair ferrous chelating activity at 200 μg/mL with 70.8%, 76.5% and 67.1%, respectively. Additionally, four pK_a values for each product were calculated following the procedure given in the text. These overall data reported here provides detailed information that might be critical for further studies on these type of compounds.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128453.

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