Synthesis and Biological Activity of New Triazole Compounds

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Abstract: Six new N,N-bis(1,2,4-triazol-1-ylmethyl)amines have been prepared in one step by condensation of 1-(hydroxymethyl)triazole with a series of amines. These reactions were carried out in refluxed CH_3CN for 4 hours. The products were recuperated with excellent and good yields (75-89.5%). Compounds (**3a-f**) were screened for their antifungal activity against the budding yeast *Saccharomyces cerevisiae* and their antibacterial activity against *Escherichia coli*.

Keywords: Synthesis, Antifungal activity, Antibacterial activity, Tridentate ligand, Nitrogen-rich and Triazole.

1. INTRODUCTION

The chemistry of nitrogen containing multipodal molecules is attracting current interest for the building of polynuclear metal complexes, as a model for bioinorganic chemistry, materials science, transport and activation of small molecules, as well as for the discovery of new catalyst precursors [1, 4]. For example, polypyrazolyl compounds have been used as mimics of active sites in copper oxidase [5]. The triazolyl ring seems to play a key role as antifungal drugs, for example, fluconazole **I** is effective against oropharyngeal and esophageal candidiasis [6, 7], although fosfluconazole **II** is less active than **I** *in vitro* but has similar efficacy clinical profile in animal models and patients [8, 9] (Fig. **1**).

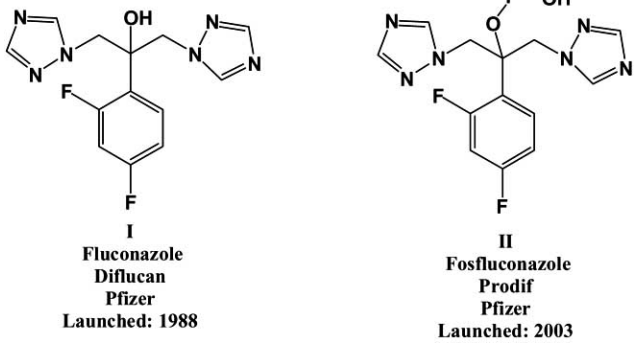

Fig. (1).

Meanwhile, these compound derivatives have been involved in several types of chelating ligands**,** classified as a - 6 and 8 - electron donating tridentate ligands with two N-donor sites of triazolyl rings and one N-donor site of amine. The property behaviour of these new ligands and their reactivity is strongly dependent on the electronic richness of the nitrogen atoms and on the steric hindrance of the substituents [10]. Thus it seems crucial to synthesise a diversity of this type of tridentate ligands (Scheme **1**). One of our research interests is the synthesis of new tridentate nitrogen donor compounds which have biological activities such as antifungal [11] and anticancer [12]. In addition, we are very interested in the study of their catalytic activities such as isomerisation [13] and oxidation reaction [14]. To our knowledge the 1,2,4-triazole unit has never been incorporated into a tridentate symmetrical structure type (-N-CH_2-N- junction) or screened for biological activities. We report here an easy and facile synthesis of six new N,N-bis(1,2,4-triazolyl-1-ylmethyl)amines containing new bulky alkyl, aromatic, amino acid groups at the central nitrogen atom with diverse substituents and their antifungal and antibacterial biological activities.

2. RESULTS AND DISCUSSION

2.1. Synthesis

The synthesis of N,N-bis(1,2,4-triazol-1-ylmethyl)amines **3a-f** as outlined in Scheme **1** was carried out by refluxing one equivalent of an amines **2a-f** and two equivalent of 1-(hydroxymethyl)-1,2,4-triazole **1** in CH_3CN for 4 hours.

The electronic effect of the substituents R (alkyl group or aryl group) as an electro-donating or electro-accepting group represents only one way to modify the coordination properties of donor sites from the triazolyl rings. The nature of the substituents R, particularly the electronic effect of alkyl versus aryl group R, and the steric hindrance of R are also important factors. Thus, six bulky amines **2a-f**, differently substituted in ortho, meta and para position, were condensed with the precursors **1** [15] (Scheme **1**). The electronic impact of the different groups R is visible on the chemical shift in proton and carbone NMR of the methylene bridge between the central nitrogen atom and the triazolyl rings as well as on the two –CH triazolique. As expected the hydrogen atoms of the triazolyl rings are more strongly influenced by the nature of R according to the observed values for **3e** 8.00; 8.22 ppm (amino acid); **3a 7**.75; 8.17 ppm (phenyl with attracting group on para and ortho) and for **3f** 7.84; 8.18 ppm (alkyl group). Compounds **3d-e** show two unequivalent methylene groups with partially overlapping signals as a consequence of the chiral carbon centre. The methylene proton

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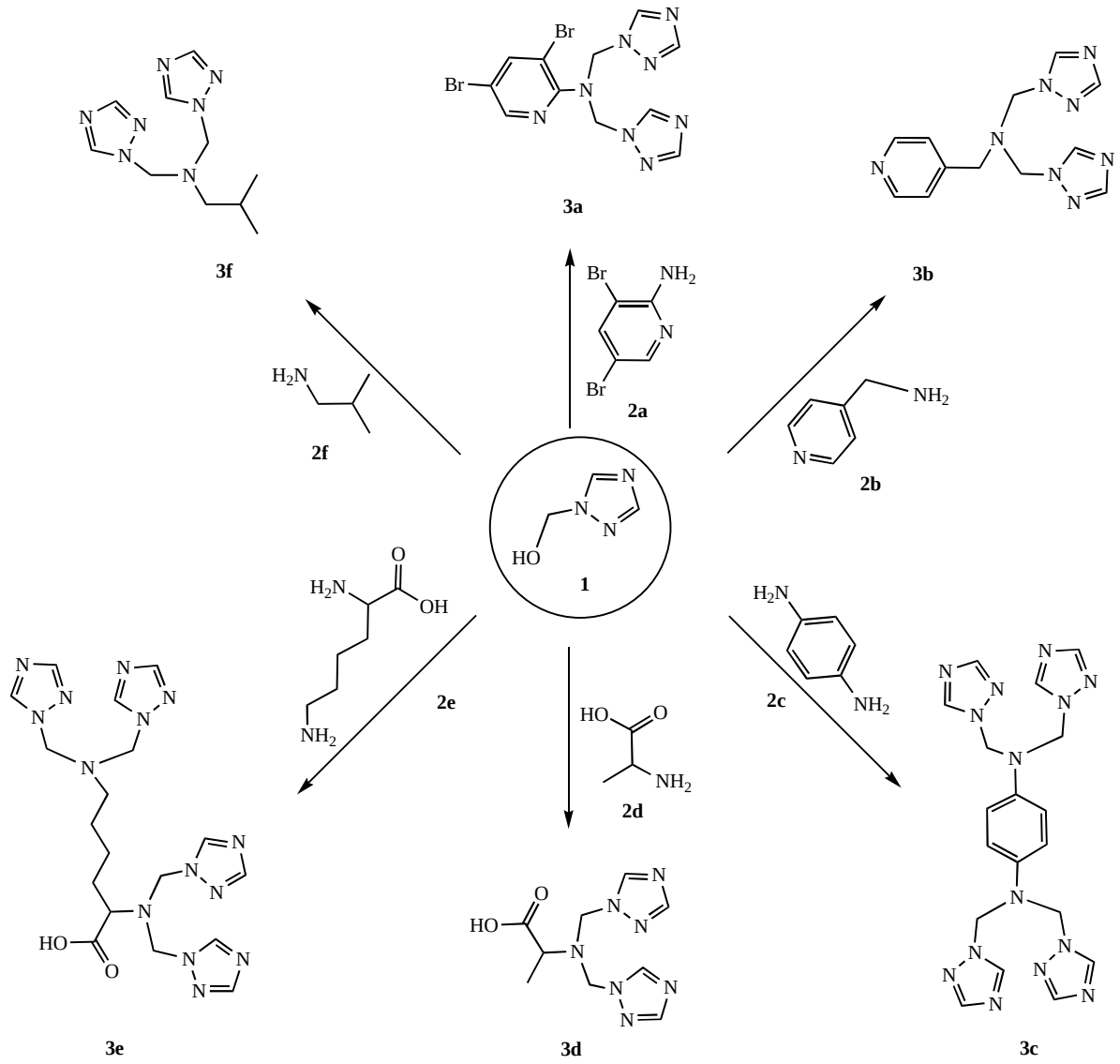

Reaction conditions: one equivalent of amine 2a-f plus two equivalents of 1 in reflux CH_3CN during 4 hours.

Scheme 1. Synthesis of N,N-bis(1,2,4-triazol-1-ylmethyl)amines **3a-f.**

Table 1. Chemical shift in ^{1}H NMR and ^{13}C NMR of the methylene bridge between the central nitrogen atom and the triazolyl rings and the -CH triazolic

Compounds	Yield	^{1}H NMR δ en ppm (N-CH_2-N)	^{13}C NMR δ en ppm (N-CH_2-N)	^{1}H NMR δ en ppm (H triazol)	^{13}C NMR δ en ppm (C triazol)
3a	77.2%	5.84	54.69	7.75; 8.17	141.72; 146.97
3b	89.5%	5.62	71.51	7.99; 8.10	144.41; 151.98
3c	85%	5.94	64.59	7.99; 8.68	144.64; 152.06
3d	84.5%	5.36	64.63	7.95; 8.56.	144.37;151.85
3e	81%	5.31; 5.65	67.36; 71.96	8.00; 8.22	146.80; 151.98
3f	86%	5.06	66.16	7.84; 8.17	143.57; 151.82

resonances changed with the variation of the nature of the substituent on amines, which agree with the literature reported [16-18]. The methylene protons are diastereotopic in the chiral ligands and their signals appear as AB systems with similar germinal coupling constants of 14.7Hz (**3d**) and 13.6Hz (**3e**) (Table **1**).

The variation of the proton and carbon's shift of the methylene groups (5.06 to 5.84) and (54.69 to 71.96) could be correlated to the tuning of the electronic richness of these ligands. One can envisaged that with the new electro attracting and/or bulky substituents, these six ligands have more potential for different coordination

Table 2. Antimicrobial Activity of the Compounds Evaluated Against *E. coli*. (+): Active, (-): Not Active

Compound\Concentrations	1 mM	2 mM
3a	-	-
3b	-	-
3c	-	-
3d	-	-
3e	+	+
3f	+	+

behaviour than reported similar ligands [19] as well as on their biological activities.

2.2. Antibacterial and Antifungal Activities

We first evaluated the compounds for their antibacterial activity against *E. coli* as described in Materials and methods. Compounds **3a, 3b, 3c** and **3d** showed no antibacterial activity when they were used at 1 mM. Even higher concentrations of these compounds (2 mM) did not inhibit growth (Table **2**). Whereas, the presence of 1 mM of compound **3e** or **3f** in the culture prevented the growth of E. coli cells, demonstrating that these compounds have antibacterial activity against E. coli (Table **2**).

Regarding the antifungal activity, compounds were tested for toxicity against the budding yeast (Saccharomyces cerevisiae) cells. Cells were cultured in the presence of 1 mM of each compound and assayed for growth inhibition in liquid culture as described in Materials and methods. Compounds **3a** and **3b** were not toxic to yeast cells, whereas compounds **3c** and **3d** showed weak antifungal activity (Fig. **2A**). Interestingly, compounds **3e** and **3f** displayed strong antifungal activity (Fig. **2A**), consistent with their antibacterial activity against *E. coli*. Further evaluation of **3e** and **3f** compounds has demonstrated that compound **3e** was more potent than compound **3f.** As a matter of fact, when these two compounds were used at 0.5 mM, **3e** was much more toxic than **3f** to yeast cells after longer incubation with the compound (Fig. **2B**). Compound **3d**, which has an acidic function, had little anti-fungal activity when used even at 1 mM, indicating that the acidic functional group of compound **3e** is not critical for the anti-fungal activity of this compound. Similarly, **3d** had no anti-bacterial activity when used at 2 mM, demonstrating that the acidic functional group of compound **3e** is not important for the anti-bacterial activity of this compound. Therefore, our results suggest that the bulky nature as well as the flexibility of the alkyl group at the central nitrogen atom of the triazole compound is important for the biological activity.

3. EXPERIMENTAL SECTION

Melting point is uncorrected, proton NMR spectra of the compounds dissolved in $CDCl_3$, and DMSO were obtained with Bruker 300 spectrometer. Infrared spectra were recorded on a Perkin-Elmer 1310 spectrophotometer. Mass spectra were determined on a Micromass LCT, where tz refers to triazole and ar refers to aromatic compounds).

3.1. General Procedure

A mixture of N-hydroxymethyl-1,2,4-triazole (10 mmol) and amine (5 mmol) in acetonitrile (25 mL) was stirred and refluxed in a closed vessel for 4 hours. Then the acetonitrile layer was dried and treated with anhydrous $MgSO_4$. After filtration the solvent was removed under vacuum and the crude products were washed with water, diethylether or dichloromethane wasthen dried. The products were analysed as such:

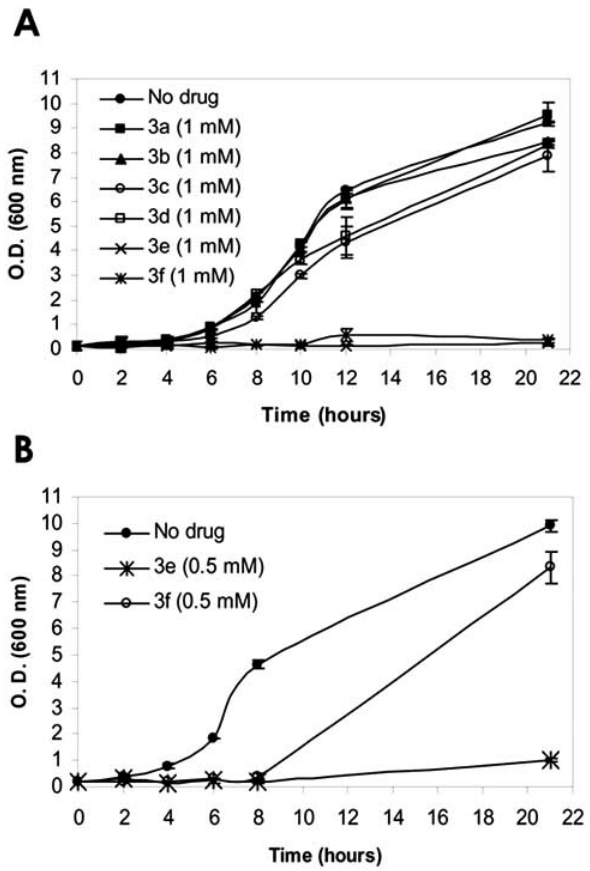

Fig. (2). Cultures of *S. cerevisiae* were grown in the presence of the compounds. Optical density was measured every 2 hours to follow cell growth.

N,N-bis((1H-1,2,4-triazol-1-yl)methyl)-3,5-dibromopyridin-2-amine 3a

White solid (77.2%), Mp = 190-192°C, ^{1}H NMR (300MHz, DMSO) δ ppm: 8.17(s, 2H, CH_{tr}); 8.03(s, 1H, CH_{Ar}); 7.80(d, 1H, CH_{Ar}); 7.75(s, 2H, CH_{tr}); 5,84(d, 4H, N-CH_2-N). ^{13}C NMR (75MHz, DMSO) δ ppm: 151.89(CH_{Ar}); 146.97(CH_{tr}); 141.72(CH_{Ar}, CH_{tr}); 108.94(C-Br); 104.78(C-Br); 54.69(N-CH_2-N). IR (KBr, ν cm^{-1}) : 3317(C-H); 1501(C=N); 1303(N-0); 1270 (C-N); 674(C-Br). MS (ES) (m/z) (%) = 411.48(100); 245.60(99); 167.64(50.92); 265.04(40.74).

N,N-bis((1H-1,2,4-triazol-1-yl)methyl)-pyridin-4-amine 3b

White solid (89.5%), Mp = 148-150°C. ^{1}H NMR (300MHz, DMSO) δ ppm: 8.68 (s, 2H, -CH_{tz}) 8.10 (d, 2H, -$CH_{ar,}$ J = 3.73 Hz); 7.99 (s, 2H, -CH_{tz}), 6.77 (d, 2H, -CH_{ar}, J = 4.83 Hz); 5.62 (s, 4H, -NCH_2N-); 5.46 (s, 2H, Pyr-CH_2N-). ^{13}C NMR (75MHz, DMSO) δ ppm: 152.21 (-CH_{ar}); 151.98 (-CH_{tz}); 150.18 (-CH_{ar}); 144.41 (-CH_{tz}); 108.57(-CH_{ar}); 71.51 (-NCH_2N-); 55.98 (Pyr-CH_2N-). IR (KBr, ν cm^{-1}): 3258.4 (-CH); 1610 (-C=C); 1507 (-C=N); 1275 (C-N); 1133; 995; 681. MS (ES) (*m*/z) (%) = 270.75(98); 189.77(73.4); 172.90(100).

N,N,N,N-tetrakis((1H-1,2,4-triazol-1-yl)methyl)benzene-1,4-diamine 3c

White solid (85%), Mp = 179-181°C; ^{1}H NMR (300MHz, DMSO) δ ppm: 8.68 (s, 4H, -CH_{tz}); 7.99 (s, 4H, -CH_{tz}); 7.08 (s, 4H, H_{ar}); 5.94 (s, 8H, -N-CH_2-N-). ^{13}C NMR (75MHz, DMSO) δ ppm: 152.06 (-N=C_{tz}); 144.64 (-N=C_{tz}); 138.56 (-CH_{ar}); 116.38 (-CH_{tz}); 64.59 (-NCH_2N-). IR (KBr, ν cm^{-1}): 3104 (-C-H); 3138; 2994; 1530 (-C=C-); 1505 (-C=N); 1265 (-C-N); 1196; 1130; 956; 801; 759;

684. MS (ES) (m/z) (%) = 432.66 (50); 340.96 (48); 282 (100); 268.98 (33).

2-(bis((1H-1,2,4-triazol-1-yl)methyl)amino)propanoic acid 3d

White solid (84.50%), Mp = 124-126°C. ^{1}H NMR (300MHz, DMSO) δ ppm : 8.56(s, 2H, CH_{tr}); 7.95(s, 2H, CH_{tr}); 5.36(q, 4H, N-CH_2-N, J = 14.7 Hz); 3.92(q, 1H, CH); 1.30(d, 3H,CH_3). ^{13}C NMR (75MHz, DMSO) δ ppm:175.17(COOH); 151.85(CH_{tr}); 144.37(CHtr); 64.63(N-CH_2-N); 58.39(CH); 14.55(CH_3). IR (KBr, ν cm^{-1}): 3109(OH); 1509(C=N); 1275(C-N); 1138; 679. MS (ES) (m/z) (%) =253.46(100); 175.84(34); 245.49(14.67); 167.73(3.6).

2,6-bis(bis((1H-1,2,4-triazol-1-yl)methyl)amino)hexanoic acid 3e

Oil yellow (81%), ^{1}H NMR (300MHz, DMSO) δ ppm: 8.22(s, 4H, -CH_{tr}); 8.00(s, 4H, -CH_{tr})

5.65(s, 4H, N-CH_2-N); 5.31(q, 4H, N-CH_2-N, J = 13.6); 3.6(t, 1H, N-CH-COOH); 2.65(t, 2H, N-C$\underline{H_2}$-CH_2); 1.5(m, 2H, CH-CH_2); 1.25(m, 4H, CH_2-$\underline{CH_2}$-$\underline{CH_2}$-CH_2). ^{13}C NMR (75MHz, DMSO) δ ppm: 175.21(C=O); 151.98(CH_{tr}); 146.80(CH_{tr}); 71.96(N-CH_2-N); 67.36(N-CH_2-N); 63.57(N-$\underline{CH}$-COOH); 49.57(N-$\underline{C}H_2$-CH_2); 29.74(N-CH-$\underline{C}H_2$); 26.52(N-CH_2-$\underline{C}H_2$); 23.23(N-CH-CH_2-$\underline{C}H_2$). IR (KBr, ν cm^{-1}): 3409(O-H); 3117(C-H); 2937(C-H); 1653; 1511(C=N); 1276(C-N); 1135; 970; 679.

N,N-bis((1H-1,2,4-triazol-1-yl)methyl)-2-methylpropan-1-amine 3f

Oil white (86%), ^{1}H NMR (300MHz, DMSO) δ ppm: 8.17(s, 2H, CH_{tr}); 7.84(s, 2H, CH_{tr}); 5.06(s, 4H, N-CH_2-N); 2.38(d, 2H, CH_2) ; 1.72 (m, 1H, CH) ; 0.75 (d, 6H, CH_3). ^{13}C NMR (75MHz, DMSO) δ ppm: 151.82(CH_{tr}); 143.57(CH_{tr}); 66.16(N-CH_2-N); 58.11(s, 1C, C^3); 26.17(CH); 20.03(CH_3). IR (KBr, ν cm^{-1}): 3116(-C-H); 2960; 2872; 1509(C=N); 1274(C-N); 1136; 1020; 964; 747; 679; 643. MS (ES) (m/z) (%) = 233.06(100); 134.08(66.66); 202.96(40).

3.2. Determination of the Antibacterial Activity

The antibacterial activity against E. coli (DH5 α strain) has been determined in liquid medium (LB, Laury Broth) using the phenol red indicator (32661, Riedel-de Haen). The bacterial isolate was cultivated overnight at 37°C under aeration. Then, a fraction of the overnight culture containing roughly 1x10^6 bacterial cells was used to inoculate the test tube. After inoculation, the test tube containing the compound and the phenol red indicator was incubated at 37°C. Twenty-four hours later, the bacterial overgrowth was determined by visual observation as the culture become turbid. Bacterial overgrowth was also determined by the colour change of the culture from pink-red to orange and then yellow following the acidification http://en.wikipedia.org/wiki/Acidof the medium. In the presence of a compound with antibacterial activity, the culture stays limpid and the phenol red indicator remains pink-red. Each test was repeated three times at least.

3.3. Determination of the Antifungal Activity

The yeast (*Saccharomyces cerevisiae*) strain BY4741 [20] was used in the growth rate study. Growth rate of yeast cells was measured as the optical density of cells at 600 nm as a function of time (hours) in rich medium. Yeast cells were diluted from an overnight culture to an O.D. (600 nm) of ~0.08 and allowed to grow until the O.D. (600 nm) reached ~0.14, ensuring that the cells were in logarithmic phase. Drug was then added and growth rate was measured. All compounds were diluted in 100% DMSO, and all assays, including the "no drug" control, contained 1% DMSO.

4. CONCLUSION

In conclusion, we have synthesised new variety of tripodal compounds bearing 1,2,4-triazolyl moiety with good to excellent yields. Their biological activities have been performed; antifungal against yeast cells of (*Saccharomyces cerevisiae*) and antibacterial against (*Escherichia coli*). The structural and the electronic diversity of these products affected their biological activities. Further developments on this subject to understand their mechanistic interactions are currently in progress.

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