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New *N,N,N',N'*-tetradentate Pyrazoly Agents: Synthesis and Evaluation of their Antifungal and Antibacterial Activities

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Abstract: A new library of *N,N,N',N'*-tetradentate pyrazoly compounds containing a pyrazole moiety was synthesized by the condensation of (3,5-dimethyl-1H-pyrazol-1-yl)methanol 2a or (1H-pyrazol-1-yl)methanol 2b with a series of primary diamines in refluxed acetonitrile for 6h. The antifungal activity against the budding yeast *Saccharomyces cerevisiae*, as well as the antibacterial activity against *Escherichia coli* of these new tetradentate ligands were studied. We found that these tetradentate ligands act specifically as antifungal agents and lack antibacterial activity. Their biological activities depend on the nature of the structure of the compounds.

Keywords: Tetradentate pyrazole, synthesis, donor-groups, antibacterial, antifungal.

1. INTRODUCTION

The growing interest in finding new bioactive compound against diseases provides exciting prospects for the preparation of many functionalized heterocyclic rings such as pyrazole [1], triazole [2] and tetrazole [3]. This continuing interest is evident from the large and the enormous research in this section and from the potential applications in bioinorganic and medicinal chemistry [4-7]. In fact, most of the new drugs contain heterocyclic compounds [8]. In this paper, specially newly prepared pyrazole compounds are reported. Indeed, over the past few years, increasing attention has been paid to pyrazole and their complexes in the pharmaceutical and agro-chemical industries, and large advanced synthetic methods have been designed recently due to the non-existence of pyrazole derivatives in nature, probably, due to the difficulty in the construction of N–N bond by living organisms [9]. Actually, the pyrazole system represents an important heterocyclic template due to its long history and various applications such as antiproliferative [10], antibacterial [11], analgesics [12], antiparasitic [13], antiviral [14], antiglycemic [15] or as anti-in ammatory agents [16].

In previous work, we showed that some tridentate ligands based on pyrazole and triazole have some biological activities [17-18]. Using functional genomics technologies in yeast, we also showed that two tridentate ligands based on pyrazole and triazole act as DNA damage agents [18].

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In this paper we report the synthesis of some new *N,N,N',N'*-tetradentate pyrazole as well as their antifungal and antibacterial biological activities.

2. MATERIALS AND METHODS

2.1. Procedure of Synthesis

The following products illustrated below were prepared after a modification on the method of the synthesis reported in the literature [19-26]. The compounds were obtained by a simple condensation between (3,5-dimethyl-1H-pyrazol-1-yl)methanol **2a** or (1H-pyrazol-1-yl)methanol **2b** and various primary diamines (Scheme (**1**)).

2.2. Determination of the Antifungal Activity

The antifungal activity against the yeast (*Saccharomyces cerevisiae*) strain BY4741 [27] has been determined using liquid cell culture assay as described [18].

2.3. Determination of the Antibacterial Activity

Disc diffusion assay has been used to evaluate the antibacterial activity of the newly synthesized molecules [28-31]. In this assay, the Gram-negative bacterial strain *E. coli* (*DH5α* strain) has been used. The bacterial isolate was cultivated overnight in liquid Luria-Bertani medium (LB) at 37°C under aeration. After that, a suspension containing 10^8 CFU/mL of bacteria cells was prepared and used to inoculate Petri plates containing solid (LB) medium. The plates were then allowed to dry for 15 minutes. Then, the paper discs (6mm in diameter) which had previously been impregnated

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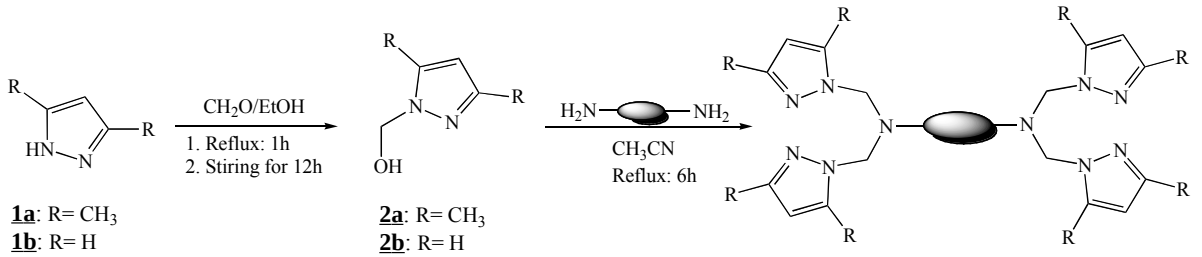

1a: R= CH_3
1b: R= H

2a: R= CH_3
2b: R= H

Comp.	R		Yield (%)
3a	-CH_3		82.21
3b	-H		70.58
4a	-CH_3		83.73
4b	-H		65.69
5a	-CH_3		84.93
6a	-CH_3		82.85
7a	-CH_3		55.28
7b	-H		44.28
8a	-CH_3		22.28
9a	-CH_3		28.03
9b	-H		60.73

Scheme 1. General pathway of synthesized pyrazolic compounds.

with the tested compounds were placed on the inoculated agar plates. The compounds were then allowed to diffuse into the medium by incubating the plates for one hour at room temperature. These were then incubated at 37°C. Twenty-four hours later, the antibacterial activity was evaluated by measuring the inhibition zone diameters in millimetre. The measurements of inhibition zones were performed three times for each drug including compound 15 as a positive control (PC) [32].

3. RESULTS AND DISCUSSION

3.1. Synthesis

From Fig. (**1**), we clearly show that the N-CH_2-N signal of our compounds is different from a singlet for **4a** (**3a** and **6a**), doublet for **7a** (**7b**, **8a** and **9a**) and a double of doublet for the compound **5a**. The difference in the multiplicity of the N-CH_2-N signal is due to the space structure of each molecule. Therefore, in the case of the singlet peak of the compound **4a** for example, the symmetry makes atoms equivalent in the molecule, which means that all the proton atoms of N-CH_2-N bond are in identical locations relative to a plane of symmetry in the molecule, then these atoms are equivalent and we see just one peak with an integration of 8 proton. The doublet peak for example **7a** means that the molecule is not symmetric relative to a plane or a center of symmetry that means the two protons are not equivalent to each other. Finally, the compound **5a** shows two unequivalent methylene groups with partially overlapping signals as a consequence of the chiral carbon centre.

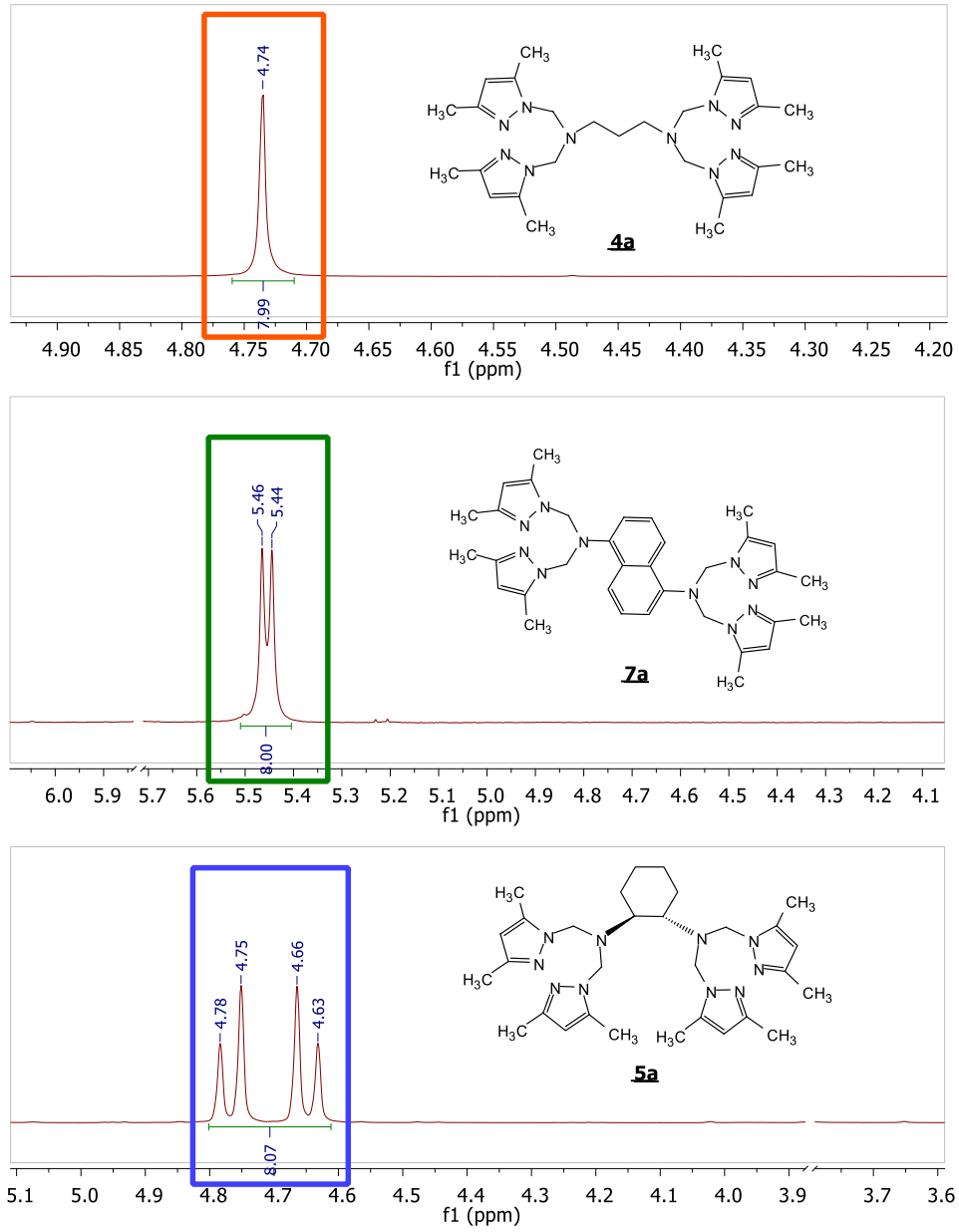

Fig (1). Multiplicity of the N-$\underline{CH_2}$-N signal in the compounds **4a**, **7a** and **5a**.

The methylene protons are diastereotopic in the chiral ligand **5a** (Fig. (**1**)) and their signals appear as AB systems with a coupling constants of 13 Hz. Very sensitive probes for changes in the environment are the methylene proton resonances which agree with the reported literature [33-35].

3.2. Antifungal Activity of Tetradentate Based on (3,5-dimethyl-1H-pyrazol-1-yl)methanol and (1H-pyrazol-1-yl)methanol Compounds

We first evaluated seven new tetradentate based on (3,5-dimethyl-1H-pyrazol-1-yl)methanol for their antifungal activity against budding yeast (*Saccharomyces cerevisiae*) cells. Using liquid cell culture assay, yeast cells were grown in the presence of 500 μM of each compound and assayed for growth inhibition (Fig. **2A**,**2B**). Compound **7a** did not inhibit yeast growth, however compounds **8a** and **9a** were slightly toxic, but compounds **3a**, **4a** and **6a** showed moderate antifungal activity. Interestingly, compound **5a** displayed strong antifungal activity. Overall, these results suggest that the 3,5-dimethyl-1H-pyrazoly group of these tetradentate ligands is not important for the antifungal activity of these compounds. However, the central diamine group is critical for bioactivity in yeast. In addition, compound **5a** was more potent than the other tetradentate ligands, indicating that the central cyclohexane-1,2-diamine group of **5a** is the most active followed by propane-1,3-diamine of **4a**, ethane-1,2-diamine of **3a**, Benzene-1,2-diamine of **6a**, aniline of **9a** and then 9H-fluorene-2,7-diamine of **8a**. Because compound **7a** showed no antifungal activity, the central naphthalene-1,5-diamine group is inactive.

We next assessed four tetradentate compounds based on (1H-pyrazol-1-yl)methanol for toxicity against budding yeast

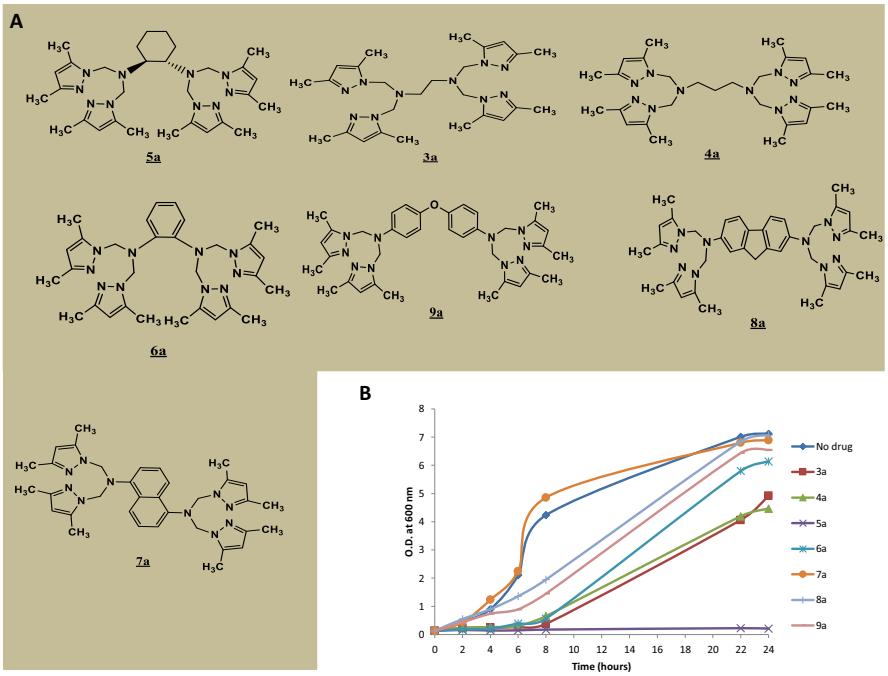

Fig (2). Antifungal activity of tetradentate ligands based on (3,5-dimethyl-1H-pyrazol-1-yl)methanol. **A.** Structures of the tetradentate ligands analyzed. **B.** Yeast cells were cultured in the presence of 500 µM of the compounds shown in 2A. Optical density was measured every 2 hours to follow cell growth.

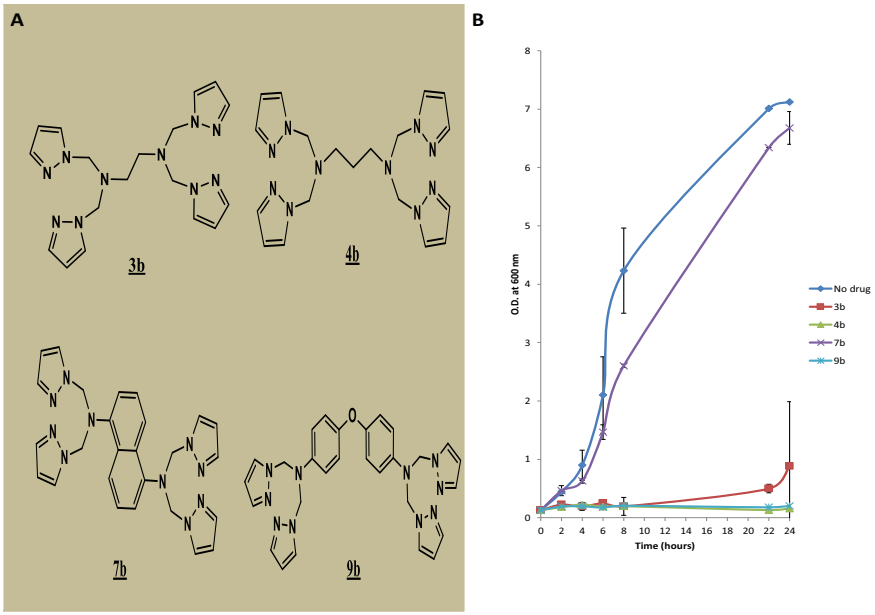

Fig (3). Antifungal activity of tetradentate ligands based on (1H-pyrazol-1-yl) methanol. **A.** Structures of the tetradentate ligands analyzed. **B.** Yeast cells were cultured in the presence of 500 µM of the compounds shown in 3A. Optical density was measured every 2 hours to follow cell growth.

cells. Cells were grown in the presence of 500 µM of each compound and assayed for growth inhibition in liquid culture (Fig. **3A**, **3B**). Compounds **7b** showed weak antifungal activity, whereas compounds **3b**, **4b** and **9b** displayed strong bioactivity. Interestingly, these results suggest that tetradentate ligands based on (1H-pyrazol-1-yl)methanol are much more bioactive than tetradentate ligands based on (3,5-dimethyl-1H-pyrazol-1-yl)methanol. As a matter of fact, **3b**, **4b**, **7b** and **9b** were much more toxic to yeast cells than **3a**, **4a**, **7a** and **9a,** respectively.

3.3. Antibacterial Activity of Tetradentate Based on (3,5-dimethyl-1H-pyrazol-1-yl)methanol and (1H-pyrazol-1-yl)methanol Compounds

It has been previously shown that tridentate ligands have antibacterial activity against *E. coli* [17]. Therefore, it is

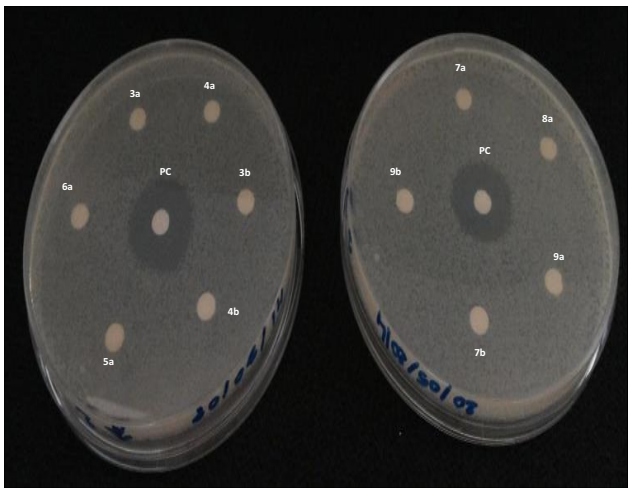

Fig (4). Antibacterial activity of tetradentate based on (3,5-dimethyl-1H-pyrazol-1-yl)methanol and (1H-pyrazol-1-yl)methanol compounds. *E. coli* cells were first spread on petri plates containing solid LB medium. Then, the paper discs (6mm in diameter) which had previously been impregnated with the tested compounds were placed on the inoculated agar plates. These were then incubated at 37°C. Twenty-four hours later, the antibacterial activity was assessed by measuring the diameter of the growth-inhibition zone in millimetre. The tested compounds including the positive control (PC) are indicated.

likely that these tetradentate ligands have antibacterial activity against *E. coli*. To test this hypothesis, we evaluated these compounds for their antibacterial activity against *E. coli* as described in materials and methods. Interestingly, all the tetradentate compounds tested **3a, 3b, 4a, 4b, 5a, 6a, 7a, 7b, 8a, 9a** and **9b** showed no antibacterial activity when they were used at 500 µM (Fig. **4**); Data not shown). Thus, together with the antifungal activity analysis, these results suggest that these tetradentate ligands act specifically as antifungal agents and lack antibacterial activity. Indeed, specific inhibitory compounds are extremely valuable because they are leading compounds for designing new drugs. Further investigation is required to determine the biological activity of these compounds against more fungal and bacterial strains, and to better understand their mode of action.

4. CONCLUSION

New series of *N,N,N',N'*-tetradentate pyrazole with N-C-N junction were synthesized using the condensation between a pyrazole moiety and a primary diamine. Their structures were characterized by ^{1}H, ^{13}C NMR, Infrared and mass spectroscopy. The nature of the multiplicity of the N-$\underline{CH_2}$-N signal was also discussed. We found that some tetradentate compounds have strong antifungal activity and that the central diamine group is critical for bioactivity in yeast. As matter of fact, the central cyclohexane-1,2-diamine group of 5a is the most active. In addition we found that tetradentate ligands bearing pyrazol-1-yl methyl moiety are much more active than those bearing (3,5-dimethyl-pyrazol-1-yl)methyl moiety. Further investigation is required to better understand the mode of action of this class of compounds.

5. EXPERIMENTAL

5.1. General

3,5-dimethyl-1H-pyrazole **1a**, 1H-pyrazole **1b**, formaldehyde and used solvent were commercially available, were of sufficient purity, and were used without further treatment. Melting points were obtained using the capillary tube method with an Electrothermal 9100 apparatus. Proton and carbon nuclear magnetic resonance spectra were recorded on a Bruker 300 instrument (operating at 300.13 MHz for ^{1}H, 75.47 MHz for ^{13}C) spectrometer. Chemical shifts are reported in parts per million (ppm). The band positions on Infrared Spectra (IR) are reported in reciprocal centimeters (cm^{-1}) on a Shimadzu infrared spectrophotometer using the KBr disc technique. Mass spectra (MS) were obtained by using electrospray ionization (ESI) technique. All analysis has been done in the Catholic University of Louvain platform, Belgium and in University of Rennes 1 France.

5.2. General Experimental Protocol

A solution of primary diamine (one equiv.) in acetonitrile (20 mL), was slowly added to a solution of **2a** or **2b** (four equiv.) in 60 mL of acetonitrile. The reaction mixture was stirred under refluxed for 6h then dried over $MgSO_4$. The solvent was removed under reduced pressure. The solid was dried using a Schlenk line.

5.3. Spectral Data

For abbreviations we took: pz = pyrazole; bz = benzene; cyh1 = CH of cyclohexane; cyh2 = CH_2 of cyclohexane; cyp = cyclopentadiene; naph = naphthalene.

The compounds **3a**-**b** and **6a** are already described in the literature, but the method of their synthesis is different from the reported one [24].

5.3.1. N^1,N^1,N^3,N^3-tetrakis((3,5-dimethyl-1H-pyrazol-1-yl)methyl)propane-1,3-diamine 4a

White solid, yield = 86.73%. **MP**: 64-66 °C. **IR** (ν (cm^{-1})): 3200 - 2864 (CH); 1556 (C=N); 1458(C=C); 1303 (C-N aromatic); 1145 (C-N aliphatic).**^{1}H NMR** (300 MHz, $CDCl_3$, δppm): 5.78 (s, 4H, pz), 4.74 (s, 8H, N-$\underline{CH_2}$-N), 2.68 (t, 4H, N-$\underline{CH_2}$-CH_2 of amine, J = 6 Hz), 2.24 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 1.71 – 1.64 (m, 2H, N-CH_2-$\underline{CH_2}$ of amine). **^{13}C NMR** (75 MHz, $CDCl_3$, δppm): 147.31

($C^{(3)}$ pz),139.70($C^{(5)}$ pz),105.57(pz),70.45(N-$\underline{CH_2}$-N),49.17 (N-$\underline{CH_2}$-CH_2 of amine), 22.43 (N-CH_2-$\underline{CH_2}$ of amine), 13.43(CH_3), 11.08 (CH_3).

5.3.2. N^1,N^1,N^3,N^3-tetrakis((1H-pyrazol-1-yl)methyl)propane-1,3-diamine 4b

Transparent oil, yield = 65.69 % .**IR** (ν (cm^{-1})): 3200 - 2858 (CH); 1514 (C=N); 1440 (C=C); 1280 (C-N aromatic); 1143 (C-N aliphatic). **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm): 7.49 – 7 .36 (m, 8H, $pz^{(5)}$ /$pz^{(3)}$), 6.19 (t, 4H, pz, J = 3 Hz), 4.85 (d, 8H, N-$\underline{CH_2}$-N, J = 3 Hz), 2.50 (t, 4H, N-$\underline{CH_2}$-CH_2 of amine, J = 3 Hz),1.57 – 1.51 (m, 2H, N-CH_2-$\underline{CH_2}$ of amine). **^{13}C NMR** (75 MHz, $CDCl_3$, δppm): 139.59 ($C^{(3)}$pz), 129.57 ($C^{(5)}$pz), 105.91(pz), 67.69(N-$\underline{CH_2}$-N), 48.85 (N-$\underline{CH_2}$-CH_2 of amine), 22.96 (N-CH_2-$\underline{CH_2}$ of amine). **MS** [M^+] (m/z): calculated 394,48, found 420,12 ([M^++ Na+3]).

5.3.3. (1S,2S)-N^1,N^1,N^2,N^2-tetrakis((3,5-dimethyl-1H-pyrazol-1-yl)methyl)cyclohexane-1,2-diamine 5a

Yellow solid, yield = 84.93 %. **MP**: 120-122°C. **IR** (ν (cm^{-1})): 3100- -2860 (CH); 1556 (C=N); 1460(C=C); 1303(C-N aromatic); 1195 (C-N aliphatic). **^{1}H NMR** (300 MHz, $CDCl_3$, δppm): 5.77 (s, 4H, pz), 4.72 (dd, 8H, N-$\underline{CH_2}$-N, J = 13Hz), 2.38 – 2.30 (m, 2H, cyh1), 2.22 (s, 12H, CH_3), 2.17 (s, 12H, CH_3), 1.90 – 1.14 (m, 8H, cyh2). **^{13}C NMR** (75 MHz, $CDCl_3$, δppm): 147.33($C^{(3)}$ pz), 139.47($C^{(5)}$pz), 105.70(pz), 71.90(N-$\underline{CH_2}$-N), 66.13(cyh1), 28.87(cyh2), 4.14(cyh2), 13.48(CH_3), 11.11(CH_3). **MS** [M^+] (m/z): calculated 546.77, found 586.9 ([M^++K+1]).

5.3.4. N^1,N^1,N^5,N^5-tetrakis((3,5-dimethyl-1H-pyrazol-1-yl)methyl)naphthalene-1,5-diamine 7a

Purple solid, yield= 55.28 %. **MP**: 196-198 °C. **IR** (ν (cm^{-1})): 3336- -2941 (CH); 1589 (C=N); 1438(C=C); 1384(C-N aromatic); 1161 (C-N aliphatic). **^{1}H NMR** (300 MHz, DMSO, δppm): 7.48 – 7.02 (m, 6H, naph), 5.76 (s, 4H, pz), 5.45 (d, 8H, N-$\underline{CH_2}$-N, J = 6.1 Hz), 2.31 (s, 12H, CH_3), 2.08 (s, 12H, CH_3). **^{13}C NMR** (75MHz, DMSO, δppm): 142.23 ($C^{(3)}$ pz), 138.79 ($C^{(5)}$pz),105.10(pz), 57.83(N-$\underline{CH_2}$-N), 13.34(CH_3), 10.81(CH_3). **MS** [M^+] (m/z): calculated 590.36, found 613.34 ([M^++Na]).

5.3.5. N^1,N^1,N^5,N^5-tetrakis((1H-pyrazol-1-yl)methyl)naphthalene-1,5-diamine 7b

Purple solid, yield = 44.28%. **MP**: 194-196°C. **IR** (ν (cm^{-1})): 3444- -2903 (CH); 1593 (C=N); 1435(C=C); 1396(C-N aromatic); 1145 (C-N aliphatic). **^{1}H NMR** (300 MHz, DMSO, δppm): 7.80 –7 .17 (m, 14H, $pz^{(5)}$ /$pz^{(3)}$/naph), 6.22 (t, 4H, pz, J = 3 Hz), 5.62 (d, 8H, N-$\underline{CH_2}$-N, J=6.8 Hz). **^{13}C NMR** (75MHz, DMSO, δppm): 142.29 ($C^{(3)}$ pz), 138.66 ($C^{(5)}$pz), 105.85(pz), 60(N-$\underline{CH_2}$-N). **MS** [M^+] (m/z): calculated 478.23, found 501.22 ([M^++Na]).

5.3.6. N^2,N^2,N^7,N^7-tetrakis((3,5-dimethyl-1H-pyrazol-1-yl)methyl)-9H-fluorene-2,7-diamine 8a

Brown solid, yield = 22.28%. **MP**: 216-218 °C. **IR** (ν (cm^{-1})): 3298- -2811 (CH); 1616 (C=N); 1485(C=C); 1265(C-N aromatic); 1164 (C-N aliphatic). **^{1}H NMR** (300 MHz, DMSO, δppm): 7.36 – 6.63 (m, 6H, bz), 5.76 (s, 4H, pz), 5.30 (d, 8H, N-$\underline{CH_2}$-N, J = 6.7 Hz), 3.63 (s, 2H, cyp), 2.28 (s, 12H, CH_3), 2.08 (s, 12H, CH_3). **^{13}C NMR** (75MHz, DMSO, δppm): 143.08 ($C^{(3)}$ pz), 138.44 ($C^{(5)}$ pz), 105.20 (pz), 57.69(N-$\underline{CH_2}$-N),36.37(cyp),13.31(CH_3), 10.71(CH_3). **MS** [M^+] (m/z): calculated 628,38, found 675,13 ([M^++2Na+1]).

5.3.7. 4,4'-oxybis(N,N-bis((3,5-dimethyl-1H-pyrazol-1-yl)methyl)aniline) 9a

Beige solid, yield = 28.23%. **MP**: 78-80°C. **IR** (ν (cm^{-1})): 2954- 2920 (CH); 1556 (C=N); 1456(C=C); 1317 (C-N aromatic); 1232 (C-O); 1161 (C-N aliphatic). **^{1}H NMR** (300 MHz, DMSO, δppm): 7.08 – 6 .40 (m, 8H, bz), 5.78 (s, 4H, pz), 5.22 (d, 8H, N-$\underline{CH_2}$-N, J = 7.4 Hz), 2.23 (s, 12H, CH_3), 2.08 (s, 12H, CH_3). **^{13}C NMR** (75 MHz, DMSO, δppm): 145.93($C^{(3)}$ pz), 138.68 ($C^{(5)}$pz), 105.31(pz), 70.35(N-$\underline{CH_2}$-N),13.33(CH_3), 10.52(CH_3). **MS** [M^+] (m/z): calculated 632.37, found 655.35 ([M^++Na]).

5.3.8. 4,4'-oxybis(N,N-bis((1H-pyrazol-1-yl)methyl)aniline) 9b

Beige solid, yield = 60.73%. **MP**: 68-70°C. **IR** (ν (cm^{-1})): 3310- 2816 (CH); 1504 (C=N); 1400(C=C); 1273 (C-N aromatic); 1215 (C-O); 1165 (C-N aliphatic). **^{1}H NMR** (300 MHz, $CDCl_3$, δppm): 7.57(d, 4H, bz, J = 3 Hz), 7.45(d, 4H, bz, J = 3 Hz), 7.04(d, 4H, $pz^{(5)}$, J = 9Hz), 6.88(d, 4H, $pz^{(3)}$, J = 6Hz), 6.27 (t, 4H, pz, J = 3 Hz), 5.39 (s , 8H, N-$\underline{CH_2}$-N). **^{13}C NMR** (75 MHz, $CDCl_3$, δppm): 140.40 ($C^{(3)}$ pz), 129.52 ($C^{(5)}$ pz),106.22(pz), 66.94 (N-$\underline{CH_2}$-N). **MS** [M^+] (m/z): calculated 520,29, found 523,01 ([M^++3]).

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

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