Synthesis and cytotoxic studies of N-heterocyclic Mannich Bases

Mamona Waris[1], Freeha Hafeez[1*], Rabia Ali[2], Hira Akmal[1], Muhammad Suleman[1]

[1]Department of Chemistry, Riphah International University Faisalabad

[2]Department of Chemistry, University of Agriculture Faisalabad

Abstract

The *N*-heterocyclic derivatives containing nitrogen atom have sustained enthusiasm of scientists and emphasizing the numerous applications made possible by their unique structure in diverse field. A variety of *N*-heterocyclic piperazine derivatives 5a-e, were synthesized in this study. Spectro-analytical methodologies were used to fully characterize each of the synthesized compounds. The structures of synthesized derivatives were determined using the Proton NMR, melting points, and FT-IR. Among these synthesized derivatives, the compound 5e was presented the Hemolysis of (0.8 %) with lowest toxicity and had greater thrombolytic potential, exhibited lysis of (62.5%). So, the compound 5e was the best one, whereas the other derivatives were showed lower to moderate cytotoxicity effects.

Introduction:

The N-heterocycles play a significant role due to their biologic, [1] Pharmacologic, [2] and therapeutic, [3] importance in addition to their abundance. They play a crucial part in the biological assessments of anti-inflammatory, [4]. antibacterial, [5] antiviral, [6] and anti-tumor [7] measures. Researchers have continued to be interested in nitrogen-containing heterocyclic compounds, and their unusual structures have led to a number of applications in numerous sectors [8]. Due to the intense interest in heterocycles, the synthesis of these compounds has traditionally been one of the most important study areas in synthetic chemistry [9].

The most significant category of N-heterocyclic derivatives is mannich bases. Beta-amino ketone-containing compounds are referred to as mannich bases. It is an outcome of the mannich reaction. A specific kind of nucleophilic addition process known as the mannich reaction produces carbon-carbon bonds. It's an essential stage in the creation of a variety of organic molecules, medicines, and other compounds. The mannich interactions are essential for the formation of nitrogen-containing compounds. There are numerous mannich bases with amino alkyl chains that are potent medicinal drugs, including fluoxetine, atropine, ethacrynic acid, and trihexyphenidyl, along with others [10].

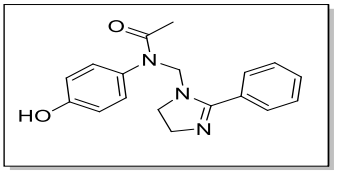


Fig 1: Antioxidant Mannich bases

The mannich bases also acted as important functional determinations or pharmacological intermediates that were used in the subsequent synthesis of numerous biologically active chemicals containing amino-alkyl chains. It is common practice to synthesize molecules with an atom of nitrogen via the mannich process. The importance of the mannich bases has increased due to their application in cytotoxic and anticancer drugs [11]. Additionally, the mannich bases have antifungal, antibacterial, and antitubercular effects [12]. In their work, Koksal et al. described the biological, anti-HIV, anti-inflammatory, and analgesic properties of mannich bases. It is also recognized that the mannich bases are used as resins, surface-active agents, detergent additives, and polymers [13]. There have been created mannich bases of a number of active chemicals to get around the limitations. The mannich bases of 2-naphthol "optically pure chiral" are utilized to make enantioselective carbon-carbon bonds by ligand-accelerated and metal-mediated catalysis [14]. The mannich bases and their derivatives are used as intermediates in the synthesis of bioactive chemicals and compounds [15].

MATERIALS AND METHODS

The chemicals and solvents used in the research were purchased from E. Merck Germany and were at least once distilled before use. 1H-NMR spectra were obtained using a 400 MHz nuclear magnetic resonance spectrometer (model AV-400). The aforementioned solvent was CDCl3. The solvent signal was measured at 7.26 ppm.

The procedure for synthesis of 1-(methylsulfonyl)piperazine 3

Methanesulfonyl chloride **2** (0.12 g, 1.1 mmol) and Piperazine **1** (0.1 g, 1.1 mmol), was permitted to react, in presence of DCM (10 mL). With constant stirring at room temperature the reaction was carried out for 24-48 hours. With TLC (Ethyl acetate: Pentane: Triethylamine in 3:2:0.5 ratios) the reaction progress was checked. With the help of filtration the product **3** was achieved. Warm n-hexane was utilized during recrystallization to purify the crude product.

The procedure for the synthesis of *N*-mannich bases 5a-e

1-(methylsulfonyl)piperazine **3** (1.0 equiv.), and various *N*-heterocyclic secondary amines **4a-e** (1.0 equiv.) were permitted to react in 37% formalin solution (2.0 equiv.). the solvent ethanol was used. For 24 hours, the reaction solution was kept at 80°C. TLC confirmed that the response had finished and offered information about its continuation. The mixture was cooled to room temperature before the end product was separated. The products were then purified by flash column chromatography in a ratio of 3:2:0.5 of ethyl acetate, pentane, and triethylamine.

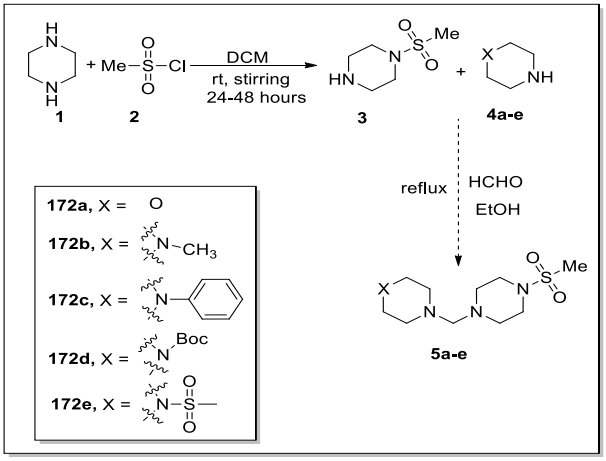


Fig 2: General outline scheme for the synthesis of 1-(methylsulfonyl)piperazine 3 and its derivatives 5a-e

Hemolysis Assays:

5mL of fresh blood from a healthy donor was collected and placed in an EDTA tube. Blood was collected into microcentrifuge tubes and then centrifuged for 5 minutes at 1000 rpm to separate red blood cells (RBCs). After that, the supernatant was eliminated, and the RBC pellet underwent three PBS (phosphate buffer saline) washes. The RBC pellet was recovered after washing, and 20 L of the sample solution in DMSO was added. The tubes were incubated at 37°C for 60 minutes. The tubes were again centrifuged for 5 minutes at a speed of 13000 rpm after being removed from the incubator. The recovered supernatant should be diluted with cooled phosphate buffer saline solution. evaluated the absorbance at 517 nm. In this method, DMSO functioned as the adverse control and ABTS as the favorable control. The proportion of RBC lysis in tests was determined using the latter formula, and experiments were performed in triplicate.

The RBC's Lysis (%) =

$$\frac{absorbance\ of\ sample - absorbance\ \ of\ negative\ control}{absorbance\ of\ positive\ control}\ \mathbf{100}$$

Thrombolysis Assays:

The thrombolytic assays were carried out utilizing the literature approach. 500 L of blood was then placed to eppendorf tubes that had already been cleaned 56 and weighed after a 3 mL sample from a healthy human donor was acquired. Fill these tubes with blood once more, and then incubate them for an hour at 37°C to look for clot development. The tube containing the clot was then weighed after the serum was removed. The tubes were then incubated once more for 3 hours at 37 °C to ascertain the results of the lysis after the clot was infused with 40 L of the sample solution in DMSO. Although ABTS served as the negative control in this test, DMSO was used instead. The formula below was used to calculate the percentage of lysis in the studies, which were done in triplicate.

The percentage of clot lysis =

$$\frac{initial\ clot\ weight - final\ clot\ weight}{initial\ clot\ weight} \times \mathbf{100}$$

Table 1: The hemolytic and thrombolytic activity of synthesized derivatives 5a-e

Sr. #	Derivatives	Percentage of Hemolysis ± SD	Percentage of Thrombolysis ± SD
1	173a	2.06 ± 0.121	44.2 ± 0.081
2	173b	1.33 ± 0.004	46.1 ± 0.081
3	173c	3.90 ± 0.005	43.29 ± 0.008
4	173d	5.6 ± 0.041	42.2 ± 0.081
5	173e	0.8 ± 0.009	62.5 ± 0.081

Table 2: Spectral data of compounds 5a-e

Comp	**M.P** °C	**Yield**	

5a	112	71	Off-white amorphous: m. p: 112°C. FT-IR, (C-N) = 1130 cm^{-1}, (C-C) = 600 cm^{-1}, (S=O) = 1273 cm^{-1}, (C-O) = 1015 cm^{-1}, (C-H) = 2821 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$), 3.65(t, 4H, *J* = 8 Hz), 3.43(s, 2H, CH_2), 2.95(s, 3H, CH_3), 2.50(t, 4H, *J* = 8 Hz), 2.45(t, 4H, *J* = 8 Hz), 2.37(t, 4H, *J* = 8 Hz, piperazine).
5b	120	69	Off-white amorphous: m. p: 120°C. FT-IR, (C-N) = 1100 cm^{-1}, (C-C) = 600 cm^{-1}, (S=O) = 1200cm^{-1}, (C-H) = 2821 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$), 3.43(s, 2H, CH_2), 2.95(s, 3H, CH_3), 2.45(t, 4H, *J* = 8 Hz), 2.37(t, 4H, *J* = 8 Hz, piperazine), 2.35(t, 8H, *J* = 8 Hz, piperazine), 2.26(s, 3H, CH_3)
5c	145	70	Off-white amorphous: m. p: 145oC. FT-IR, (C-N) = 1100 cm-1, (C-C) = 600 cm-1, (S=O) = 1260cm-1, (C-H) = 2821 cm-1, (C=C) = 1645 cm-1. 1H NMR (400 MHz, CDCl3), 7.27(t, 2H, J = 8 Hz), 6.94(d, 2H, J = 8 Hz), 6.79(t, 1H, CH, benzene), 3.44(t, 4H, J = 8 Hz), 3.43(s, 2H, CH2), 2.95(s, 3H, CH3), 2.48(t, 4H, J = 8 Hz), 2.45(t, 4H, J = 8 Hz), 2.37(t, 4H, J = 8 Hz)
5d		73	Off-white amorphous: m. p: 130oC. FT-IR, (C-N) = 1100 cm-1, (C-C) = 600 cm-1, (S=O) = 1250cm-1, (C=O) = 1650 cm-1, (C-O) = 1020 cm-1, (C-H) = 2835 cm-1. 1H NMR (400 MHz, CDCl3), 3.43(s, 2H, CH2), 3.20(t, 4H, J = 8 Hz), 2.95(s, 3H, CH3), 2.51(t, 4H, J = 8 Hz), 2.45(t, 4H, J = 8 Hz), 2.37(t, 4H, J = 8 Hz), 1.38(s, 9H, CH3)
5e		74	Off-white amorphous: m. p: 129oC. FT-IR, (C-N) = 1100 cm-1, (C-C) = 600 cm-1, (S=O) = 1273cm-1, (C-H) = 2845 cm-1. 1H NMR (400 MHz, CDCl3), 3.43(s, 2H, CH2), 2.95(s, 6H, CH3), 2.45(t, 8H, J = 8 Hz), 2.37(t, 8H, J = 8 Hz)

Cytotoxic evaluation:

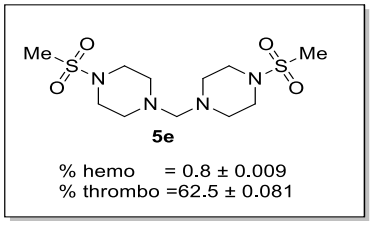
Hemolysis and thrombolysis:

The chemical 5e demonstrated the best molecule among all the produced derivatives and had the lowest levels of toxicity and hemolysis (0.8).

The highest thrombolysis value was also displayed by compound 5e, which was 62.5.

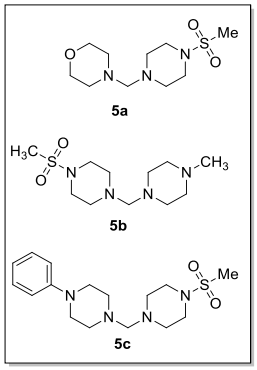
Discussion:

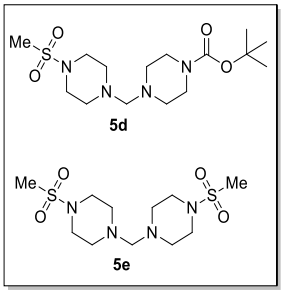
The compound 5e is the best among these synthesized derivatives 5a-e; it had the lowest toxicity and the best percentage of RBC lysis (0.8%); other derivatives had cytotoxic effects that ranged from low to moderate; and 5e had a higher potential for thrombolysis; testing it against ABTS revealed the highest lysis of (62.5%).



Spectro-analytic methods were used to fully characterize each of the produced compounds 5a-e.

For the purpose of determining the structures of synthesized derivatives, 1H NMR, FT-IR, and melting points were used. By analyzing hemolysis and thrombolysis in vitro, synthetic derivatives' cytotoxic effects were assessed.





Conclusion:

Based on the results of the current investigation, it is advised that the 5e derivative be used as a cytotoxic agent. In conclusion, the current study gave us the chance to carry out further research in these areas by designing and creating novel bioactive compounds by modifying the Mannich bases' scaffold with different structural moieties. Through in-vivo study, it may be possible to determine the full potential and usefulness of these substances for the treatment of malignant illnesses.

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