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Assessment of Anti-bacterial Activity of (E)-2-(Naphthalen-1-yldiazenyl) Malononitrile Against Pathogens Bacteria.

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(نفتالين-1-يل ديازينيل) مالونونيتريل -2-(E) تقييم النشاط المضاد للبكتيريا لمركّب ضد بعض البكتيريا المسببة للمرض

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Abstract

(E)-2-(naphthalen-1-yldiazenyl) malononitrile (NDMN) was synthesized via a diazotization–coupling reaction and evaluated for its antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli*. The compound showed strong, concentration-dependent antibacterial activity, with significantly higher efficacy against MRSA than *E. coli*, as confirmed by disc diffusion, MIC, and MBC assays. These results suggest that NDMN is a promising synthetic antimicrobial agent, particularly against Gram-positive resistant bacteria, and merits further biological and toxicity investigations.

Keywords:
Azo compounds; Malononitrile; Antibacterial activity; MRSA, *Escherichia coli*.

الملخص

في ظل التزايد المستمر لظهور البكتيريا المقاومة المتعددة للمضادات الحيوية، يهدف هذا البحث إلى تحضير وتقييم النشاط المضاد للبكتيريا لمركّب −2−(E)(نفتالين−1−يل ديازينيل) مالونونيتريل .(NDMN) تم تحضير المركّب باستخدام تفاعل الديازوتة والازدواج القياسي، ثم اختُبر نشاطه المضاد للبكتيريا ضد بكتيريا *Staphylococcus aureus* المقاومة للميثيسيلين (MRSA) وبكتيريا *Escherichia coli* أظهرت النتائج نشاطًا مضادًا للبكتيريا يعتمد على التركيز، مع فعالية أعلى بشكل ملحوظ ضد بكتيريا MRSA مقارنةً بـ *E. coli*، وذلك وفق اختبارات الانتشار بالأقراص وتحديد التركيزين المثبط الأدنى (MIC) والقاتل الأدنى (MBC). تشير هذه النتائج إلى أن المركّب المحضَّر يُعد مرشحًا واعدًا كمضاد بكتيري صناعي، لا سيما ضد البكتيريا موجبة الجرام، مع التأكيد على ضرورة إجراء دراسات السمية والسلامة الحيوية قبل التطبيقات العلاجية المحتملة.

الكلمات المفتاحية:
مركبات الآزو؛ المالونونيتريل؛ النشاط المضاد للبكتيريا؛ المكورات العنقودية الذهبية المقاومة للميثيسيلين(MRSA) ، بكتيريا *Escherichia coli*.

Introduction:

The increasing multidrug-resistant (MDR) pathogens that contain bacteria lead to an expansive dissemination issue in the management of different infections. As a result, interest in studying underutilized sources of antimicrobial compounds, specifically secondary metabolites of medicinal plants as well as synthetic compounds, has reemerged [1,2]. The main aim in medicinal chemistry, however, has been to design and produce bioactive compounds that can be utilized as therapeutic agents with reduced side effects [3], but the increasing problem of resistant infections has significantly diminished the efficacy of most antibiotics [4,5]. hence, the emergence of resistant infections has stimulated researchers to develop new analogues as alternatives which can be used with reduced side effects [6]. Azo-dyes remain the subject of considerable interest due to their wide range of applications in both traditional coloring industries and more complex fields such as medicine, biotechnology, cosmetics, and materials science. Specifically, the far more widespread example of azo-dyes are aromatic azo-derivatives, due to their greater stability, which is a result of the extensive resonance delocalization of the functionality -N=N-groups as well as the aromatic system. Malononitrile is one of the building blocks that are very reactive and versatile, and is important in the production of azo dyes. Due to an activated methylene group and strong electron-withdrawing

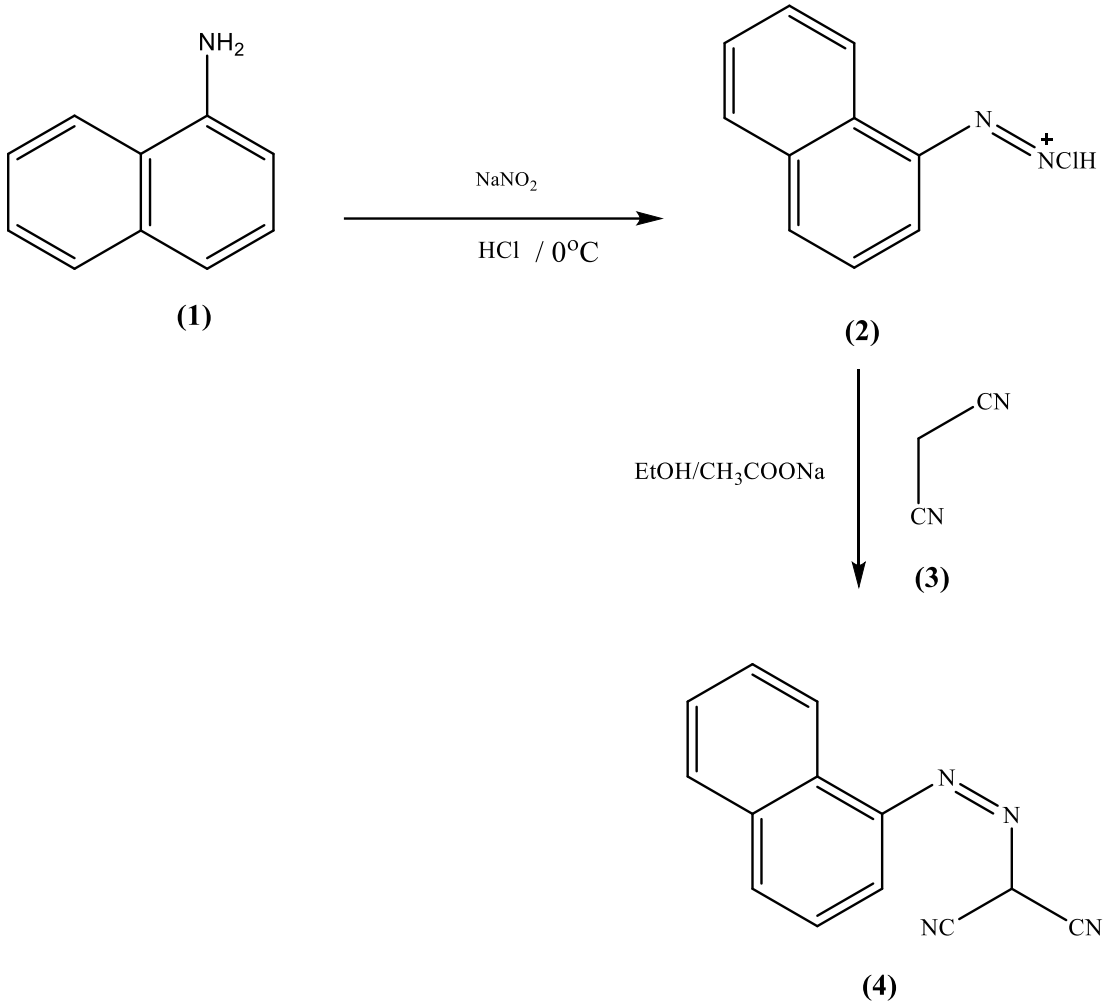
cyano substituents, it easily reacts in condensation and coupling reactions. In addition to its synthetic usefulness, the nitrile functional group has also been linked to such interesting biological characteristics as anticancer activity. This reactivity and multifunctionality make malononitrile an indispensable reagent in the dye chemistry as well as pharmaceutical, agricultural and industrial research. [5, 6]. Based on this consideration, the present study reports the synthesis of ***(E)-2-(naphthalen-1-yldiazenyl) malononitrile*** and explores its biological activity.

Material and methods

Synthesis of ***(E)-2-(naphthalen-1-yldiazenyl) malononitrile (NDMN)***

The 1-naphthylamine (**1**) (17.0 mmol) of 2.3 g was dissolved in 10 ml of 34% hydrochloric acid, and a cold solution of sodium nitrite (1.2 g, 17.0 mmol in 20 mL water) was added dropwise while stirring at 0-5 ̊C. The resulting diazonium salt (**2**) solution was added slowly to a (10 mL) ethanolic solution of malononitrile (**3**) (1.2 g, 17.0 mmol) and CH_3COONa (1.4 g, 17.0 mmol) while stirring. The precipitated product (**4**) was collected by filtration, washed thoroughly with distilled water, and dried under vacuum for 24 h. recrystallization from 50%aqueous ethanol afforded dark brown shiny plates in 80% yield; m. p. (150 _152̊ C).

The target compound was synthesized following the standard diazotization-coupling methodology used for the preparation of arylazo-malononitrile [7, 8]. To the best of our knowledge, this specific derivative **(NDMN)** has not been previously reported in literature, although it is indexed in chemical databases such as PubChem and ChemSpider without experimental data. The synthetic route is outlined in Scheme 1



Scheme 1

Preparation of Bacterial Inoculum

Methicillin-resistant *Staphylococcus aureus* (MRSA SA121) and *Escherichia coli* (*E. coli* EC49) are the bacterial strains that are procured by the Biotechnology Research Centre (BRC) in Tripoli. Bacterial strains preparation was done based on the Clinical and Laboratory Standards Institute (CLSI) guidelines: M02-A11. In short, the two bacteria were re-cultured in nutrient Mueller Hinton agar (MHA; Difco, Sparks, USA) for 24 h at 37 °C [9]. Thereafter, two or three colonies of bacteria then transferred in 1 mL of Mueller Hinton broth (MHB; Difco, Sparks, USA) with a sterile cotton swab, vortexed the bacterial suspension 10 min and then left to develop for one day at 37 °C. Subsequently, 10 µL of bacterial suspension was moved into 10 mL of MHB. A standard broth microdilution [9] and inoculum quantification

techniques [10] were used to dilute the turbidity of inoculum to about to above 10^6 CFU/mL. The quantification of inoculum was done by inoculating 20 µL of bacterial suspension into MHA and counting of the number of colonies grown that incubated in 24h at 37 °C.

Antimicrobial Disc Diffusion Test

The antimicrobial effect of **(NDMN)** was tested in the disc diffusion method [10]. In a nutshell, a sterile cotton swab was used to evenly spread the bacterial inoculum on the surface of Mueller-Hinton agar (MHA) plates. Filter paper discs (6 mm) (Whatman, Germany) were wetted with 10 µL of the **(NDMN)** solution of 10, 5, and 1 mg/mL. The discs were then placed carefully on the inoculated agar plates, but with enough spacing between the two discs. The plates were also placed with positive controls (10 µg Streptomycin against Gram-negative bacteria and 10 µg Vancomycin against Gram-positive bacteria) and a negative control (10% DMSO). After incubating at 37 °C, 24 hours were taken and the inhibitory zones were measured in millimetres.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Values

The MICs and MBCs were identified based on CLSI standards [11]. Measuring the MICs and MBCs of malononitrile at a concentration of 1, 5, and 10 mg/mL against MRSA and *E. coli* was done in a 96-well microtiter plate, with 10^6 CFU/mL of bacterial inoculum, by the standard two-fold serial microdilution technique. Each concentration of the **(NDMN)** (100 µL) was combined and further concentrated in 2-fold of the test bacteria using MHB (100 µL). The highest concentration of the compound took place in column 12 of the microtiter plate, and the lowest concentration in column 3. Column 2 was the positive growth control (MHB with the inoculum only), and column 1 was the negative control (MHB only, no inoculum or antibacterial agent). The incubation of the microtiter plate in aerobic conditions of 37 °C was then done over 24 hours. The minimum concentration of the antibacterial agent at which visible bacterial growth was completely suppressed was termed as the MIC. The values of MBC of the different bacterial species were obtained by subculture to MHA plates by eliminating the media in wells that had no visible growth. The plates were then left to incubate at 37 °C for 24 hours, when visible growth was observed in the control plates. The lowest concentration that is able to kill the microorganisms fully was considered to be the MBC [12].

Statistical Analyses

Microsoft Excel 2010 and the findings were stated as mean ± SD of 3 replicates.

Results and discussion

Table 1 and Figures 1 and 2 show the results of the antibacterial disc diffusion test of malononitrile versus MRSA and *E. coli*. The data shows that malononitrile showed significant antibacterial activity against MRSA at the concentration of 1, 5, and 10 mg/mL with the resultant inhibition zones of 34.6, 36.3, and 41.3 mm respectively. By contrast, significantly less inhibitory activity was observed against E. coli by the same concentrations with an inhibition zone of 14.3, 16.0, and 16.0 mm, respectively. Compared to these, the typical positive controls generated inhibition zones of 16 to 21 mm of MRSA and *E. coli*.

Table 1: Disc diffusion of (NDMN) against tested bacteria

Concentration of *(NDMN)*	MRSA (SA121) clear zone(mm) ± SD	*E. coli* (EC49) clear zone(mm) ± SD
1 mg/mL	34.6±0.57	14.3±0.57
5 mg/mL	36.3±1.15	16.0±1.00
10 mg/ mL	41.3±1.52	16.0±1.00
Control +	16.0±0.00	21.0±0.00
Control -	NA	NA

Positive control: Vancomycin for MRSA and Streptomycin for *E. coli*, Negative control: DMSO, NA: No Activity, Results were expressed as means ± sd. Significant differences in means (triplicate).

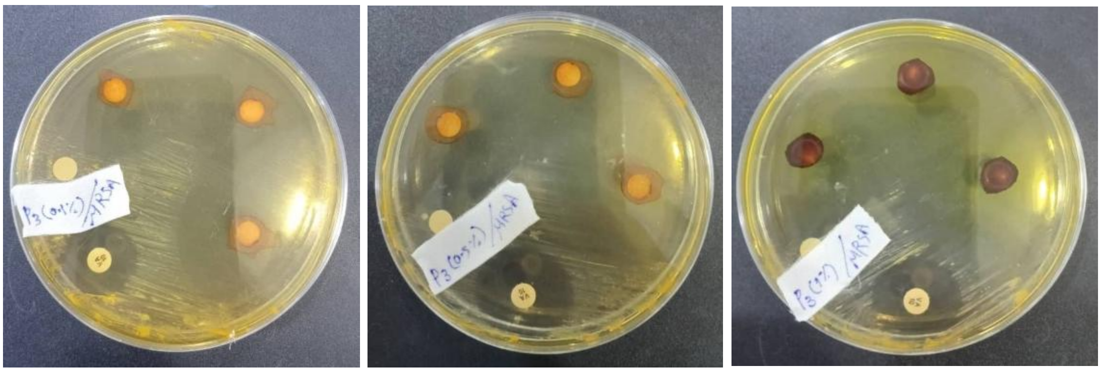


Fig 1: Disk diffusion test of *(NDMN)* at different concentrations, Vancomycin control positive and control negative DMSO 10% against MRSA

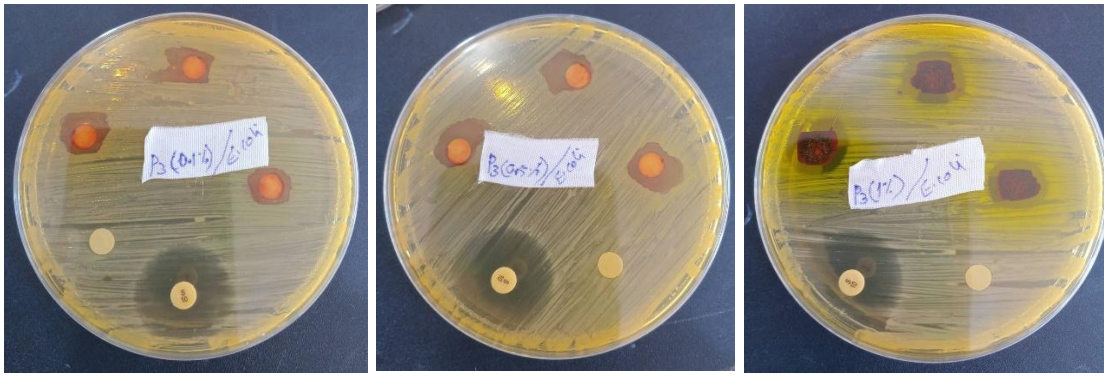


Fig 2: Disk diffusion test of (NDMN) at different concentrations, Streptomycin control positive and control negative DMSO 10% against *E. coli*

The vulnerability of the MRSA and *E. coli* to malononitrile with varying concentrations were determined on the ground of their minimum inhibitory concentration (MIC) and their minimum bactericidal concentration (MBC) value. Table: 2, figure 3 and 4 present the values of the MIC and MBC respectively.

Table 2: Minimum inhibitory concentrations (MICs) and Minimum bactericidal concentration (MBCs) of (NDMN)

Concentration of *(NDMN)*	**MRSA (SA121)**		***E. coli* (EC49)**	
	MIC	**MBC**	**MIC**	**MBC**
1 mg/mL	19.5	39.0	125	250
5 mg/mL	4.8	9.7	31.3	62.5
10 mg/mL	0.9	1.9	7.8	15.6

MIC and MBC values are expressed in µg/mL

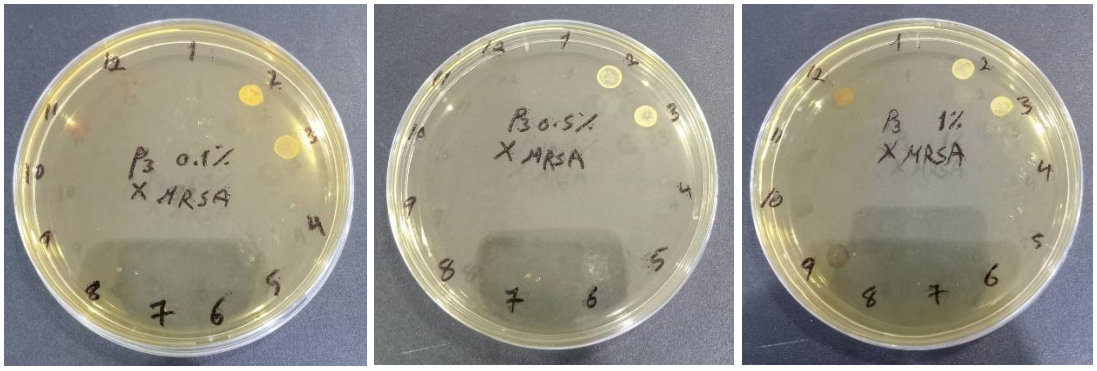


Fig 3: MIC and MBC values of (NDMN) at different concentrations against *MRSA*.

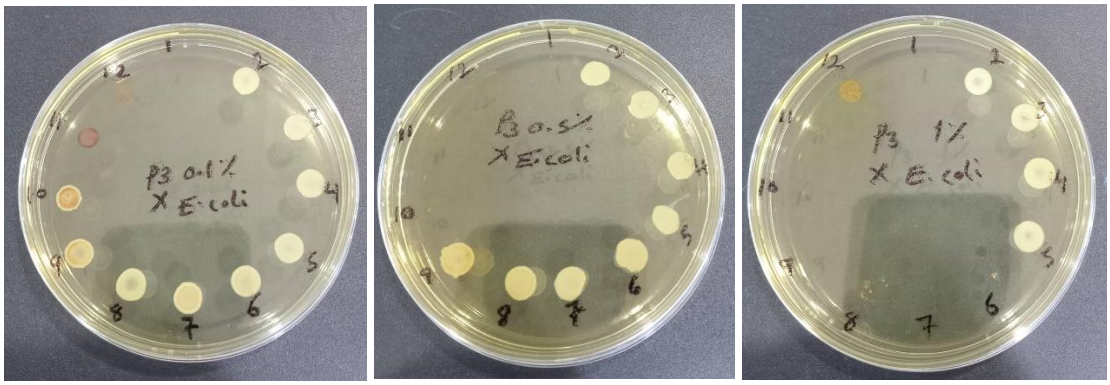


Fig 4: MIC and MBC values of (NDMN) at different concentrations against *E. coli*.

The two bacteria were susceptible to malononitrile. It produced the lowest MIC values (0.9 µg/mL) at a concentration of 10mg/ mL at MRSA compared to *E. coli*, which was inhibited at 7.8mg/ mL at the same concentration, and inhibited MRSA at 1 and 5 mg/ mL, respectively, at concentrations of 1 mg/mL and 5 mg/mL, respectively. In general, the MBC values were between 39.0 and 1.9 µg/mL, with various concentrations of the MBC using MRSA, as it was a little weaker, with 250 to 15.9 µg/mL being the MBC values against *E. coli*. As it could be observed in the results, this compound produced a clear impact on both types of bacteria. It is interesting, however, to note that its effect was more on MRSA (SA121), which is a Gram-positive bacterium, than on *E. coli* (EC49), which was an agent representative of Gram-negative bacteria. This variation in the effect of the compound on the two bacterial types could be as a result of the varying effect of the compound on the cell wall of the two bacteria, since the structure of the cell wall differs in these two bacteria. It can be caused by the effect of **(NDMN)*)*** on some internal cellular elements, or it can be caused by the inhibition of some essential biological process in the life of the bacteria, and hence this consequently causes the inhibition of the growth of these bacteria, depending on the type of bacteria.

Conclusion

Finally, **(NDMN)** exhibited an effect in all its concentrations on the two types of bacteria that were employed by this study. It is also to be mentioned that it showed a higher effect on MRSA (SA121) than *E. coli* (EC49). Thus, it is suggested that this compound may be employed as synthesized antimicrobial agent in case the suggested toxicity tests are carried out and no adverse effects appear when handled with this substance. It is also recommended to concentrate on its application in fighting Gram-positive bacteria.

REFERENCES

[1] J. Vinodkumar, I. Keshavayya, C. T. Pushpavathi, M. R. Keerthikumar, B. N. Maliyappa, Struct. Chem. 2020, 31(4), 1317.

[2] Y. Ali, S. A. Hamid, U. Rashid, Mini Rev. Med. Chem. 2018, 18(18), 1548.

[3] S. Benkhaya, S. M'rabet, A. El Harfi, Heliyon 2020, 6(1), e03271.

[4] Q. Zhang, J.-L. Liu, X.-X. Feng, J.-C. Liu, New J. Chem. 2021, 45(11), 5221.

[5] G. S. Deora, C. Karthikeyan, N. Moorthy, V. Rathore, A. K. Rawat, A. K. Tamrakar, A. K. Srivastava, P. Trivedi, Med. Chem. Res. 2013, 22(11), 5344

[6] E. A. Hassan, A. M. Elmaghraby, Int. J. Innov. Scient. Res. 2015, 16, 11.

[7] El-Menyawy, E. M., Nawar, H. H., & Zedan, I. T. (2021). *Surfaces and Interfaces*, *23*. https://doi.org/10.1016/j.surfin.2021.101040

[8] Elbadawy, H. A., Ali, A. E. D., Elkashef, A. A., Foro, S., & El-Sayed, D. S. (2022). *Applied Organometallic Chemistry*, *36*(9). https://doi.org/10.1002/aoc.6793

[9] Rukayadi, Y., Lau, K. Y., Zainin, N. S., Zakaria, M., & Abas, F. (2013). *International Food Research Journal*, 20(5), 2905.

[10] Indira, G. (2014). *Clin Microbial*, *3*(3).

[11] Clinical and Laboratory Standards Institute (CLSI).(2003). Reference method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically.*Approved standard M7-A6*. National Committee for Clinical Laboratory Standards, Wayne, Pennsylvania, USA.

[12] Rukayadi, Y., Shim, J. S., & Hwang, J. K. (2008). *Mycoses*, *51*(4), 308-312.