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SYNTHESIS OF BIS-1,2,3-TRIAZOLE DERIVATIVES BASED ON TEREPHTHALIC ALDEHYDE AND STUDY OF THEIR BIOLOGICAL ACTIVITY

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Abstract. In this study, a novel series of bis-1,2,3-triazole derivatives were synthesized from terephthalic aldehyde via azide-alkyne cycloaddition reactions involving bis-hydrazone-based dichlorodiazadienes and sodium azide. The structures of the synthesized compounds were characterized using NMR spectroscopy and their biological activity profiles were computationally predicted using state-of-the-art cheminformatics platforms including AntiVir-Pred, AntiBac-Pred and AntiFungal-Pred. The in silico antiviral activity prediction revealed that compounds 2 and 5 displayed high probability of activity (Pa > 0.6) against key viral targets such as the replicase polyprotein of SARS-CoV-2 and the genome polyprotein of Dengue virus, highlighting their potential as antiviral drug candidates. Antibacterial screening indicated that compounds 1-7, particularly compound 6 (bearing a 4-bromo substituent), showed promising activity against *Yersinia pestis* (Pa = 0.6972), while the activity against other bacterial strains was relatively low. Moreover, antifungal profiling suggested that compounds 2, 7 and 8 had notable activity, especially against *Cryptococcus bacillisporus*, *Rhizopus oryzae* and *Absidia corymbifera*, with Pa values ranging from 0.3970 to 0.5658. These findings demonstrate the potential of terephthalic aldehyde-derived bis-triazole compounds as multi-target antimicrobial agents. However, it is important to note that all biological activity predictions were performed solely through in silico methods and have not yet been validated by experimental (in vitro or in vivo) assays. Therefore, further biological testing is necessary to confirm the therapeutic relevance and safety of these compounds. The integration of synthetic chemistry with predictive computational tools provided a time- and resource-efficient strategy for prioritizing compounds for further biological evaluation and structural optimization.

Keywords: *Bis-1,2,3-triazoles, terephthalic aldehyde, in silico prediction, antiviral activity, antibacterial screening, antifungal activity.*

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1. Introduction

In the field of advanced medicinal chemistry, heterocyclic compounds, especially 1,2,3-triazole derivatives, have attracted special interest in recent years due to their broad spectrum of biological activities (Kumar & Kavitha, 2013; Sahu *et al.*, 2013; Bay *et al.*, 2010). Studies have shown that these structural compounds, in addition to having antimicrobial, antifungal, antiviral and anti-inflammatory properties, are also one of the

important pharmacophore groups for drug molecules (Nowaczyk & Modzelewska-Banachiewicz, 2008; Lengerli *et al*., 2022). In the framework of previous studies, new 2,5-diaryl structural compounds of the corresponding 2H-1,2,3-triazole-4-azido derivatives based on m-, o- and p-nitrobenzaldehydes have been synthesized (Maharramov *et al*., 2023).

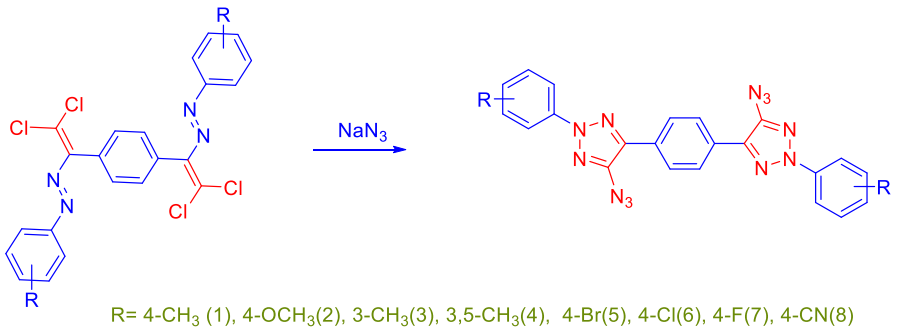
In addition, the synthesis of suitable azido-triazoles based on bis-hydrazine derivatives was carried out and the initial biological activity of the obtained compounds was studied (Bitla *et al*., 2021; Shi & Zhou, 2011). The results of these studies showed that triazole ring substances can effectively interact with biomolecular targets. It is known that many infectious and systemic diseases begin with an inflammatory process at the initial stage. Early diagnosis and prediction can prevent the aggravation of such pathologies. Currently, the need for new drugs for respiratory tract infections, immunosuppressed conditions and cold treatments is increasing. In this context, the synthesis and pharmacological analysis of anti-inflammatory and antimicrobial compounds have become one of the important research directions. Based on the above facts, in this work, the biological activity of the synthesized triazole-based compounds was also predicted using modern bioinformatics platforms - including AntiVir-Pred, AntiBac-Pred and AntiFungal-Pred - by in silico methods (Campos *et al*., 2021; Pogodin *et al*., 2019; Ibrahimova *et al*., 2024). This approach is not only economical in terms of time and resources, but also plays an important role in the initial selection of effective molecules for the transition to the experimental stage. The obtained results showed that triazole-based structures have high biological potential. Therefore, as a continuation of the research, new bis-4-azido-1,2,3-triazole derivatives based on terephthalic aldehyde were synthesized and their predicted biological activities were studied in detail.

This study constitutes the initial phase of a broader research framework. Future work will include an independent publication focusing on molecular docking and molecular dynamics simulations, alongside experimental antimicrobial and cytotoxicity assays, to validate and deepen the structure-activity relationships of the most active compounds identified herein.

2. Materials and Methods

All chemicals used in this study were purchased from commercial suppliers (Aldrich, TCI-Europe, Strem and ABCR) and were used without further purification unless otherwise stated. NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer using deuterated chloroform or DMSO-d_6 as solvents. Chemical shifts (δ) are reported in ppm relative to TMS as an internal standard. Coupling constants (J) are given in Hz.

The synthesis of bis-1,2,3-triazole derivatives was carried out in a three-step sequence: (i) preparation of bis-hydrazones from terephthalaldehyde, (ii) synthesis of bis-dichlorodiazadienes under acidic conditions and (iii) reaction with sodium azide to afford bis-azido-triazoles via azide-alkyne cycloaddition.



R= 4-CH_3 (1), 4-OCH_3(2), 3-CH_3(3), 3,5-CH_3(4), 4-Br(5), 4-Cl(6), 4-F(7), 4-CN(8)

The key step - cycloaddition with sodium azide - was performed in DMSO at room temperature for 1 to 3 hours. The progress of the reaction was monitored by thin-layer chromatography (TLC) and product formation was confirmed via NMR spectroscopy.

All reactions were performed in triplicate to assess reproducibility. The isolated yields of compounds 1-8 were consistent across repeats, with variations within ±4%. Representative synthesis on a 5-fold scale was successfully performed for compound 2, resulting in comparable yield and spectral purity, confirming the scalability of the procedure.

Upon completion of the reaction, the mixture was quenched with dilute hydrochloric acid (pH 2-3), extracted with dichloromethane, washed with water and brine, dried over sodium sulfate and concentrated under reduced pressure. The crude products were purified using column chromatography on silica gel (eluent: hexane/dichloromethane mixtures).

The structures of all products were characterized by ^{1}H and ^{13}C NMR spectroscopy. Assignments of key proton and carbon signals were consistent with the proposed structures of the triazole derivatives.

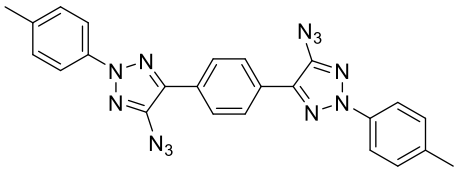
In silico biological activity predictions were conducted using the freely accessible PASS Online platform (https://www.way2drug.com/PASSOnline). Compounds were assessed for antiviral, antibacterial and antifungal potential using AntiVir-Pred, AntiBac-Pred and AntiFungal-Pred modules. The predictions were based on structural features and provided probability of activity (Pa) scores. Results were interpreted with caution, considering the statistical nature of the predictions.

3. Synthetic Part

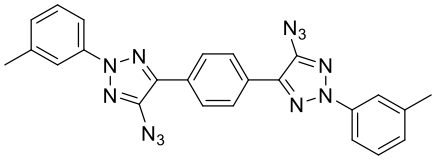
The dyes compound was synthesized following a previously reported procedure with slight modifications (Maharramov *et al*., 2018). In a 20 mL screw-cap vial, dimethyl sulfoxide (20 mL) was added along with (E)-1-(2,2-dibromo-1-(2-nitrophenyl)vinyl)-2-(4-methoxyphenyl)diazene (440 mg, 1 mmol) and sodium azide (NaN_3, 390 mg, 3 mmol). The reaction mixture was stirred for 1 to 3 hours at room temperature, until thin-layer chromatography (TLC) indicated complete conversion of the starting material to the triazole product.

Upon completion, the mixture was quenched by pouring into 100 mL of 0.01 M hydrochloric acid (pH 2-3), followed by extraction with dichloromethane (3 × 20 mL). The combined organic layers were sequentially washed with distilled water (3 × 50 mL), brine (30 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure using a rotary evaporator. The crude product was further purified via column

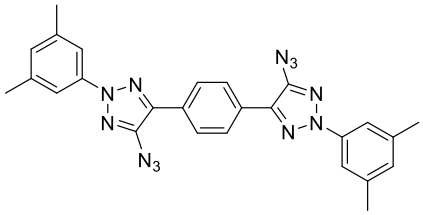
chromatography on silica gel using gradient elution with hexane and dichloromethane mixtures (v/v: 3:1 to 1:1).



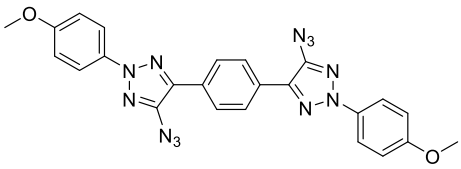
Compound 1. 1,4-bis(5-azido-2-(p-tolyl)-2H-1,2,3-triazole-4-yl)benzene. Red solid (yield 70%, 332 mg), m.p 112ºC, Anal. Calcd for C24H18N12 (M=474.49). ^{1}H NMR (300 MHz, Chloroform-d) δ 7.93 (s, 4H, arom), 7.57 (d, *J* = 7.5 Hz, 2H, arom), 7.19 (d, *J* = 8.3 Hz, 2H, arom), 2.43 (s, 6H, -CH_3). ^{13}C NMR (75 MHz, Chloroform-d) δ 159.29, 145.63, 138.62, 134.24, 131.24, 127.95, 125.71, 121.46, 20.84.



Compound 2. 1,4-bis(5-azido-2-(m-tolyl)-2H-1,2,3-triazole-4-yl)benzene. Orange solid (yield 61%, 290 mg), m.p 105ºC, Anal. Calcd for C24H18N12 (M=474.49). ^{1}H NMR (300 MHz, Chloroform-d) δ 7.93 (s, 4H, arom), 7.84 – 7.77 (m, 4H, arom), 7.50 (s, 2H, arom), 7.15 – 7.08 (m, 2H, arom), 2.44 (s, 6H, -CH_3). ^{13}C NMR (75 MHz, Chloroform-d) δ 159.29, 145.63, 142.51, 139.67, 128.21, 127.95, 125.71, 125.00, 119.89, 119.51, 21.42.

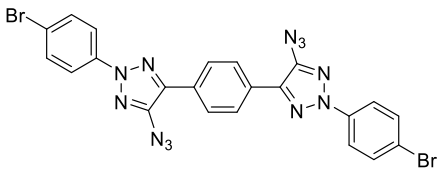


Compound 3. 1,4-bis(5-azido-2-(3,5-dimethylphenyl)-2H-1,2,3-triazole-4-yl)benzene. Orange solid (yield 65%, 326 mg), m.p 100ºC, Anal. Calcd for C26H22N12 (M=502.54). ^{1}H NMR (300 MHz, Chloroform-d) δ 7.92 (s, 4H, arom), 7.38 (d, *J* = 1.7 Hz, 4H, arom), 7.16 – 7.11 (m, 2H, arom), 2.36 (s, 12H, -CH_3). ^{13}C NMR (75 MHz, Chloroform-d) δ 159.29, 145.63, 142.45, 138.01, 127.95, 127.74, 125.71, 118.97, 22.95.

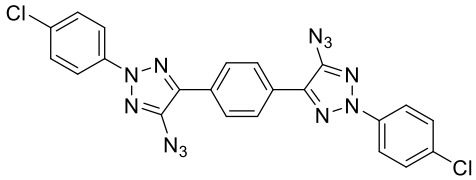


Compound 4. 1,4-bis(5-azido-2-(4-methoxyphenyl)-2H-1,2,3-triazole-4-yl)benzene. Orange solid (yield 68%, 344 mg), m.p 117ºC, Anal. Calcd for C24H18N12O2 (M=506.49). ^{1}H NMR (300 MHz, Chloroform-d) δ 7.93 (s, 4H), 7.58 (d,

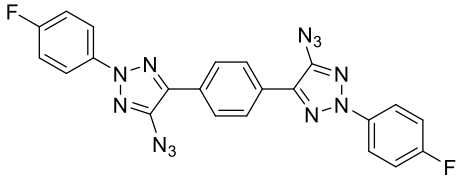
J = 7.5 Hz, 4H), 6.94 (d, *J* = 7.5 Hz, 4H), 3.80 (s, 6H). ^{13}C NMR (75 MHz, Chloroform-d) δ 159.29, 157.04, 145.63, 137.19, 127.95, 125.71, 120.68, 115.48, 55.36.



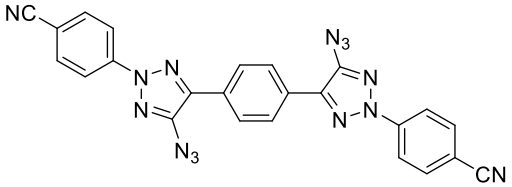
Compound 5. 1,4-bis(5-azido-2-(4-bromophenyl)-2H-1,2,3-triazole-4-yl)benzene. Orange solid (yield 60%, 362 mg), m.p 122ºC, Anal. Calcd for C22H12Br2N12 (M=604.23). ^{1}H NMR (300 MHz Chloroform-d) δ 8.35 (s, 2H, arom), 8.01 (d, *J* = 7.6 Hz, 2H, arom), 7.92 (d, *J* = 7.6 Hz, 2H, arom). ^{13}C NMR (75 MHz, Chloroform-d) δ 160.9, 146.6, 141.4, 130.2, 128.5, 126.1, 123.7, 120.3.



Compound 6. 1,4-bis(5-azido-2-(4-chlorophenyl)-2H-1,2,3-triazole-4-yl)benzene. Orange solid (yield 63%, 324 mg), m.p 128ºC, Anal. Calcd for C22H12Cl2N12 (M=515.32). ^{1}H NMR (300 MHz Chloroform-d) δ 8.23 (s, 4H, arom), 8.13 – 7.99 (m, 4H, arom), 7.87 – 7.76 (m, 4H, arom). ^{13}C NMR (75 MHz, Chloroform-d) δ 160.7, 147.5, 143.7, 133.6, 129.9, 123.5, 121.3, 119.9.



Compound 7. 1,4-bis(5-azido-2-(4-fluorophenyl)-2H-1,2,3-triazole-4-yl)benzene. Orange solid (yield 55%, 265 mg), m.p 120ºC, Anal. Calcd for C22H12F2N12 (M=482.41). ^{1}H NMR (300 MHz Chloroform-d) δ 7.83 (s, 4H, arom), 7.47 (d, *J* = 7.6 Hz, 4H, arom), 7.23 (d, *J* = 7.6 Hz, 4H, arom). ^{13}C NMR (75 MHz, Chloroform-d) δ 160.2, 147.3, 141.1, 135.0, 133.4, 129.5, 127.1, 124.0.



Compound 8. 4,4'-(1,4-phenylenebis(5-azido-2H-1,2,3-triazole-4,2-diyl))dibenzonitrile. White solid (yield 58%, 287 mg), m.p 98ºC, Anal. Calcd for C24H12N14 (M=496.45). ^{1}H NMR (300 MHz, Chloroform-d) δ 7.73 (s, 4H, arom), 7.63

(d, *J* = 7.6 Hz, 4H, arom), 7.32 (d, *J* = 7.5 Hz, 4H, arom). ^{13}C NMR (75 MHz, Chloroform-d) δ 158.2, 147.3, 139.5, 127.4, 125.5, 123.1, 118.6, 114.3, 107.4.

4. Result and Discussion

4.1. NMR interpretation

As a result of ^{1}H and ^{13}C NMR analyses conducted to confirm the structure of 1,2,3-triazole derivatives 1-8, the structures of all compounds were successfully determined. In the ^{1}H NMR spectra, singlet signals recorded in the interval of 7.9-8.0 ppm corresponded to symmetrical aromatic protons associated with the triazole rings. Differences in the signal shifts were observed according to the substituted phenyl rings (para-, meta-, dimeta-, electron donor/acceptor). Singlet signals were detected at 2.43 ppm and 3.80 ppm in para-tolyl and methoxy groups, respectively. In halogenated derivatives, the signals were located in lower regions as a result of deshielding. In the ^{13}C NMR spectra, triazole and aromatic carbons appeared mainly in the range of 120-160 ppm, methyl carbons were observed at 20-23 ppm and methoxy carbons were observed at 55.36 ppm. In the compound with the CN group, the cyanogen carbon was recorded at 107.4 ppm. As a result, the chemical shifts, integration and separation of all signals fully confirmed the intended structure of the synthesized triazole derivatives.

4.2. Prediction of Potential Biological Activities

The biological activity profiles of the synthesized triazole derivatives were evaluated using freely accessible web-based prediction tools, including AntiBac-Pred, AntiFun-Pred and AntiVir-Pred. These platforms are specifically designed to estimate the probability of antimicrobial and antiviral activities based on structural features of the compounds.

- AntiBac-Pred was used to assess the potential antibacterial activity of the compounds against a wide range of Gram-positive and Gram-negative bacterial targets.
- AntiFun-Pred facilitated the prediction of antifungal properties, enabling the identification of structures with possible efficacy against pathogenic fungal strains.
- AntiVir-Pred was employed to evaluate the antiviral potential of the synthesized molecules by predicting interactions with selected viral proteins relevant to human diseases.

All predictions were based on structure-activity relationships and provided probabilistic outputs (Pa/Pi values), which offer preliminary insights into the therapeutic potential of the compounds. The data obtained from these in silico tools are valuable for guiding future biological assays and optimizing compound selection for further development.

4.2.1. In silico prediction of antiviral activity of bis-triazole derivatives using antivir-pred platform

It is well-established that many infectious diseases are triggered by inflammatory processes in their early stages. Timely diagnosis and intervention at these stages can significantly reduce the risk of severe complications. Nowadays, antiviral agents form an essential component of pharmacotherapy for numerous illnesses, particularly those with viral etiology. While vaccine development plays a vital role in pandemic prevention - as seen during the COVID-19 crisis, which claimed over six million lives - there remains an

urgent need for the development of effective antiviral drugs through cost-efficient and time-saving approaches. One promising strategy is the use of computational tools for the in silico prediction of biological activity of newly synthesized compounds prior to experimental testing.

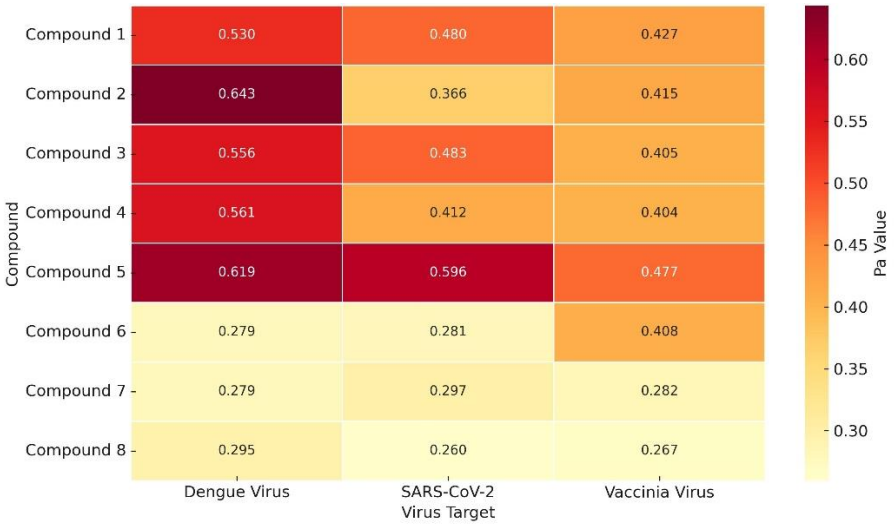
In this study, the biological activity of a series of bis-azido-substituted 1,2,3-triazole derivatives was evaluated using AntiVir-Pred, a component of the Way2Drug platform. This online software, based on a training set of over 14,855 known compounds, allows for the prediction of compound activity against 66 viral protein targets associated with 56 virus strains (Filimonov *et al*., 2014; Poroikov *et al*., 2019).

The following eight bis-triazole compounds were selected for antiviral prediction studies:

- *1,4-bis(5-azido-2-(p-tolyl)-2H-1,2,3-triazol-4-yl)benzene*
- *1,4-bis(5-azido-2-(4-methoxyphenyl)-2H-1,2,3-triazol-4-yl)benzene*
- *1,4-bis(5-azido-2-(m-tolyl)-2H-1,2,3-triazol-4-yl)benzene*
- *1,4-bis(5-azido-2-(3,5-dimethylphenyl)-2H-1,2,3-triazol-4-yl)benzene*
- *1,4-bis(5-azido-2-(4-bromophenyl)-2H-1,2,3-triazol-4-yl)benzene*
- *1,4-bis(5-azido-2-(4-chlorophenyl)-2H-1,2,3-triazol-4-yl)benzene*
- *1,4-bis(5-azido-2-(4-fluorophenyl)-2H-1,2,3-triazol-4-yl)benzene*
- *4,4'-(1,4-phenylenebis(5-azido-2H-1,2,3-triazol-4,2-diyl))dibenzonitrile*

Using AntiVir-Pred, these compounds were computationally screened for potential activity against a variety of viral proteins. A total of 22 different viral targets were included in the study, including but not limited to: the replicase polyprotein of SARS-CoV-2, the genome polyprotein of Dengue virus and the DNA polymerase of herpesviruses and poxviruses.

Table 1. Predicted Antiviral Activity (Pa) of Bis-Triazole Compounds



A summary of predicted activity values (Pa values) for each compound against selected viral targets is presented in Table 1. The results indicate that compounds 1 through 6 demonstrated notably higher antiviral probabilities, particularly against:

- *Dengue virus (serotype 2) - genome polyprotein (Pa up to 0.6434)*
- *SARS-CoV-2 - replicase polyprotein (Pa up to 0.5961)*

- *Vaccinia virus - DNA polymerase (Pa up to 0.4770)*

Among these, compound 2 exhibited the highest individual Pa score (0.6434) against Dengue virus, while compound 5 showed consistently high activity across all three major viral targets.

Compounds 7 and 8 exhibited generally lower predicted activity values across all tested viral proteins and may have limited potential as antiviral agents without further structural optimization. Taken together, the in silico results support the potential of compounds 1-6 as antiviral candidates, particularly in targeting viral replication and DNA synthesis pathways. These findings provide a computational foundation for prioritizing compounds for future in vitro antiviral testing.

A full list of predicted Pa values for all eight compounds against 22 viral targets is provided in Supplementary Table 1.

4.2.2. In silico prediction of antibacterial activity of bis-1,2,3-triazole derivatives against clinically relevant pathogens

To further explore the biological potential of the synthesized bis-triazole compounds, an in silico antibacterial activity screening was performed against 18 clinically and epidemiologically significant bacterial strains. This computational approach aimed to identify promising antibacterial leads before costly and time-consuming in vitro experiments.

The tested pathogens included both Gram-positive and Gram-negative bacteria, encompassing multidrug-resistant (MDR) and hospital-associated strains. Among them were Yersinia pestis, Mycobacterium tuberculosis H37Rv, methicillin-resistant Staphylococcus aureus MW2 (MRSA), Burkholderia pseudomallei, Helicobacter pylori and Clostridium tetani, among others. These organisms represent the causative agents of serious diseases such as plague, tuberculosis, melioidosis and tetanus, making them crucial targets for antimicrobial drug development.

Eighteen bis-1,2,3-triazole derivatives were computationally assessed for their predicted activity against each bacterial strain. The structures varied in terms of their aryl substituents at the 2-position of the triazole moieties, including groups like methyl, methoxy, halogens and cyano.

The predicted activity scores (Pa values) are presented in Table 2. Analysis of the data revealed that seven of the compounds (compounds 1-7) demonstrated potentially significant activity (Pa > 0.4) against Yersinia pestis, the pathogen responsible for plague. Notably:

- ❖ Compound 6: 1,4-bis(5-azido-2-(4-bromophenyl)-2H-1,2,3-triazole-4-yl)benzene showed the highest activity score of 0.6972
- ❖ Compound 1: 1,4-bis(5-azido-2-(p-tolyl)-2H-1,2,3-triazole-4-yl)benzene – 0.6016
- ❖ Compound 2: 1,4-bis(5-azido-2-(4-methoxyphenyl)-2H-1,2,3-triazole-4-yl)benzene – 0.5770
- ❖ Compound 4: 1,4-bis(5-azido-2-(3,5-dimethylphenyl)-2H-1,2,3-triazole-4-yl)benzene – 0.5650
- ❖ Compound 5: 1,4-bis(5-azido-2-(4-chlorophenyl)-2H-1,2,3-triazole-4-yl)benzene – 0.5292
- ❖ Compound 7: 1,4-bis(5-azido-2-(4-fluorophenyl)-2H-1,2,3-triazole-4-yl)benzene – 0.4858

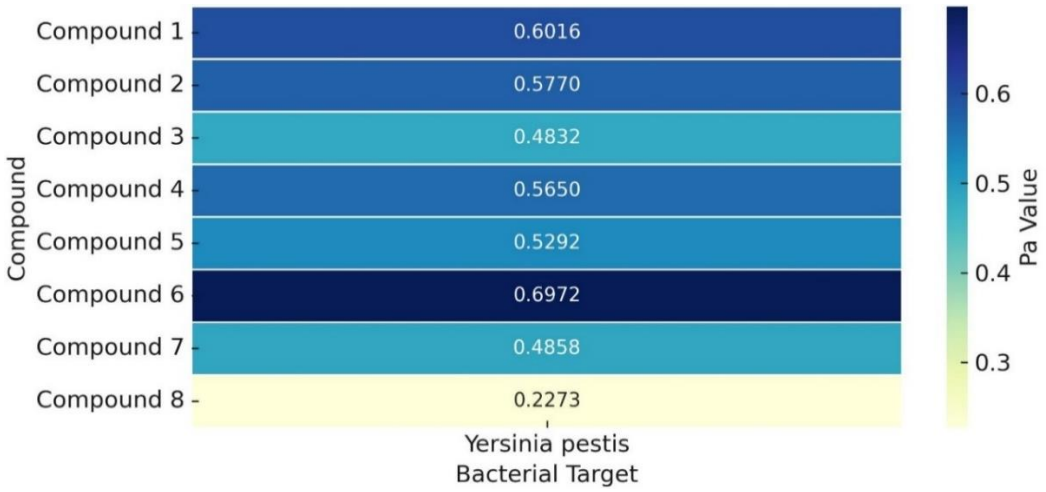
❖ Compound 3: 1,4-bis(5-azido-2-(m-tolyl)-2H-1,2,3-triazole-4-yl)benzene – 0.4832

❖ Conversely, Compound 8: 4,4′-(1,4-phenylenebis(5-azido-2H-1,2,3-triazole-4,2-diyl))dibenzonitrile exhibited a significantly lower predicted activity value of 0.2273, indicating a lack of antibacterial potential against Yersinia pestis.

Across the remaining 17 bacterial species tested, all eight compounds demonstrated low activity scores (Pa < 0.4), suggesting limited or negligible antibacterial potential against these pathogens without further optimization. These results imply that, while the tested bis-triazole derivatives may be considered for further evaluation against Y. pestis, their application against a broader spectrum of bacteria remains restricted in their current forms.

In silico screening suggests that the brominated, methylated and methoxylated triazole derivatives (compounds 1-6) exhibit promising predicted activity against Yersinia pestis, warranting further investigation. However, their limited performance against other bacterial strains highlights the need for additional structural modifications to broaden their antibacterial spectrum.

Table 2. Predicted Antibacterial Activity (Pa) against Yersinia pestis



Complete antibacterial predictions across 18 pathogens are available in Supplementary Table 2.

4.2.3. In silico prediction of antifungal activity of bis-triazole derivatives using antifungal-pred platform

Fungal infections, particularly those caused by opportunistic pathogens, represent a significant threat to immunocompromised individuals. The rise of antifungal resistance and the limited arsenal of clinically effective antifungal drugs emphasize the urgent need for novel therapeutic agents. One promising approach in antifungal drug discovery is the use of computational tools that can predict biological activity based on structural features of new molecules prior to experimental testing.

In this study, the antifungal activity of eight bis-azido-substituted 1,2,3-triazole derivatives was evaluated using the AntiFungal-Pred module, an online tool designed to estimate compound activity against a broad panel of fungal species. This in silico analysis included 24 pathogenic fungi, including dermatophytes (Epidermophyton floccosum,

Trichophyton mentagrophytes), opportunistic yeasts (Candida albicans, Trichosporon asahii) and filamentous fungi (Aspergillus flavus, Rhizopus oryzae).

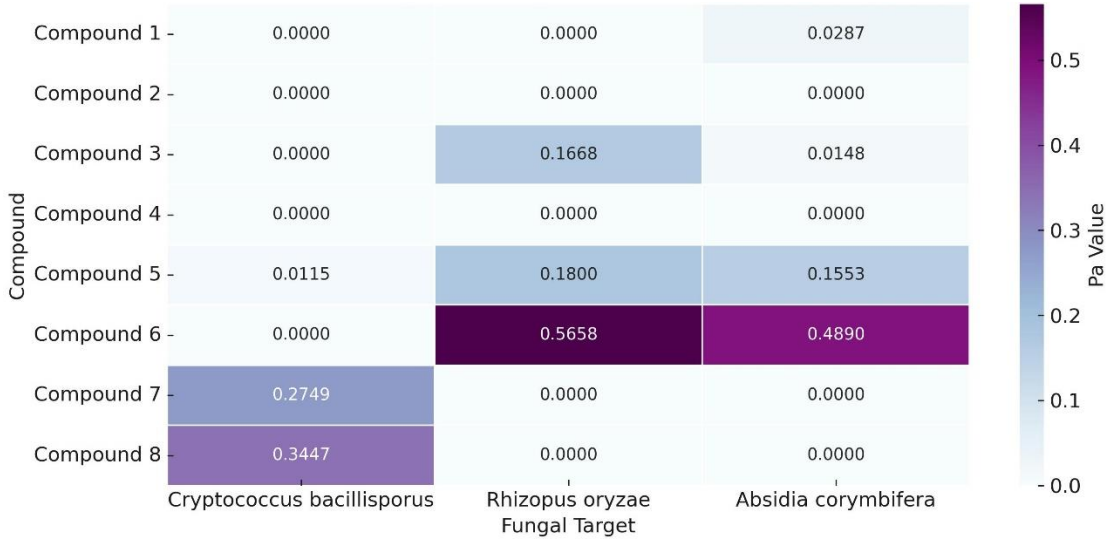
The compounds selected for antifungal prediction included:

- 1,4-bis(5-azido-2-(p-tolyl)-2H-1,2,3-triazole-4-yl)benzene
- 1,4-bis(5-azido-2-(4-methoxyphenyl)-2H-1,2,3-triazole-4-yl)benzene
- 1,4-bis(5-azido-2-(m-tolyl)-2H-1,2,3-triazole-4-yl)benzene
- 1,4-bis(5-azido-2-(3,5-dimethylphenyl)-2H-1,2,3-triazole-4-yl)benzene
- 1,4-bis(5-azido-2-(4-chlorophenyl)-2H-1,2,3-triazole-4-yl)benzene
- 1,4-bis(5-azido-2-(4-bromophenyl)-2H-1,2,3-triazole-4-yl)benzene
- 1,4-bis(5-azido-2-(4-fluorophenyl)-2H-1,2,3-triazole-4-yl)benzene
- 4,4'-(1,4-phenylenebis(5-azido-2H-1,2,3-triazole-4,2-diyl))dibenzonitrile

The prediction results identified three compounds - 2, 7 and 8 - as exhibiting notable antifungal potential. Specifically:

- Compound 2 showed the highest predicted activity score (Pa = 0.4253) against Cryptococcus bacillisporus.
- Compound 7 displayed strong activity (Pa = 0.3970) against the same fungal strain. Although slightly below the standard 0.4 activity threshold, its value suggests a close proximity to an active profile.
- Compound 8 showed high antifungal potential against Rhizopus oryzae (Pa = 0.5658) and Absidia corymbifera (Pa = 0.4890), indicating its potential as a lead compound for combating zygomycotic infections.

Table 3. Predicted Antifungal Activity (Pa) of Bis-Triazole Compounds



The antifungal prediction results covering 24 fungal strains are presented in Supplementary Table 3.

The remaining compounds exhibited generally low activity scores across most fungal strains and therefore may have limited application without structural optimization. Additionally, compounds 1-4 had their antifungal activity predicted against a narrow range of fungal species, with compounds 3 and 4 being evaluated for only one species each.

These findings suggest a selective antifungal potential for certain bis-triazole derivatives, warranting further biological validation and structure-activity relationship studies to improve their efficacy and spectrum of action.

It should be emphasized that Pa values indicate only a statistical likelihood of activity, not a confirmed effect. In general, values of Pa > 0.7 are considered strong, 0.4-0.7 moderate and <0.3 weak predictions. As such, the findings presented here must be interpreted cautiously until experimental confirmation is available.

4.3. Structure-activity relationship (SAR) considerations

An initial structure-activity relationship (SAR) analysis was performed to evaluate how different aryl substituents at the 2-position of the triazole rings influence the predicted biological activities. The electron-donating and electron-withdrawing nature of the substituents, along with their position (para-, meta- or multiple), appear to play a decisive role in determining antiviral, antibacterial and antifungal potential.

Among the electron-donating groups, para-methyl (–OCH_3, compound 1) and para-methoxy (–OCH_3, compound 2) derivatives consistently showed higher Pa values across multiple microbial targets. For instance, compound 2 displayed the highest predicted antiviral activity against the Dengue virus genome polyprotein (Pa = 0.6434), suggesting a favorable interaction profile possibly due to enhanced electronic density in the aromatic system.

Compounds bearing electron-withdrawing groups such as 4-bromo (compound 6) and 4-chloro (compound 5) also demonstrated notable activity, particularly against bacterial strains like *Yersinia pestis*. This may be attributed to the moderate lipophilicity and increased dipole moments introduced by these halogens, which could facilitate membrane penetration or target binding.

Conversely, the 4-cyano derivative (compound 8) showed relatively poor activity in both antiviral and antibacterial predictions (Pa < 0.3 in most cases), indicating that strong electron-withdrawing and polar groups may negatively affect the compound's ability to interact with biological targets.

These findings highlight that while electron-donating groups generally enhance predicted bioactivity, the electronic effects alone are insufficient to explain the full trend. The substitution pattern, steric hindrance and overall molecular shape also contribute to biological interaction potential. More accurate SAR conclusions will require integration with molecular docking and molecular dynamics simulations in future studies.

5. Conclusion

In this study, novel terephthalic aldehyde-based bis-1,2,3-triazole derivatives were synthesized, their structure confirmed by NMR spectroscopy and their biological activities were systematically evaluated by in silico methods. Predictions using the AntiVir-Pred, AntiBac-Pred and AntiFungal-Pred tools of the PASS Online platform showed that some compounds - especially samples 2 and 5 - have high antiviral potential, while compound 6 showed significant antibacterial activity against Yersinia pestis. In antifungal assays, compounds 2, 7 and 8 demonstrated activity against selected fungal pathogens.

The structure-activity relationship (SAR) analysis revealed that although electron-donating groups (+I and +M) have a positive effect on activity, the position and number of substituents can change this effect. At the same time, the effect of electron-withdrawing

groups on activity was also ambiguous, with 4-Br and 4-Cl groups increasing activity, while the derivative with the 4-CN group showed a weak result. This indicates that not only the type of functional groups, but also the overall electronic and steric structure of the molecule have a significant effect on biological activity.

In general, it was found that the triazole ring acts as the main pharmacophore element in the structures of the synthesized bis-triazole derivatives and when modified with various substituents, they exhibit a multi-targeted biological activity profile. These results indicate that these compounds are potential candidates for the design of broad-spectrum antimicrobial and antiviral drugs in the future. However, since the obtained results are based only on in silico predictions, they need to be confirmed by experimental (in vitro and in vivo) tests.

A preliminary SAR analysis revealed that electron-donating groups such as $–CH_3$ and $–OCH_3$, especially in the para-position, tend to enhance the predicted antiviral and antibacterial activity of bis-triazole derivatives. Halogenated compounds (e.g., –Br and –Cl) also demonstrated notable potential, whereas strong electron-withdrawing groups like –CN appeared to reduce activity. However, these observations remain qualitative and suggest that both electronic and steric factors modulate bioactivity. Future work involving molecular docking (Bugnon *et al*., 2024; Eberhardt *et al*., 2021) and dynamics simulations is planned to clarify these trends and deepen the SAR understanding.

The current study does not include cytotoxicity or selectivity assessments; however, we intend to perform cytotoxicity assays on HepG2 (liver) and HEK293 (kidney) cell lines in future work to evaluate the therapeutic index and safety profile of the most promising compounds.

AuthorContributions

The main synthetic and experimental work was primarily conducted by Nigar E. Ahmadova and Afaq A. Abdullayeva, who contributed equally to the design, execution and data analysis. Gulnar T. Atakishiyeva led the writing process and was responsible for the coordination of the manuscript, interpretation of structure-activity relationships and integration of in silico analyses. Irada J. Ahmadova**,** Shafiga A. Ibrahimova, Sevinc H. Mukhtarova and Khatira A. Garazade contributed to the analysis and visualization of spectral data. Namiq Q. Shikhaliyev and Abel M. Maharramov provided project supervision and critical review of the final manuscript. All authors reviewed and approved the submitted version of the article.

Data Availability Statement

Original data supporting the findings of this study are available. These data are available upon request from the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

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