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Novel triazole-pyrazine as potential antibacterial agents: Synthesis, characterization, Antibacterial activity, drug-likeness properties and molecular docking studies

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Abstract: Through Williamson ether synthesis in tetrahydrofuran at room temperature, new triazole derivatives containing 3,6-di(pyridin-2-yl)pyridazin-4-yl)methanol were created. The obtained alkyne and azide derivatives were then put through a clickable reaction using copper catalyzed azide-alkyne cycloaddition reaction (CuAAC) under more environmentally friendly conditions. Through the use of NMR and IR spectroscopy and the High Resolution Mass-Spectrometry technique (HRMS), the produced compounds were examined for their antibacterial properties against both gram-positive and gram-negative bacteria. The antibacterial tests performed with these pyrazine derivatives reveal that compound **4** showed the greatest activity among the investigated compounds against the negative gram-positive *Staphylococcus aureus* and *Streptococcus fasciens*, and positive gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*. Furthermore, research on drug similarity and docking studies were carried out to define their mechanism of action for their antibacterial activities on the theory that these chemicals can be a valuable paradigm for the invention and synthesis of more potent antibacterial possibilities.

Keywords: *Triazole-pyrazine; CuAAC; N-alkylation; Antibacterial activity; Molecular docking.*

1. Introduction

Despite the rarity of pyridazines in nature, their popularity began to increase, which eventually led to the synthesis of a variety of derivatives (Barmade *et al.*, 2016), that are successfully used in pharmaceutical and agrochemical products. Pyridazine and its derivatives are the most important heterocyclic compounds in view of their numerous pharmacological activities and their potentially usefulness in agriculture and medicine as well as anticorrosion of metals in aggressive media (Ahmer *et al.*, 2004; Saddik *et al.*, 2012; Ghazoui *et al.*, 2013; Anand *et al.*, 2014; Ali *et al.*, 2015). Click chemistry has been successful in the preparation of 1,2,3-triazoles by 1,3-dipolar cycloaddition.

Triazoles constitute a particular range of heterocyclic compounds exhibiting a wide panel of biological activities (Bassler *et al.*, 1999; Bay *et al.*, 2010; Arora *et al.*, 2012; Sathish *et al.*, 2013). One of the most important features of triazoles is that they may be used as a linker and exhibit bioisosteric effects on peptide bond, aromatic ring, double bonds and an imidazole ring. Some unique features like the formation of hydrogen bonds, dipole-dipole interactions and π-stacking of triazole compounds have elevated their importance in the field of medicinal chemistry as they attach to the biological target with high affiliation due to their better solubility.

Isoxazoline compounds have been employed as important building blocks for the synthesis of organic molecules, which are largely used in the chemical industry and life sciences (Benzai *et al.*, 2019). They have a range of biologically interesting properties and they constitute a unique class of pharmacophores that can be found in many therapeutic agents. Since there is a broad spectrum of protein targets that isoxazole compounds can interact with, isoxazole compounds have a variety of biological activities including anticancer, antibacterial, antifungal, antiviral, antimicrobial, antitubercular, anti-inflammatory and corrosion (Hachim *et al.*, 2022; Byadi *et al.*, 2020; Filali *et al.*, 2020; Filali *et al.*, 2019; Kang *et al.*, 2018; Sysak *et al.*, 2017; Brito *et al.*, 2013; Zarrouk *et al.*, 2013; Samanta *et al.*, 2011; Mihit et al., 2006; Frieser *et al.*, 2004; Joseph *et al.*, 2004; Kang *et al.*, 2000; Bassler *et al.*, 1997;). For example, Joseph has synthesized a number of compounds derived from isoxazole and isooxazoline (Joseph *et al.*, 2004). These are active against 3 bacteria, namely Bascillus cirroflagellosus, *E. coli* and *Pseudomonas putida*. The successful development of isoxazole compounds has led to the commercialization of many corresponding drugs on the market.

Starting from some considerations that attest the importance of pyridine ring, in this, we are presented the synthesis of new pyrazine derivatives containing 3,6-di(pyridin-2-yl)pyridazin-4-yl)methanol and their antibacterial activities. Moreover, drug-likeness and molecular docking studies were used to predict the compounds' binding interactions at the selected putative bacterial targets.

2. Materials and methods

2.1. Chemistry

2.1.1. Procedure of O–alkylation

2 g (7.6 mmol) of 3, 6-di(pyridin-2-yl)pyridazin-4-yl)methanol and 10 mL of THF are introduced into a flask, after which (3 eq) of NaH, (0.01 eq) of 18,6-crown ether and (1.3 eq) of propargyl or allyl bromide are added. The reaction mixture is stirred at room temperature for two days and the reaction is followed by thin layer chromatography. Then the solvent is evaporated, followed by liquid-liquid extraction with dichloromethane. The organic phase is dried, evaporated and purified by chromatography on silica gel column.

2.1.1.1. Synthesis of 4-((prop-2-yn-1-yloxy)methyl)3,6-di(pyridin-2-yl)pyridazine

White solid. The yield is 80%. Rf (ethyl acetate/hexane 2:1): 0.3. Mp (ºC): 127. IR (KBr): ν, cm-1): 1576 (C=Caromatics); 2137 (C≡C). 1H NMR (CDCl3): δ, ppm 2,46 (s, 1H); 4,39 (s, 2H); 5,21 (s, 2H); 7,38-7,44 (m,2Har); 7,88-7,95(m,2Har); 8,45-8,48 (dd,1Har); 8,74-8,80 (m,3Har); 8,95 (s,1Har). 13C NMR (CDCl3): δ, ppm 58,38 (CH2--alkyne); 68,12(CH2-O); 75,01 (C-alkyne); 121,77 ; 123,02 (CH-alkyne); 123,83; 124,33; 124,64; 136,90; 137,08; 148,46; 149,59 (CHar); 139,16; 153,67; 155,79; 156,12; 157,74 (Car).. HRMS (FAB+): m/z Calcd for C18H14N4O 303.1242; Found 303.1240.

2.1.1.2. Synthesis of 4-((allyloxy)methyl)-3,6-di(pyridin-2-yl)pyridazine

Chestnut solid. The yield is 87%. Rf (ethyl acetate/hexane 2:1): 0.51. Mp (ºC): 113. IR (KBr): ν, cm-1): 1500-1600 (C=Caromatics); 3080(CH2-alkene). 1H NMR (CDCl3): δ, 4,17-4,19 (d, 2H, CH2-O); 5,20 (s, 2H, O-CH2); 5,23-5,38 (d, 2H, CH2- alkene) ; 5,93-6,04 (m, 1H, CH- alkene); 7,37-7,41 (m, 2H, CHar) ; 7,86-7,92 (m, 2H, CHar) ; 8,40-8,43 (dd, 1H, CHar) ; 8,69-8,73 (m, 3H, CHar); 8,96 (s, 1H, CHar) .. 13C NMR (CDCl3): δ, ppm 68,38 (2CH2-O); 72,08 (CH2- alkene); 117,35 (CH-alkene), 121,73; 123,04; 123,77; 124,33; 124,60; 136,87; 137,03; 148,39; 149,56 (CHar) ; 139,88; 153,71; 155,86; 156,27; 157,77 (Car).HRMS (FAB+): m/z Calcd for C18H16N4O 305.1393; Found 305.1395.

2.1.2. Synthesis of 1,2,3-triazoles

In a 100 ml flask, 1 eq of alkyne and 1 eq of azide are dissolved in an ethanol/water mixture at room temperature. Copper chloride 0.1 eq is added and the reaction mixture is stirred at room temperature for 12 hours, then the ethanol is evaporated under reduced pressure. Distilled water is then added to the residual product which is then extracted three times with dichloromethane. The organic phase is dried over sodium sulfate, then filtered and evaporated under vacuum. The crude is then purified by chromatography on silica gel column, eluted by a mixture of hexane and ethyl acetate.

2.1.2.1. Synthesis of 2-(acetoxymethyl)-6-(4-(((3,6-di(pyridin-2-yl)pyridazin-4-yl)methoxy) methyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

White solid. The yield is 84%. R_f (ethyl acetate/hexanel 2:1): 0.77. Mp (ºC): 184. IR (KBr)**:** ν, cm^{-1}): 1581,31 (C=$C_{aromatics}$); 1742,67 (C=0). ^{1}H NMR ($CDCl_3$): δ, ppm 1,83; 2,032; 2,075 (3s, 12 H, 4CH3, OAc); 3,1-3,8 (m, 1H); 4,1-4,20 (dd, 1H); 4,25-4,36 (dd, 1H); 4,86 (s, 2H) ; 5,18 (s, 2H); 5,22-5,29 (m, 1H); 5,42-5,45 (d, 2H); 5,87-5,90 (m, 1H);7,39-7,42 (m, 2Har); 7,88-7,93 (m, 3Har); 8,42-8,45 (d,1H); 8,70-8,74 (m, 1Har); 8,77-8,78 (m, 2Har); 8,94 (s, 1Har). ^{13}C NMR ($CDCl_3$): δ, ppm 20,05; 20,47; 20,50; 20,64 (4 CH3, OAc); 61,57 (CH_2-OAc); 64,47 (CH2-N); 67,75 (CH2-O). 68,68; 70,33; 72,66; 75,12; 85,79 (5CH-$_{cycleglugosyl}$); 121,10 (CH-$_{Triazol}$); 121,79; 123,02; 123,79; 124,29; 124,61; 136,85; 137,06; 148,45; 149,51 (9 CHar); 139,27; 145,72; 153,71; 155,80; 156,18; 157,75 (Car) 168,77-169,30; 169,85; 170,43 (COAc). HRMS (FAB^+): *m/z* Calcd for $C_{32}H_{33}N_7O_{10}$ 676.2415; Found 676.2355.

2.1.2.2. Synthesis of 4-(((1-benzyl-1H-1,2,3-triazol-4-yl) methoxy) methyl)-3,6-di (pyridin-2-yl)pyridazine

White solid. The yield is 88%. R_f (ethyl acetate/hexanel 2:1): 0.75. Mp (ºC): 142. IR (KBr)**:** ν, cm^{-1}): 1437 (C=N); 1577 (C=$C_{aromatics}$). ^{1}H NMR ($CDCl_3$): δ, ppm 4.83; 5.17 (2s, 4H, 2CH_2-O); 5,53 (s, 2H, CH_2-N); 7.21-7.49 (m, 8Har); 7,56 (s, 1H, $CH_{triazolic}$); 7.81-7.93 (m, 2Har); 8.40-8.43 (d, 1Har); 8.61-8.83 (m, 3Har); 8.9 (s, 1Har)..^{13}C NMR ($CDCl_3$): δ, ppm 54.53 (CH2-N); 64.46, 68.57 (2 CH2-O); 121.74($CH_{Triazol}$); 122.66; 123.003; 124.30; 128.08; 129.07 (CHar); 145.20 (CT_{raizol}); 134.49; 139.41; 153.64; 155.67; 156.25; 157.69 (Car). HRMS (FAB^+): *m/z* Calcd for $C_{25}H_{21}N_7O$ 436,1880; Found 436.1880.

2.1.3. Synthesis of isoxazolines and isoxazoles

In a flask fitted with a bromine bulb 1.2 eq of aldoxime (38, 39) are dissolved with 1 eq of compound 1 or 2 in 10 ml of chloroform. The mixture is brought to -5 °C under stirring for 10 minutes,

then 8 ml of sodium hypochlorite NaOCl is added dropwise. After 3 h of stirring the organic phase is extracted and dried with sodium sulfate Na2SO4, after removal of the solvent the product is purified by silica gel column chromatography.

2.1.3.1. Synthesis of 3-(p-chlorophenyl)-5-(((3,6-di(pyridin-2-yl)pyridazine -4-yl) methoxy) methyl) isoxazole

Beige solid. The yield is 79%. R_f (ethyl acetate/hexanel 2:1): 0.3. Mp (ºC): 177. IR (KBr)**:** ν, cm^{-1}): 1113(C-0); 1575 ($C=C_{aromatics}$). ^{1}H NMR (DMSO): δ, ppm 4,95; 5,17 (2s, 4H, $2CH_2$-O); 7,2 (s,1H $CH_{isoxazol}$); 7,52-7,54 (d, 4H, CHar); 7,86-7,89 (d, 2Har, CHar); 8,021-8,072 (m, 2H, CHar); 8,30-8,32 (d,1H, CHar); 8,62-8,65 (d,1H, CHar); 8,70-8,77 (dd, 2H, CHar); 8,81 (s,1H, CHar).^{13}C NMR (DMSO): δ, ppm 63,45 ; 68,92 ($2CH_2$-O); 102,48 ($CH_{isoxazol}$); 121,59; 122,33; 124,56; 124,79; 125,72; 128,85; 128,85; 129,64; 129,64; 137,83; 138,11; 149,11; 150,11 (CHar); 128,85; 135,44; 139,09; 153,11; 155,46; 156,41; 157,55 (Car); 161,43 ; 170,26 ($2C_{ioxazol}$).HRMS (FAB^+): *m/z* Calcd for $C_{25}H_{18}ClN_5O_2$ 456,1222; Found 456.1214.

2.1.3.2. Synthesis of 5-(((3,6-di(pyridin-2-yl)pyridazin-4-yl)methoxy)methyl)-3-(4-nitrophenyl) isoxazole

Yellow solid. The yield is 79%. R_f (ethyl acetate/hexanel 2:1): 0.3. Mp (ºC): 177. IR (KBr)**:** ν, cm^{-1}): 1113(C-0); 1575 ($C=C_{aromatics}$). ^{1}H NMR (DMSO): δ, ppm 4,91; 5,2 (2s, 4H, $2CH_2$-O); 7,21 (s,1H, $CH_{isoxazole}$); 7,66-7,88 (dd,2H, CHar); 7,95-8,06 (m,4H, CHar); 8,43-8,5 (t,3H, CHar); 8,73-8,86 (d,3H, CHar); 8,88(m,1H, CHar).^{13}C NMR (DMSO): δ, ppm 63,43; 68,96 (2CH2-O) ; 102,94 ($CH_{isoxazole}$); 121,61; 122,36; 124,56; 124,56;124,81; 124,81;125,72; 128,81; 128,42; 128,42; 129,64; 137,94; 138,22; 149,17; 150,32 (CHar) ; 134,85; 139,10; 148,86; 153,08; 155,45; 156,52; 157,56 (Car); 160,97; 170,98 ($2C_{isoxazole}$).HRMS (FAB^+): *m/z* Calcd for $C_{25}H_{18}N_6O_4$ 467,1462; Found 467.1460.

2.1.3.3. Synthesis of 3-(p-chlorophenyl)-5-(((3,6-di(pyridin-2-yl) pyridazin-4 yl)methoxy)methyl)-isoxazoline

Beige solid. The yield is 81%. R_f (ethyl acetate/hexanel 2:1): 0.74. Mp (ºC): 114. IR (KBr)**:** ν, cm^{-1}): 1362 (N-O);1480 (C=N). ^{1}H NMR ($CDCl_3$): δ, ppm 3,21-3,43 (m, 2H, $CH_{2\text{-isoxazoline}}$); 3,80-3,85 (m, 2H, O-$CH_2$);5,01 (m,1H,$CH_{isoxzoline}$); 5,16-5,29 (m,2H, CH_2-O);7,2-7,41 (m, 4H, CHar);7,45-7,62 (m,2H, CHar); 7,80-8,01 (m, 2H, CHar);8, 2 (s,1H, CHar); 8,6-8,67 (t,3H, CHar); 8,84 (s,1H, CHar).^{13}C NMR ($CDCl_3$): δ, ppm 37,18 ($CH_{2\text{-isoxazolinique}}$) ; 69,99 ($CH_2$-O); 79,92 ($CH_2$-Cycl); 72,08 ($CH_{isoxazolinique}$); 121,75; 122,88; 123,87; 124,31; 124,63; 127,97; 127,97; 128,89; 128,89; 135,95; 136,92; 148,43; 149,47 (CHar) ; 128,01; 135,95; 137,06; 153,55; 155,54; 155,72; 156,08; 157,73 (Car). HRMS (FAB^+): *m/z* Calcd for $C_{24}H_{20}ClN_5O_2$ 458,1378; Found 458.1375.

2.1.3.4. Synthesis of 5-(((3,6-di(pyridin-2-yl) pyridazin-4-yl)methoxy) methyl)-3-(p-nitrophenyl)-isoxazoline

Yellow solid. The yield is 79%. R_f (ethyl acetate/hexanel 2:1): 0.66. Mp (ºC): 175. IR (KBr)**:** ν, cm^{-1}): 1560 (NO2) ; 1350 (N-O); 1577 (C=Car). ^{1}H NMR ($CDCl_3$): δ, ppm 3,39-3,53 (m, 2H, $CH_{2\text{-isoxazoline}}$); 3,86-3,87 (m, 2H, CH2-O); 5,21 (m,2H, CH2-O); 5,20 (m,1H, $CH_{isoxazoline}$); 7,36-7,40 (m, 2H, CHar); 7,79-7,93 (m,4H, CHar); 8,17-8,20 (d, 2H, CHar);8,42-8,45 (d,1H, CHar); 8,62-8,64 (m,3H, CHar); 8,80 (s,1H, CHar).^{13}C NMR ($CDCl_3$): δ, ppm 36,62 ($CH_{2\text{-isoxazoline}}$), 70,07 (CH2-O);

80,82 (CH2-Cycle) ; 71,94 ($CH_{isoxazoline}$); 121,77; 121,77; 122,76; 123,88; 123,88; 124,32;1 24,66; 127,42; 127,42; 137,12; 137,21; 148,38; 148,42 (CHar) ; 135,60; 136,96; 149,39; 153,52; 155,00; 155,70; 156,03; 157,68 (Car). HRMS (FAB^+): *m/z* Calcd for $C_{24}H_{20}ClN_5O_2$ 469,1619; Found 469.1619.

2.2. Antibacterial activity

2.2.1. Microorganisms and inoculum preparation

The tested microorganisms included the following bacteria: *Escherichia coli* ATCC 4157; *Pseudomonas aeruginosa* ATCC 27853; *Staphylococcus aureus* ATCC 25923 and *Streptococcus fasciens* 29212. All pathogenic microroganisms isolated from patients were stored at the culture collection of the Biology Department (Microthec Unity) at the Faculty of Science, Rabat, Morocco. They were maintained in Brain Heart Infusion (BHI) at -80°C. Prior to the experiment, cultures were prepared by subculturing 1 ml of each culture stock in 9 ml of BHI broth.

2.2.2. Agar Disc Diffusion Method

The Agar Disc Diffusion Method (ADD) was employed for the determination of antibacterial activities of the tested products as described previously. The test samples were first dissolved in Dimethylsulfoxid (1%) (DMSO), who did not affect the microbial growth. Briefly, the test was performed in sterile petri plates containing medium agar. 30ml of sterilized medium was poured into sterile petri plates. After solidification, 100 µl of fresh cultures of bacteria species (one microorganism per petri plates). Sterile filter paper disc (6 mm in diameter) were impregned with 6 µl of the test samples 20 mg/ml. All plates were sealed with sterile laboratory films avoid eventual evaporation of the test samples, and then incubated at 37°C for 24h. The diameter of inhibition zone was measured in millimeters. In addition, the antibacterial activities of the samples on bacteria were compared with the commercially available antibiotics. The antibiotic discs such Ampicillin and Chloramphenicol were placed on the surface of the plates. DMSO 1% was used as negative control. The plates were incubated at 37°C for 24 h after incubation. The diameter of inhibition were measured in mm and recorded.

2.2.3. Determination of the Minimum Inhibition Concentration (MIC)

We tested six (6) serial concentration of the high active products at concentration (20 mg/ml; 10 mg/ml, 5 mg/ml, 2,5 mg/ml; 1,25 mg/ml, 0,625 mg/ml), diluted in BHI broth. For MIC assessed, 5 ml of culture medium was inoculed with 0,1 ml of bacteria species. The MIC is the lowest concentration of samples, for which no growth was detected for 24h at 37°C (Lahyaoui *et al.*, 2024).

2.3. Drug-likeness prediction

We see that many potential therapeutic drugs never reach clinical trials because of their negative ADMET characteristics (Islam *et al.*, 2021; Tabti *et al.*, 2022). The drug-likeness method is the most recent way for discovering compounds that are recommended for use in treatments that must comply to important requirements, such as Lipinski's, Veber's, and Igan's guidelines. The Lipinski, Veber, and Igan recommendations are based on research on the ADME properties of human drugs. These recommendations are particularly helpful in the development of drugs based on the 2D small molecule structure and oral bioavailability (Netzeva *et al.*, 2005; Hansch *et al.*, 2004). Less than 10% of drugs that advance to the clinical trial stage do so without meeting one or more of these

requirements. We also evaluate the Topological Polar Surface Area (TPSA) and the number of Rotatable Bonds (n-ROTB) (Jin *et al.*, 2020). Based on the prediction of these parameters, we can establish whether a chemical interacts with a receptor in a flexible or rigid manner (Chtita *et al.*, 2020). We evaluate the compounds chosen for this investigation's drug-likeness in silico feature using the online SwissADME service.

2.4. Molecular docking Study

Computational biology organizes our understanding of life, makes biological principles robust and testable, and offers a reference map that connects individual insights (Lahyaoui *et al.*, 2023), (Lahyaoui *et al.*, 2023; Lahyaoui *et al.*, 2023). Molecular docking is currently the most commonly used method for rationalizing ligand behavior against a target of interest and performing structure-based virtual screening campaigns.

The scores that were obtained during the molecular docking process were calculated and reported using autodock software. The atomic structures were created using ChemDraw (18.2). Using the Protein Data Bank (https://www.rcsb.org/pdb/welcome.do), the target crystal structure was built and retrieved (PDB code = 6V78). According to molecular docking studies, the type of attached substituents and how they alter the interactive characteristics determine the binding interaction of a molecule with an enzyme's active site. The preparation of the protein and the ligand, as well as the reduction of energy, were the first steps in performing molecular docking studies. The water was then taken out and polar hydrogen, Kollman, and Gasteiger charges were added in the auto dock, where protein and ligand were exchanged. Following completion, the protein, ligand, and their X, Y, and Z coordinates were each stored in text and PDBQT formats. The molecular docking study was completed in the final stage by setting the location of the docking folder and utilizing command prompt. Last but not least, DSV was employed to investigate the binding interaction **(Table 2)** in the form of a 3D structure as depicted in **Figure 1**.

Analogs **1**, **2**, **4**, **6**, **7** and **8**'s docking scores were determined because to their diverse functionalities and positions, which resulted in different effects that either increased or decreased the binding interaction. As a result, their docking scores and interaction ranges differ from one another. The existence of functional groups, positions, and numbers of substituents in these compounds may increase or decrease the binding interactions of molecules in a complex, which in turn may affect their potency.

3. Results and discussion

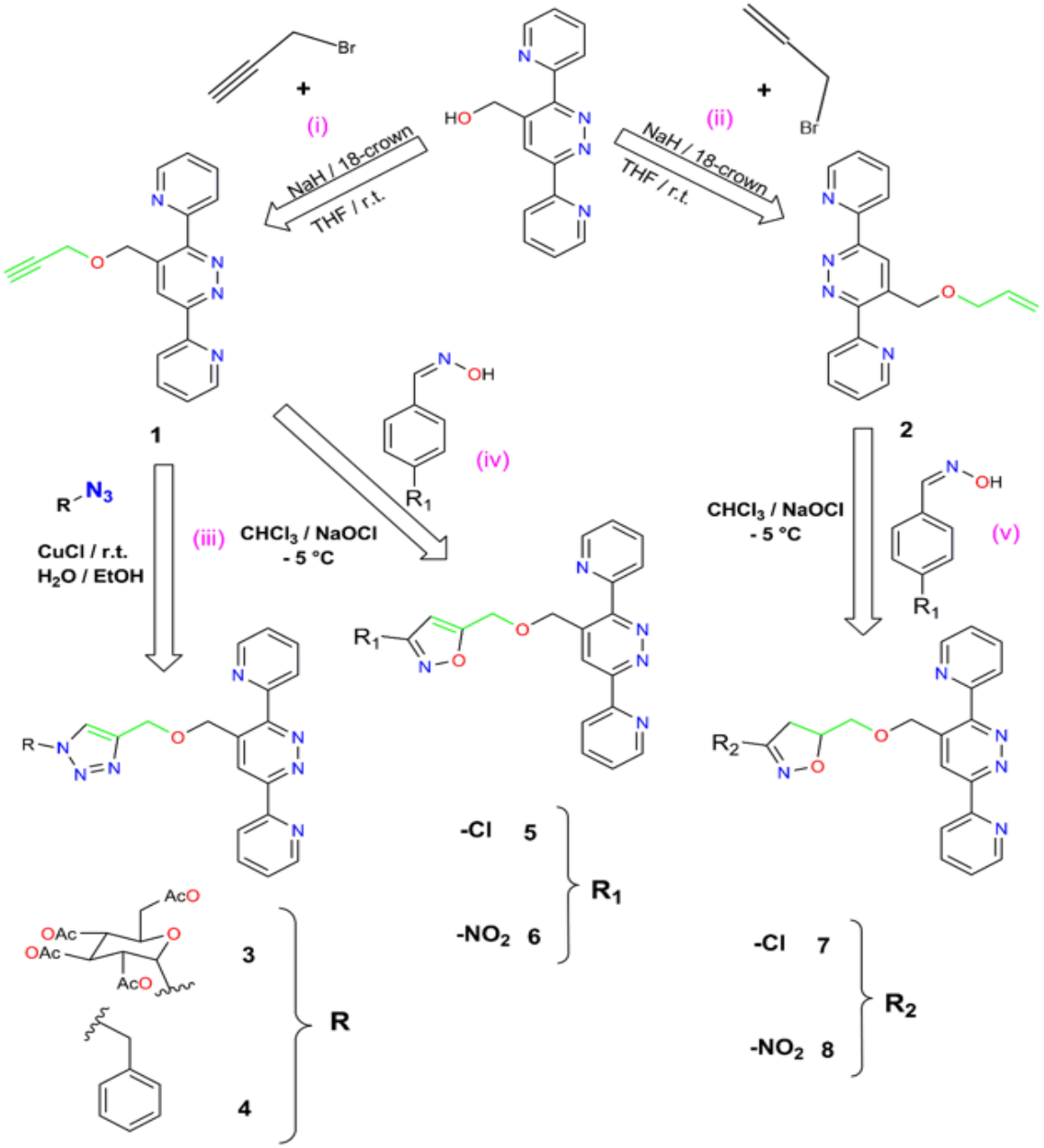
3.1. Synthesis and characterization

Two new (3,6-di(pyridin-2-yl)pyridazin-4-yl)methanol (DPPNM) (compounds **1** and **2**) were synthesized via Williamson ether synthesis in tetrahydrofuran (THF) at room temperature as shown in Scheme . The yields of these reactions were very good (ca. 80%) for both DPPNM derivatives. The obtained products were characterized by means of the melting point, NMR spectroscopy, and High Resolution Mass-Spectrometry technique (HRMS). The clickable reaction between obtained alkyne (compound 1) and three azide derivatives were performed using copper catalyzed azide-alkyne cycloaddition reaction (CuAAC) under greener conditions **(Scheme 1)** , resulting in the desired compounds (**3**, **4** and **5**). The NMR spectroscopy was investigated to confirm the regioselective synthesis of 1,4-disubstiteted 1,2,3-triazoles. The prepared compounds **1** and **2** were also investigated

in the synthesis of isoxazoles and isoxazolines by the appearance of the chemical shift of the $CH_{isoxazoles}$ group at 7.2 ppm and the $CH_{isoxazoline}$ at 5.01 ppm in the 1H NMR spectra, respectively.

3.2. Antibacterial activity

The minimum inhibition concentration (MIC) study was then carried out by the solid-state dilution method against different bacterial strains. The results of the two series tested are shown in **Table 1**. According to the results obtained, the tested products seem to be endowed with an inhibitory activity on certain classes of tested micro-organisms. The compound 1 and 4 shows a very strong activity against four bacterial strains (Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus fasciens).



Scheme 1. Synthetic route for new (3,6-di(pyridin-2-yl)pyridazin-4-yl)methanol derivatives (1-8)

Further analysis of the results, it can be deduced that the compounds examined for their antimicrobial effects, presented activities against all microbial strains. The antibacterial activity of compounds 1,

3, 4 and 8 is higher than that of ampicillin against Escherichia coli. Pseudomonas aeruginosa proved to be very sensitive towards the tested compounds which is higher than the inhibition diameter of ampicillin. Compound 4 was the only one that showed an inhibition diameter greater than the diameter of the two controls ampicillin and Chloramphenicol. The majority of the products of the synthesized compounds presented an antibacterial effect, against the two types of strains, namely gram positive and negative. The two compounds 1 and 4 showed the highest MIC against E. Coli compared to the synthesized products subject of this test, with a MIC of 5µg/ml. Compound 3 showed the highest MIC value (5µg/ml) against Pseudomonas aeruginosa (**Table 1**). The tested compounds showed a good antibacterial activity against gram positive bacterial strains (Staphylococcus aureus and Streptococcus fascien), with inhibition zone diameters greater than that of ampicillin. The compounds 7 and 8 have an antibacterial activity inferior to the ampicillin against Staphylococcus aureus and compound 3 against Streptococcus fascine.

Table 1. Minimum inhibition concentration (µg/ml) of synthesized molecules against different bacterial strains

Compounds	**MIC (µg/ml)**			
	Gram negative		***Gram positive***	
	Escherichia coli	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Streptococcus fasciens***
1	5	10	10	5
2	20	20	20	20
3	10	5	10	10
4	5	10	10	5
5	20	20	20	20
6	20	10	5	10
7	10	20	20	20
8	20	10	10	20
Chloramphenicol	12.5	25	12.5	6.25

3.3. Evaluation of drug-likeness properties

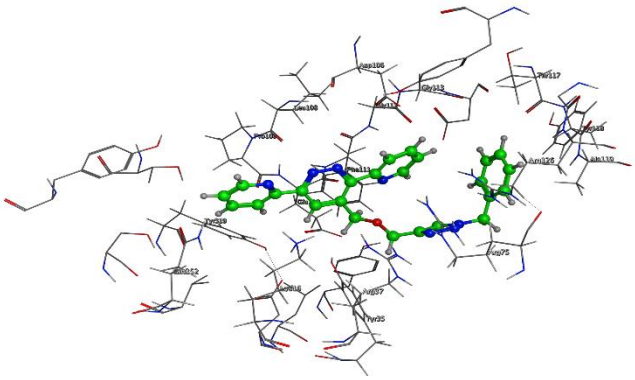
The aim of this study is to predict the drug-like properties of the studied molecules in order to define the biological activity of these compounds and to evaluate their favorable or dangerous effects on the organism if they are used in pharmaceutical applications. We are also looking for substances with drug-like properties to study the docking of molecules. **Table 3** displays the evaluation results for the drug-like properties obtained by using the SwissADME online server. The results demonstrate that all compounds (with the exception of compound **3**) have oral bioavailability that complies with all Lipinski, Veber, and Egan recommendations and has a high absorption capacity. It is acknowledged that compound **5** strayed from the Lipinski, Veber, and Egan regulations' high lipid affinity level (LogP > 5). Compounds 3 and 5 will therefore not be considered as possible therapeutic candidates. These results enable us to select more compounds that display drug-like properties and do not present any oral bioavailability concerns. However, when it is less than 140 A^2 and the number of rotatable bonds is lewer than 10, the TPSA becomes more flexible and is better able to engage with the target receptor.

3.4. Molecular docking

Docking scores are used to categorize the outcomes of our molecular docking process. The best docking poses for the targeted protein were chosen mainly based on docking ratings. Binding affinities can be related to interaction energy between proteins and ligands. The binding locations and docking scores of the chosen compounds for the encoded protein enzymes 6V78 were determined using autodock modeling software. According to the ranking poses produced by the scoring functions listed in **Table 2**, the lowest binding energy among all the estimated energies had the maximum activity. Compound **4** achieved the highest score with a result of -7.53 kcal/mol. **Table 2** also include a list of the hydrogen bonds that exist between the chemicals and the chosen protein coenzymes.

Table 2. Docking score and interaction between the compounds and 6V78 protein.

compounds	Binding energy (Kcal/mol)	Ligand	receptor	interaction	distance	E (kcal/mol)
1	-5.95	N	ARG 75	H-acceptor	3.31	-3.7
			ARG 126		3.79	-0.6
2	-6.11		ARG 75		3.51	-2.2
4	-7.53		LYS 16		3.31	-1.9
			ARG 126		3.07	-0.8
			ARG 126		2.98	-3
			ARG 75		3.46	-3.5
			ARG 37		3.32	-0.8
6	-6.74		ARG 37		2.92	-1.6
			ARG 126		3.16	-2
7	-6.73		ARG 126		3.62	-1.1
		6-ring		pi-cation	4.17	-1.1
8	-6.82		ARG 37		3.88	-0.6
			ARG 126		3.99	-1.3



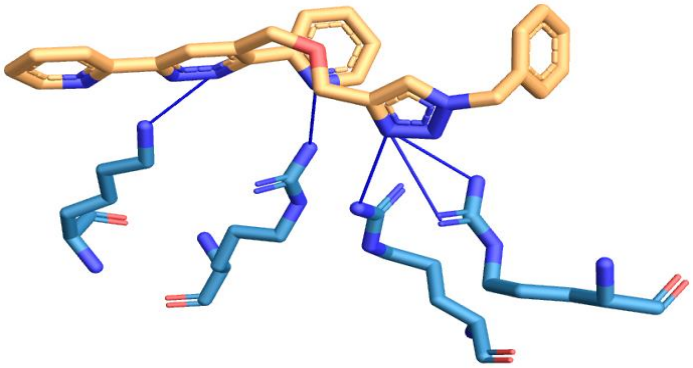


Figure. 1 2D and 3D docking of compound 4 and 6V78 protein.

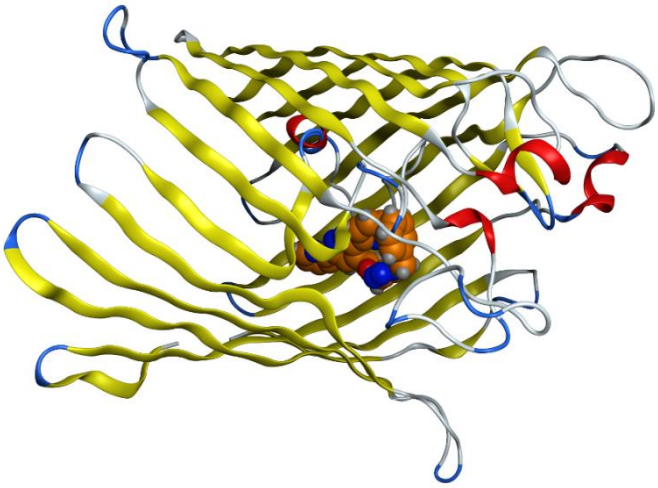


Table 3. The compounds' ADME characteristics.

MOLECULE	ABS	TPSA (A2)	n-ROTB	MW	LogP	n-OHN acceptors	n-OHNH donors	Lipinski's violations	Veber Violations	Egan Violation	S.A
rule	-	-	-	<500	≤5	<10	<5	≤1	≤1	≤1	0< S.A<10
1	high	60.79	5	302.33	2.26	5	0	0	0	0	3.11
2	high	60.79	6	304.35	2.52	5	0	0	0	0	3.03
3	low	205.93	16	675.65	1.18	16	0	2	2	1	6.29
4	high	91.50	8	435.48	2.82	7	0	0	0	0	3.66
5	high	91.50	8	435.48	8.82	7	0	0	0	0	3.66
6	high	132.64	7	390.35	1.77	9	0	0	0	1	3.53
7	high	82.38	6	381.82	2.45	7	0	0	0	0	3.98
8	high	132.64	7	390.35	1.77	9	0	0	0	1	3.53

Figure 1 shows the enzyme-calmed chemical in the position that suits it best. The primary molecular docking process, known as Autodock, is used to identify a precise docking study between drugs and target proteins. Through H-bonds with Lys16, Arg126, Arg75, and Arg37, compound **4** had a high docking score. The energy stabilization ranged from 0.8 to 3.5 Kcal/mol, and the interaction distance varied from 2.98 to 3.46A˚.

The target receptor's structure was stabilized by the bonds formed with the important amino acid residues of the binding pockets that were discovered based on the studies discussed above. Due to the strong affinity of all docked poses with the lowest binding energy, they are all considered to be in the best-docked conformation. The docking tool also made it feasible to match the experimentally discovered binding modes, thereby determining the specific conformation of the target and ligand, in light of these crucial molecular interactions.

4. Conclusion

New triazoles derivatives containing 3,6-di(pyridin-2-yl)pyridazin-4-yl)methanol were synthesized and characterized by different techniques by means of the NMR spectroscopy and High Resolution Mass-Spectrometry technique (HRMS) and subsequently tested for their antibacterial activities. The biological tests carried out with these pyrazine derivatives show that the compound **4** exhibits the best activity among the studied compounds against the negative gram, *Escherichia coli* and *Pseudomonas aeruginosa*, and positive gram *Staphylococcus aureus* and *Streptococcus fasciens*. To explain their mechanism action for their antibacterial activities, drug-likeness properties and molecular docking studies of the most activated compound were also performed.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.
Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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