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Synthesis, characterization, *in vitro* antibacterial, and molecular modeling study of some pyrazole and pyranopyrazole derivatives

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ABSTRACT:

A facile and convenient protocol is developed for designing new heterocyclic compounds using nontoxic, simple, and eco-friendly methods. A group of novel compounds containing either pyrazole or pyranopyrazole moieties was synthesized using 2-(5-oxo-4, 5-dihydro-1*H*-pyrazol-3-yl)-*N*-phenylacetamide (**1a,b**) as a precursor. The structure of the prepared compounds; namely pyranopyrazole derivatives **2** and pyrazole derivatives **3-6** was elucidated using different spectroscopic methods (such as FTIR, ^{1}H-NMR, Mass spectroscopy, and ^{13}C-NMR) as well as elemental analyses. Consequently, the newly prepared compounds were all evaluated against different human pathogenic bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Bacillus subtilis* using the commercial bactericide chloramphenicol as a reference. Most of the tested compounds showed moderate to high antibacterial activity. Molecular docking study was performed to predict binding affinities and the modes of interactions between the synthesized ligands and the active site of the topoisomerase IV. Presumably, the obtained results provide valuable information for designing, developing, and synthesizing novel heterocyclic scaffolds with enhanced pharmacological applications as antibacterial materials.

KEYWORDS: Synthesis, Pyranopyrazole, Pyrazole, Antibacterial activity, Molecular docking.

1. INTRODUCTION:

Nitrogen-containing heterocyclic compounds and their derivatives have historically been invaluable as a source of therapeutic agents. Pyrazole nucleus was among the important *N*-based heterocyclic frameworks, which is also considered a "biologically privileged" *N*-heteroaromatic five-membered compound. Currently, pyrazole and its derivatives have attracted attention due to their broad spectrum of pharmacological and agricultural applications (Ebenezer et al., 2022; Jeschke, 2016; Joshi et al., 2016; Karrouchi et al., 2018; Naim et al., 2016; Varghese et al., 2017). An arsenal of new synthetic protocols has been developed to synthesize structurally diversified pyrazole derivatives (Maddila et al., 2017; Sadeghpour et al., 2021). There are several FDA-approved drugs, insecticides, herbicides, and molluscicidal products containing the pyrazole scaffold (Amnerkar et al., 2010; Bao et al., 2017; Berghot et al., 2003; Havrylyuk et al., 2009; Kahriman et al., 2017; Nassar, 2010; Sadashiva et al., 2017; Sarkar et al., 2017; Karrouchi et al., 2018; Naim et al., 2016).

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On the other hand, the fused ring system pyranopyrazoles have been the core of interest for chemists and pharmacologists (Biswas et al., 2022; Emtiazi et al., 2022; Ganta et al., 2021; Musa et al., 2023; Nguyen et al., 2022). The innumerable applications of fused pyrazole analogs have stimulated researchers for developing new synthetic routes to prepare structurally diverse derivatives (Bekhit et al., 2022; Biswas et al., 2022; Chakraborty et al., 2023; Emtiazi et al., 2022; Sadeghpour et al., 2021) In part, molecular docking studies attempted to reveal the wide range of drug-like properties of the synthesized derivatives in order to create opportunities to harness the full potential of these compounds. Molecular docking investigation was performed on the newly synthesized compounds against *E.coli* In this work, the synthesis of biologically important compounds using simple, efficient, non-toxic, readily available catalysts and eco-friendly methods was developed. The structure elucidation of the different novel pyrazole, pyranopyrazole, and pyridopyrazole derivatives has been undertaken. Consequently, the prepared compounds were screened for their antibacterial activity against gram-positive and gram-negative human strains of bacteria as an example of pathogenic microorganisms. topoisomerase IV receptors to determine the various modes of bonding and interactions between the selected ligands and the target protein. The theoretical calculations indicated that the synthesized compounds are promising candidates for further biomedical applications.

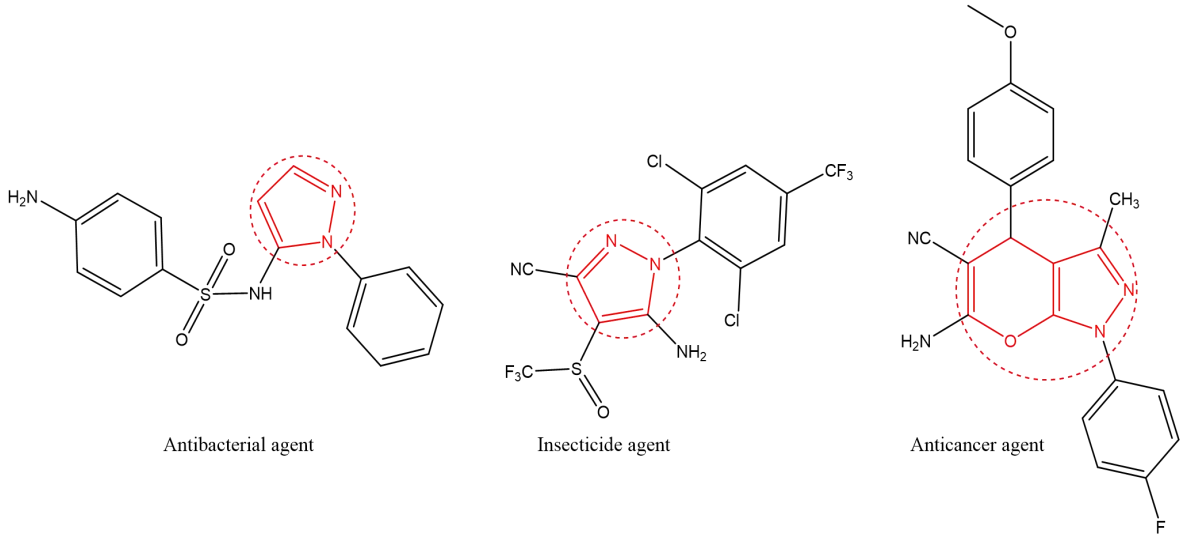


Figure 1. Different reported drugs bearing pyrazole scaffold.

2. MATERIALS AND METHODS:

The melting points were determined with an electrothermal melting point device and are uncorrected. The Pye-Unicam IR spectrophotometer SP 2000 was used to

record the IR (KBr) spectra (Faculty of Science, Fayoum University). At the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Nasr City, Cairo, mass spectroscopy was performed on a Direct Inlet part to mass analyzer in a Thermo Scientific GCMS model ISQ. The purity of the compounds was verified using mass spectrometry, which was also utilized to investigate the distinctive fragmentation and the anticipated molecular weight. The Mass spectroscopy was performed in Electron Impact mode. Nuclear magnetic resonance (NMR) spectra were measured in DMSO-d_6 (TMS, ^{1}H δ=0; DMSO-d_6, ^{1}H δ = 2.50) on a BRUCKER AVANCE III (^{1}H at 400-MHz) magnetic resonance spectrometer at NMR unit, Faculty of Pharmacy, Beni-suef University, Egypt. Chemical shifts (δ) and coupling constants (J) were recorded in parts per million (ppm) and Hertz (Hz), respectively. Molecular docking investigations were performed using Autodock-4 package software to determine the various modes of bonding, electrostatic, and hydrophobic interactions that can occur between the selected ligands and the target protein.

2.1. Organic Synthesis

2.1.1. Synthesis of *N*-(2-aryl)-2-(6-oxo-3-(2-oxo-2-(phenylamino) ethyl)-1-phenyl-1,6-dihydropyrano[2,3-c] pyrazol-4-yl) acetamide (2a,b):

3-oxo-N^1,N^5-diphenylpentanediamide (2.96gm, 0.01 mol) was dissolved in 100 ml absolute ethanol, and 2-(5-oxo-4,5-dihydro-1*H*-pyrazol-3-yl)-*N*-phenylacetamide (**1a,b**) (0.01 mol), as well as sodium hydroxide (0.8 gm, 0.02 mol), were added. The reaction mixture was refluxed for 6 h, cooled, and added to ice/HCl. The produced solid was filtered out, washed with water, dried, and purified by recrystallization from acetic acid to produce **2a,b**.

Compound **2a**: yellow crystals; mp 188-190 °C; yield 67%; IR (KBr) ν/cm^{-1}: 3247 (NH), 1704 (CO), 1658 (CO); ^{1}H-NMR (DMSO-d_6): δ 12.43 (s, 1H, NH D_2O exchangeable), 10.46 (s, 1H, NH D_2O exchangeable), 10.15 (s, 1H, NH D_2O exchangeable), 7.63 - 6.88 (m, 10H, Ar-), 6.04 (s, 1H, CH), 4.20 (s, 2H, CH_2), 3.90 (s, 2H, CH_2). Anal. Calcd for $C_{22}H_{18}N_4O_4$ (402.41): (C, 65.66; H, 4.15; N, 13.92%); Found (C, 65.23; H, 4.27; N, 13.80%).

Compound **2b**: brown crystals; mp 92-94 °C; yield 67%; IR (KBr) ν/cm^{-1}: 3135 (NH), 1708 (CO), 1662 (CO); ^{1}H-NMR (DMSO-d_6): δ 10.56 (s, 1H, NH D_2O exchangeable), 10.25 (s, 1H, NH D_2O exchangeable), 7.80 - 6.98 (m, 15H, Ar-), 5.63 (s, 1H, CH), 4.01 - 3.57 (dd, J = 15.8 Hz, 17.3, 2H, CH_2), 3.03 - 2.87 (dd, J = 15.0 Hz, 13.7, 2H, CH_2). Ms m/z (%): 478 (M^+, 15.48), 401 (16.43), 212 (100). Anal. Calcd for $C_{28}H_{22}N_4O_4$ (478.16) (C, 70.28; H, 4.63; N, 11.71); Found (C, 69.79; H, 4.44; N, 11.45).

2.1.2. Synthesis of 2-(4-(4-chlorobenzylidene)-5-oxo-4,5-dihydro-1*H*(1-phenyl)-pyrazol-3-yl)-*N*-phenylacetamide (3a,b):

4-chloro benzaldehyde (1.40 gm, 0.01 mol) was added to a solution of 2-(5-oxo-4,5-dihydro-1*H*-pyrazol-3-yl)-*N*-phenylacetamide (**1a,b**) (0.01 mol) in 25 ml ethanol in the presence of a few drops of piperidine and refluxed for 3 h. The reaction mixture was cooled and poured into ice/H_2O. The formed precipitate was filtered out, dried, and recrystallized from ethanol to yield **3a,b**.

Compound **3a**: white crystals; mp 200-202 °C; yield 77%; IR (KBr) ν/cm^{-1}: 3263 (NH), 3280 (NH), 1670 (CO); ^{1}H-NMR (DMSO-*d*$_6$): δ 10.44 (s, 1H, NH D_2O exchangeable), 9.92 (s, 1H, NH D_2O exchangeable), 8.39 (s, 1H, CH), 7.81 - 6.25 (m, 9H, Ar-), 3.89 - 3.55 (dd, J = 17.3 Hz, 10.6 Hz, 2H, CH_2). Ms m/z (%): 339 (M^+, 100). Anal. Calcd for $C_{18}H_{14}ClN_3O_2$ (339.78): (C, 63.63; H, 4.15; Cl, 10.43; N, 12.37); Found (C, 63.22; H, 3.89; Cl, 10.03; N, 12.02).

Compound **3b**: white crystals; mp 180-182 °C; yield 85%; IR (KBr) ν/cm^{-1}: 3193 (NH), 1670 (CO); ^{1}H-NMR (DMSO-*d*$_6$): δ 10.22 (s, 1H, NH D_2O exchangeable), 7.94 - 7.03 (m, 14H, Ar-), 7.67 (s, 1H, CH), 4.34 - 4.06 (dd, J = 11.6 Hz, 15.7, 2H, CH_2). Ms m/z (%): 416 (M^+, 100). Anal. Calcd for $C_{24}H_{18}ClN_3O_2$ (415.88): (C, 69.31; H, 4.36; Cl, 8.52; N, 10.10); Found (C, 68.92; H, 4.04; Cl, 8.22; N, 9.84).

2.1.3. Synthesis of 2-(4-(2-(4-methoxyphenyl)hydrazineylidene)-5-oxo-4,5-dihydro- 1*H*(1-phenyl)- pyrazol-3-yl)-*N*-phenylacetamide (4a,b):

2-(5-oxo-4,5-dihydro-1*H*-pyrazol-3-yl)-*N*-phenylacetamide (**1a,b**) (0.01 mol) and 1 gm of sodium acetate were dissolved in 80 ml ethanol. The suspension was treated with an equimolar amount of diazonium salt of p-anisidine (prepared in the usual way) at 0 - 5 °C, and the reaction mixture was stirred for 1 h. The formed precipitate was separated by filtration, washed with cold ethanol, dried, and purified by recrystallization from acetic acid to give (**4a,b**).

Compound **4a**: orange-red crystals; mp 238-240 °C; yield 92.7%; IR (KBr) ν/cm^{-1}: 3305 (NH), 1720 (CO), 1658 (CO); ^{1}H-NMR (DMSO-*d*$_6$): δ 11.95 (s, 1H, NH D_2O exchangeable), 11.74 (s, 1H, NH D_2O exchangeable), 10.28 (s, 1H, NH D_2O exchangeable), 7.62 - 7.06 (m, 9H, Ar-), 3.67 (s, 2H, CH_2), 3.14 (s, 3H, CH_3). Anal. Calcd for $C_{18}H_{17}N_5O_3$ (351.37): (C, 61.53; H, 4.88; N, 19.93%); Found (C, 60.93; H, 4.34; N, 19.53).

Compound **4b**: orange crystals; mp 240-242 °C; yield 90%; IR (KBr) ν/cm^{-1}: 3305 (NH), 1720 (CO), 1658 (CO); ^{1}H-NMR (DMSO-*d*$_6$): δ 12.69 (s, 1H, NH D_2O exchangeable), 10.22 (s, 1H, NH D_2O exchangeable), 7.94 - 7.03 (m, 14H, Ar-), 4.08 (s, 2H, CH_2), 2.88 (s, 3H, CH_3). Anal. Calcd for $C_{24}H_{21}N_5O_3$ (427.46): (C, 67.44; H, 4.95; N, 16.38); Found (C, 67.04; H, 4.52; N, 15.98).

2.1.4. Synthesis of 2-(4-(ethoxymethylene)-5-oxo-4,5-dihydro-1*H*(1-phenyl)-pyrazol-3-yl)-*N*-phenylacetamide (5a,b):

A mixture of 2-(5-oxo-4,5-dihydro-1*H*-pyrazol-3-yl)-*N*-phenylacetamide (**1a,b**) (0.01 mol) and triethyl orthoformate (1.66 ml, 0.01 mol) in 10 ml acetic anhydride was refluxed for 12 h. The excess of the solvent was evacuated, and the solid precipitate was washed with water, dried, and recrystallized from an appropriate solvent to afford **5a,b**.

Compound **5a**: brown crystals; mp 212-214 °C (acetic acid); yield 79%; IR (KBr) ν/cm^{-1}: 3259 (NH), 3197 (NH), 1662 (2CO); ^{1}H-NMR (DMSO-*d*$_6$): δ 11.88 (s, 1H, NH D_2O exchangeable), 10.31 (s, 1H, NH D_2O exchangeable), 7.90 - 7.22 (m, 5H, -Ar), 7.05 (s, 1H, -CH), 3.99 (s, 2H, CH_2), 3.04 (q, J = 9.6, 8.8, 8.0 Hz, 2H, CH_2), 1.23 (t, J = 15.0 Hz, 3H, CH_3). Anal. Calcd for $C_{14}H_{15}N_3O_3$ (273.11): (C, 61.53; H, 5.53; N, 15.38); Found (C, 61.21; H, 5.33; N, 15.06).

Compound **5b**: gray crystals; mp 280-282 °C (DMF/water); yield 67%; IR (KBr) ν/cm^{-1}: 3290 (NH), 1650 (CO); ^{1}H-NMR (DMSO-*d*$_6$): δ 10.31 (s, 1H, NH D_2O exchangeable), 7.90 - 7.00 (m, 10H, Ar-), 6.78 (s, 1H, CH), 3.99 (s, 2H, CH_2), 3.57 (q, J = 10.6, 9.9, 9.7 Hz, 2H, CH_2), 1.24 (t, J = 15.7, 13.3 Hz, 3H, CH_3). Anal. Calcd for $C_{20}H_{19}N_3O_3$ (349.39): (C, 68.75; H, 5.48; N, 12.03); Found (C, 68.39; H, 5.15; N, 11.83).

2.1.5. Synthesis of compounds 6a-d:

2-(4-(Ethoxymethylene) -5-oxo-4,5-dihydro-1*H*-pyrazol-3-yl) -*N*-phenylacetamide (**5a,b**) (0.01 mol) was dissolved in 50 ml absolute ethanol, (0.5 ml, 0.01 mol) hydrazine hydrate and/or aniline (0.93 ml, 0.01 mol) was added. The reaction mixture was refluxed for 2 h, concentrated, and the formed precipitate was isolated by filtration, washed with cold ethanol, dried, and recrystallized from a suitable solvent.

5-Amino-6-(phenylamino)-2,5-dihydro-3*H*-pyrazolo[4,3-*c*]pyridin-3-one (**6a**): brown crystals; mp > 300 °C (ethanol); yield 68%; IR (KBr) ν/cm^{-1}: 3286 (NH), 3135 (NH_2), 1646 (CO); ^{1}H-NMR (DMSO-*d*$_6$): δ 10.23 (s, 1H, NH D_2O exchangeable), 9.67 (s, 1H, NH D_2O exchangeable), 8.44 (s, 1H, -CH), 7.88 - 7.06 (m, 5H, Ar-), 7.46 (s, 1H, -CH), 6.54 (s, 2H, NH_2 D_2O exchangeable). Anal. Calcd for $C_{12}H_{11}N_5O$ (241.25): (C, 59.74; H, 4.60; N, 29.03); Found (C, 59.34; H, 4.32; N, 28.83).

5-Amino-2-phenyl-6-(phenylamino)-2,5-dihydro-3*H*-pyrazolo[4,3-*c*]pyridin-3-one (**6b**): white crystals; mp 248-250 °C (acetic acid); yield 66%; IR (KBr) ν/cm^{-1}: 3293 (NH_2), 3197 (NH), 1650 (CO); ^{1}H-NMR (DMSO-*d*$_6$): δ 9.52 (s, 1H, NH D_2O exchangeable), 8.45 (s, 1H, CH), 7.88 - 7.03 (m, 10H, Ar-), 6.87 (s, 1H, CH), 6.38 (s, 2H, NH_2 D_2O exchangeable). Ms m/z (%): 317 (M^+, 59.93), 302 (100). Anal. Calcd for $C_{18}H_{15}N_5O$ (317.35): (C, 68.13; H, 4.76; N, 22.07); Found (C, 67.89; H, 4.45; N, 21.77).

5-Phenyl-6-(phenylamino)-2,5-dihydro-3*H*-pyrazolo[4,3-*c*]pyridin-3-one (**6c**): orange crystals; mp 258-260 °C (acetic acid); yield 60%; IR (KBr) ν/cm^{-1}: 3282 (NH), 1662 (CO); ^{1}H-NMR (DMSO-*d*$_6$): δ 11.23 (s, 1H, NH D_2O exchangeable), 9.97 (s, 1H, NH D_2O exchangeable), 8.44 (s, 1H, -CH), 8.04 - 6.79 (m, 10H, -Ar), 7.00 (s, 1H, -CH). Ms m/z (%): 302 (M^+, 6.58), 273 (18.29), 245 (100). Anal. Calcd for $C_{18}H_{14}N_4O$ (302.34): (C, 71.51; H, 4.67; N, 18.53); Found (C, 71.12; H, 4.27; N, 17.97).

2,5-Diphenyl-6-(phenylamino)-2,5-dihydro-3*H*-pyrazolo[4,3-*c*]pyridin-3-one (**6d**): orange crystals; mp 180-182 °C (ethanol); yield 92%; IR (KBr) ν/cm^{-1}: 3263 (NH), 1666 (CO); ^{1}H-NMR (DMSO-*d*$_6$): δ 9.82 (s, 1H, NH D_2O exchangeable), 8.44 (s, 1H, CH), 7.88 - 7.05 (m, 16H, Ar- and -CH). Anal. Calcd for $C_{24}H_{18}N_4O$ (378.44): (C, 76.17; H, 4.79; N, 14.81); Found (C, 75.89; H, 4.19; N, 14.22).

3. RESULTS AND DISCUSSION:

3.1. Synthesis of pyranopyrazole, and pyrazole derivatives

2-(5-Oxo-4,5-dihydro-1*H*-pyrazol-3-yl)-*N*-phenylacetamide (**1a,b**) (Ali et al., 1979) were utilized as a key intermediate for the synthesis of novel pyranopyrazole derivatives **2** and pyrazole derivatives **3-6**.

Scheme 1: Synthesis of pyranopyrazole and pyrazole derivatives**.**

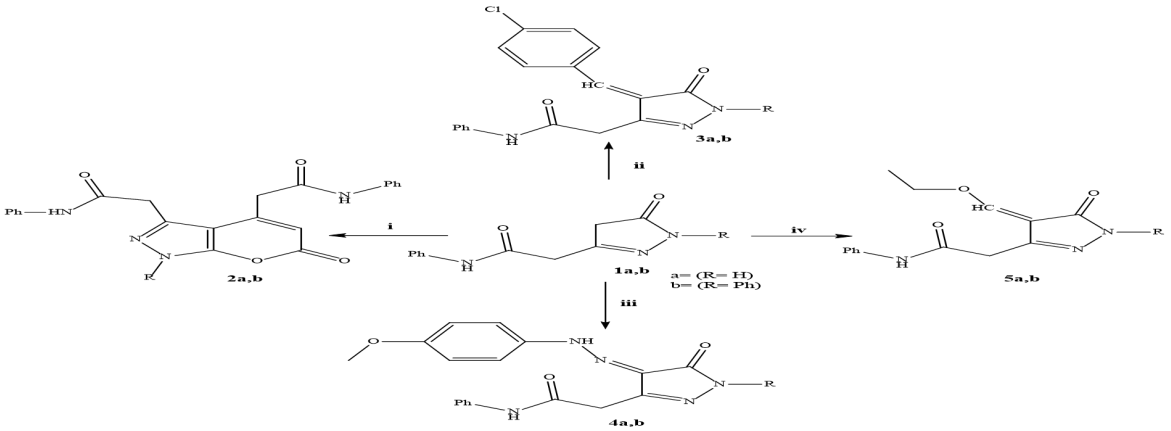
Compounds **1a,b** were allowed to react with 3-oxo-*N*^{1},*N*^{5}-diphenylpentanediamide in ethanolic sodium hydroxide solution where upon 2,2'-(6-oxo-1,6-dihydropyrano[2,3-*c*]pyrazole-3,4-diyl)bis(*N*-phenylacetamide) (**2a,b**) were formed (Scheme 1). Spectral data and analytical analysis confirmed the formation of the postulated structure (Experimental).

The reaction of compounds **1a,b** with p-chlorobenzaldehyde in ethanol containing a few drops of piperidine afforded 2-(4-(4-chlorobenzylidene) -5-oxo-4,5-dihydro- 1*H*-pyrazol-3-yl)-*N*-phenylacetamide (**3a,b**) (Scheme 1). The structure of the obtained products was established on the basis of elemental and spectral data. The IR spectra of **3a,b** displayed an -NH absorption band near 3263 - 3193 cm^{-1}, in addition to two carbonyl absorption bands at 1670 cm^{-1}.

The ^{1}H-NMR spectrum of compound **3a** showed the existence of two singlet signals at δ 10.44 and 9.92 corresponding to the two -NH groups of the amide and the pyrazole ring (D_2O exchangeable), singlet at δ 8.39 for the -CH- group, and doublet of doublet signal at δ 3.89 - 3.55 (J = 17.3 Hz, 10.6 Hz) corresponding to -CH_2 group. The aromatic protons signal appeared as a multiplet at δ 7.81 - 6.25 (9H, Ar-) in the case of **3a** and at δ 7.94 - 7.03 (14H, Ar-) for compound **3b**. The mass spectra of compounds **3a,b** were consistent with their structure (Experimental).

The coupling reaction of **1a,b** with diazotized p-anisidine in the usual manner afforded 2-(4-(2-(4-methoxyphenyl)hydrazineylidene)-5-oxo 4,5-dihydro-1*H*-pyrazol-3-yl)-*N*-phenylacetamide (**4a,b**) (Scheme 1).The IR spectra of compounds **4a,b** exhibited a strong (*NH*) absorption band at 3297 - 3289 cm^{-1}, besides other absorption bands near 1658 cm^{-1} assignable to (C=O) groups. The ^{1}H-NMR spectrum of **4a** revealed the presence of three NH singlet signals (D_2O exchangeable) at δ 11.95, δ 11.74, and δ 10.28. The aromatic protons signal appeared at δ 7.62 - 7.06 (9H, Ar-), along with the -CH_2- and -CH_3 protons singlet signals at δ 3.67 and δ 3.14, respectively. Compound **4b** showed in its ^{1}H-NMR the presence of two singlet signals at δ 12.69 and 10.22 characteristic of the NH groups (D_2O exchangeable), a multiplet at δ 7.94 - 7.03 (14H, Ar-) corresponding to the aromatic protons, in addition to the signals of CH_2 and CH_3 groups at δ 4.08 and δ 2.88, respectively.

2-(4-(Ethoxymethylene)-5-oxo-4,5- dihydro-1*H*-pyrazol-3-yl)-*N*-phenylacetamide (**5a,b**) were easily obtained by the reaction of **1a,b** with triethyl orthoformate and acetic anhydride (Scheme 1). Spectral and microanalytical analyses of **5a,b** collectively confirmed their structure. The IR spectra of compounds **5a,b** revealed the presence of absorption bands at 3259 - 3290 (NH) and 1662 - 1650 cm^{-1} (C=O). In the ^{1}H-NMR spectrum of **5a,b** the occurrence of a quartet (CH_2 protons) and a triplet (CH_3 protons), signals added further confirmation for the formation of these ethoxy methylene derivatives. Besides, other characteristic protons signals were in accordance with their structure (Experimental).



Reagents and conditions: *(i) acetone dicarboxylic dianilide, NaOH, EtOH, Reflux, 6 h; (ii) 4-chlorobenzaldehyde, EtOH, piperidine, Reflux, 3 h; (iii) p-anisidine, $NaNO_2$, HCl then sod. acetate, EtOH, 0-5°C; (iv) T.F.A.A, Ac_2O, Reflux, 12 h*

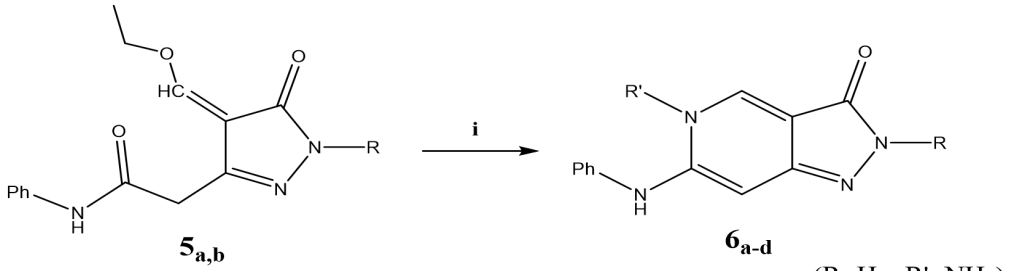
Refluxing a mixture of **5a,b** and hydrazine hydrate in absolute ethanol afforded 5-amino-6-(phenylamino)-2,5-dihydro-3*H*-pyrazolo[4,3-*c*]pyridin-3-one (**6a,b**) (Scheme 2). The identity of the obtained compounds was confirmed by spectral and elemental analysis. The IR spectrum of compound **6a** displayed absorption bands near 3286 cm^{-1} (NH_2), 3135 cm^{-1} (NH), and 1646 cm^{-1} (C=O). Meanwhile, compound **6b** showed characteristic bands at 3293, 3197, and 1650 cm^{-1} corresponding to NH_2, NH, and C=O groups, respectively. The ^{1}H-NMR spectrum of **6a** showed two singlet signals at δ 10.23 and 9.67 corresponding to the -NH- groups (D_2O exchangeable), singlet signal at δ 8.44 (1H, -CH), while the aromatic protons appeared as a multiplet at δ 7.88 - 7.06 (5H, Ar-). In addition, the singlet signals appearing at δ 7.46 for the CH group, and at δ 6.54 for the NH_2 group (D_2O exchangeable) were indicative of its structure. The ^{1}H-NMR spectrum of compound **6b** was in accordance with its structure (Experimental). The mass spectrum of **6b** exhibited a molecular ion peak at m/z= 317 (M^+, 59.93%), 302 (100%).

Scheme 2: Synthesis of compounds **6a-d.**

Alternatively, the reaction of compounds **5a,b** with aniline in place of hydrazine under the same conditions successfully produced 5-phenyl-6-(phenylamino)-2,5-dihydro-3*H* pyrazolo[4,3-c]pyridin-3-one (**6c,d**) (Scheme 2). The spectral and elemental data were in agreement with the structure of **6c,d**. The IR spectra of compounds **6c,d** displayed absorption bands near 3263 and 1666 cm^{-1} assigned to -NH- and C=O groups, respectively. The ^{1}H-NMR spectrum of **6c** showed the presence of two NH singlet signals at δ 11.23 and 9.97 (D_2O exchangeable), singlet at δ 8.44 (1H, -CH), in addition to a multiplet at δ 8.04 - 6.79 (10H, -Ar) representative of the aromatic

protons and singlet signal at δ 7.00 (1H, -CH). The mass spectrum of compound **6c** revealed a

molecular ion peak at m/z= 302 (M^+, 6.58%), 273 (18.29%), 245 (100%) in accordance with the given structure. The ^{1}H-NMR spectrum of compound **6d** was in complete agreement with its structure (Experimental).



Reagents and conditions: (i) hydrazine hydrate and/or aniline, EtOH, Reflux, 2 h.

3.2. Biological Evaluation

3.2.1. *In-vitro* antibacterial activity

The semi-quantitative disk diffusion

Table 1: In vitro antibacterial activity of compounds 1a,b, 2a,b, 3a,b, 4a,b, 5a,b, and 6a-d (Inhibition zone was in mm)[a].

Compounds	Antibacterial Activity							
	Bacterial Species (G^+)				Bacterial Species (G^-)			
	Bacillus subtilis		*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*	
	IZ	RA%	IZ	RA%	IZ	RA%	IZ	RA%
Control: DMSO	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Standard Chloramphenicol	25	100	40	100	29	100	30	100
1a	21	84	16	40	15	51.7	-	-
1b	19	76	38	95	20	68.9	-	-
2a	30	120	43	107.5	20	68.9	30	100
2b	30	120	30	75	32	110.3	22	73.3
3a	18	72	30	75	-	-	-	-
3b	29	116	40	100	17	58.6	18	60
4a	30	120	30	75	-	-	24	80
4b	22	88	31	77.5	30	103.4	23	76.7
5a	20	80	20	50	15	51.7	15	50
5b	22	88	39	97.5	23	79.3	33	110
6a	15	60	39	97.5	-	-	-	-
6b	32	128	32	80	14	48.3	30	100
6c	-	-	38	95	-	-	-	-
6d	32	128	29	72.5	24	82.8	26	86.7

[a] R.A. = Relative activity, RA= $\frac{\text{IZ of sample compound}}{\text{IZ of antibiotic}} \times 100$, IZ: Inhibition zone diameter (mm/mg sample).

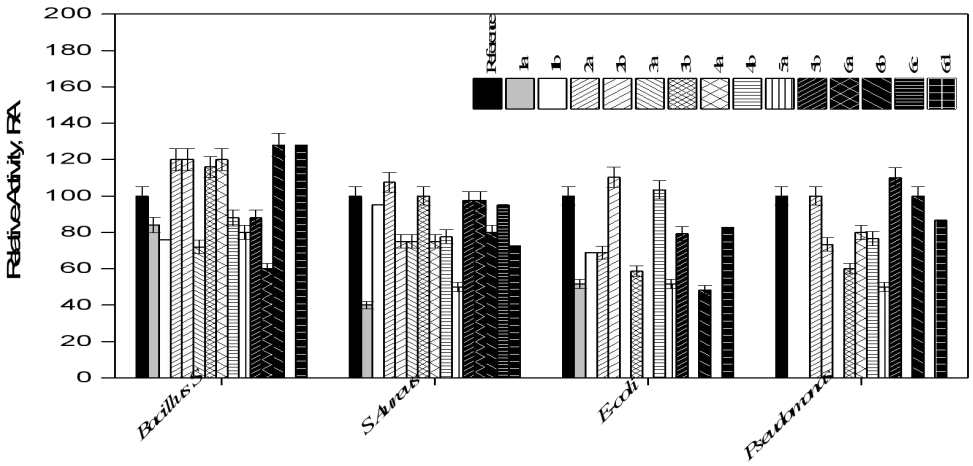


Figure 2. *In vitro* antibacterial activity of compounds *1a,b, 2a,b, 3a,b, 4a,b, 5a,b, and 6a-d* (Zone of inhibition in mm, and Reference bactericide is chloramphenicol).

Different derivatives of pyrazoles and pyranopyrazoles were synthesized and screened for their *in-vitro* antibacterial activity against gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus)* and gram-negative bacteria (*Escherichia coli, Pseudomonas aeuroginosa)*. The organisms were assessed against the activity of 50 mg/mL solutions of each compound and using the standard method for the antibacterial activity in which the inhibition zone (IZ) diameter in mm was measured. To evaluate the efficacy of the tested compounds a commercial bactericide chloramphenicol was used as a reference under the same conditions. The results in Table 1 depicted that **2a,b, 4a, 6b**,**d,** and **2a, 3b, 5b, 6b** displayed a high degree of inhibition against *Bacillus subtilis* and *Staphylococcus aureus*, respectively. Compounds **2b, 4b,** and **2a, 5b, 6b** were potent inhibitors against *Escherichia coli* and *Pseudomonas aeruginosa,* respectively. Compound **6c** exhibited no inhibition effect against *Bacillus subtilis*. Compounds **3a, 4a, 6a,c** and **1a,b, 3a, 5a, 6a,c** reflect no activity against *Escherichia coli* and *Pseudomonas aeruginosa*, respectively.

3.2.2 *In silico* molecular docking study

Autodock package software was used for performing molecular docking studies. The most stable conformers have been retained for each compound (Morris *et al*., 2009). The X-ray crystal structure of *E-coli* topoisomerase IV inhibitor was downloaded from https://www.rcsb.org/ (PDB ID: 3FV5) (Wei *et al*., 2010). The protein-ligand complex, used in the docking study was prepared in the usual manner: (1) first, the enzyme was 3D protonated; (2) Co-crystallized water molecules were deleted; (3) determination and isolation of the binding pocket took place. Discovery Studio Visualizer was used for visual analysis of complex interactions then the results were validated using Autodock-tools.

Molecular docking method was performed to determine the different modes of the binding

affinities of the synthesized compounds into the binding site of the topoisomerase IV by calculating their estimated free binding energies, the intermolecular H-bonds number along with the type and the number of interactions that may be formed with the amino acids of the topoisomerase IV into its binding site (Table 2).

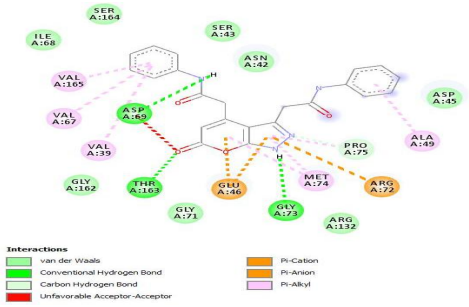
Table 2: Binding free energies, hydrogen bonds, number of interactions of the closest residues, and the docked synthesized compounds into the binding site of topoisomerase IV.

Compounds	Estimated Free binding energy (kcal/mol)	H-Bonds (HBs)	Number of interactions between the amino acids and the docked compounds into the binding site
1a	-6.84	3	6
1b	-6.99	1	8
2a	-8.74	3	15
2b	-9.44	2	14
3a	-7.62	2	11
3b	-7.84	1	11
4a	-7.32	1	15
4b	-10.87	1	16
5a	-6.60	3	13
5b	-7.32	2	10
6a	-5.21	2	6
6b	-6.35	1	7
6c	-5.84	2	5
6d	-6.30	-	7
Sulfaphenazole	-6.86	1	9
Levofloxacin	-6.55	2	9

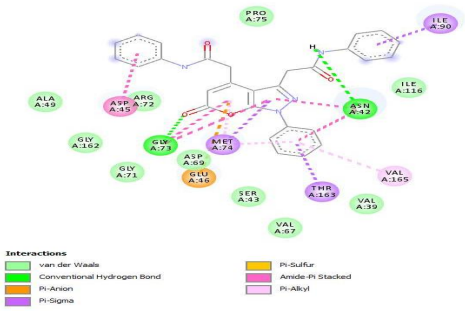
Docking studies were performed for all synthesized compounds. The synthesized compounds were well fit into the binding site of topoisomerase IV. The resulting complexes were found to be stable, and the binding energies were in the range of -5.21 to -10.87 kcal/mol (Table 2). The negative binding energies indicated that the inhibition of topoisomerase IV by those compounds was thermodynamically favorable. The docking of subset **1a**/**1b** at the topoisomerase IV active site showed that **1a** binds with the essential amino acids.

Furthermore, compound **1a** formed three H-bonds with ASP69 through the *N*-atom of the amide group with 4.14 Å bond length, VAL39 through *N*- of the pyrazole ring (3.18 Å), and with VAL165 through the C=O of the amide group (3.76 Å). Meanwhile, compound **1b** formed one H-bond with ASP69 through the *N*-atom of the amide with a bond length equal to 4.44 Å. A higher topoisomerase IV inhibition effect was observed for compound **1b** over compound **1a**, which could be interpreted by the presence of the phenyl group instead of the H atom of NH in the pyrazole ring. Subset **2a**/**2b** showed that compound **2a** formed three H-bonds with ASP69 through *NH* of the amide group with a bond length equal to 4.69 Å, GLY73 through NH of the pyrazole ring with 3.75 Å bond length, and THR163 with C=O of the pyran ring (3.95 Å) (Figure 3). On the other hand, compound **2b** revealed two H-bonds with ASN42 and GLY73 through *N*-atom of the amide and C=O of the pyran ring with 5.04 Å, and 3.26 Å bond length, respectively, with docking score (-9.44 kcal/mol) compared to (-

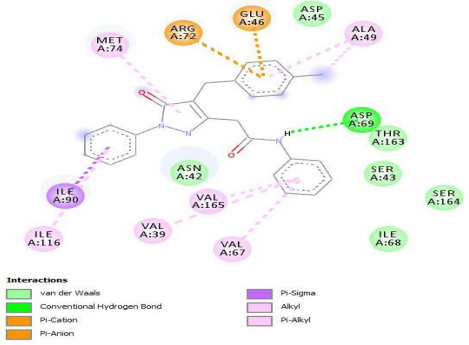
8.74 kcal/mol) for compound **2a** (Table 2). Another subset **3a/3b,** which was formed by the replacement of -NH- of the pyrazole ring with *N-Ph*, showed that **3a** formed two H-bonds with GLU46 and ARG72 through -NH- and C=O of the pyrazole ring with bond lengths equal to 4.61 and 3.27 Å, respectively. Moreover, compound **3b** appeared the existence of one H-bond with ASP69 through the -NH- of the amide group (4.36 Å) (Figure 3). The docked subset **4a/4b** revealed that compound **4a** binds through the -NH- group of the pyrazole ring with the essential amino acids forming one H-bond through GLU46 with 5.11 Å bond length. Meanwhile, compound **4b** formed one H-bond with ASN42 through the N=N group (4.63 Å) (Figure 3). In the case of subset, **5a/5b** three H-bonds in **5a** were formed, and two H-bonds in **5b**. Compound **5a** binds through C=O of the amide group forming two H-bonds with GLY73 (3.37 Å) and THR163 (4.04 Å), in addition to one H-bond through the NH group of the amide with THR163 (3.89 Å).



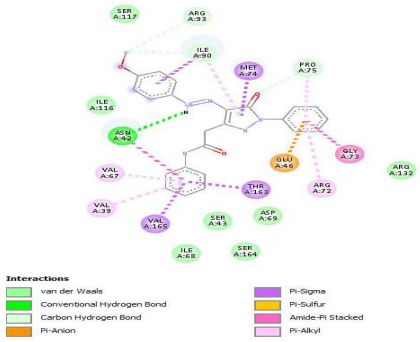
2a



2b



3b

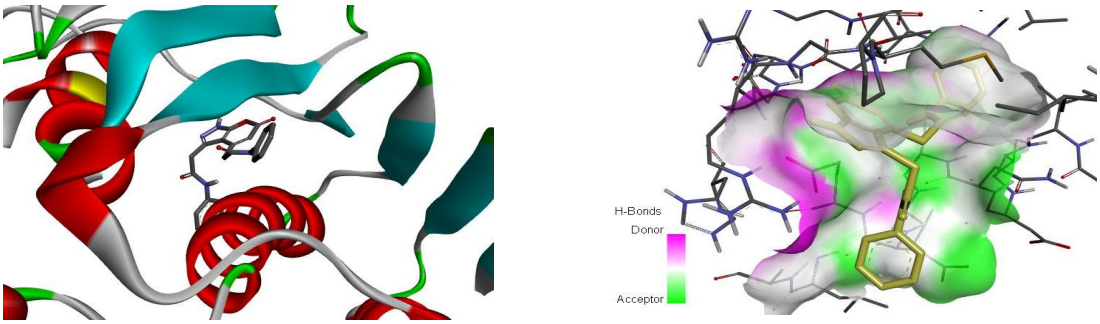


4b

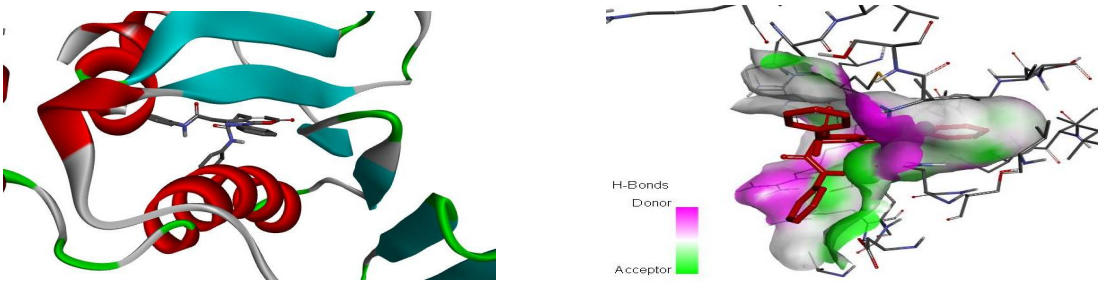
Figure 3. 2D-Closest interactions between active site residues of topoisomerase IV and the synthesized compounds 2a, 2b, 3b, and 4b.

The docked subsets **6a/6c** and **6b/6d** revealed that **6a** formed two H-bonds with SER43 and ASP69 through the NH_2 group with bond lengths equal to 3.10 Å and 4.51 Å, respectively. Furthermore, compound **6c** revealed two H-bonds with SER43 (4.44 Å) and ASP69 (4.67) through NH of the pyrazole ring. On the other hand, compound **6b** binds through the *NH_2* group forming one H-bond with ASN42 (3.57 Å) in the subset **6b/6d**, while compound **6d** showed no H-bonds with the active site of topoisomerase IV. Compared to one H-bond through VAL67 with a bond length equal to 3.38 Å for Sulfaphenazole, as well as two H-bonds with ILE90 (3.48 Å) and ILE116 (4.56 Å) for Levofloxacin with a docking score equal to -6.55 kcal/mol. The docking

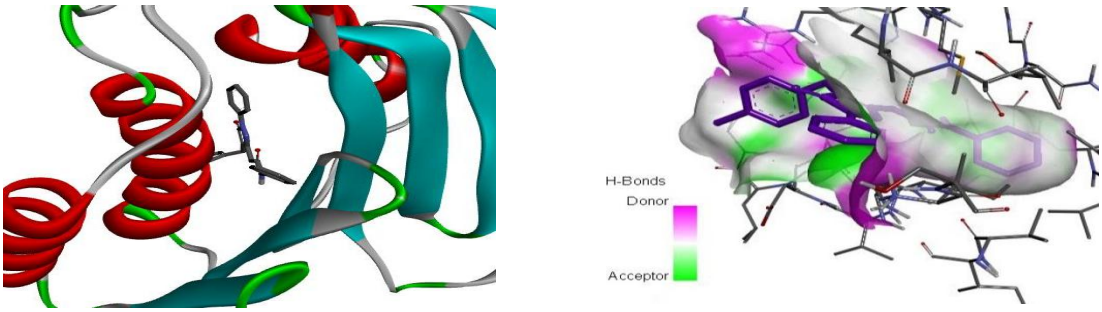
results showed that compounds **2a** and **4b** have high antibacterial activity as well as compounds **6c** and **6a** have low antibacterial activity against *E. coli,* which was in agreement with the *in vitro* study. The H-bonding and 3D structure of the synthesized compounds **2a**, **2b**, **3b,** and **4b** inside the active site of topoisomerase IV is represented in Figure 4.



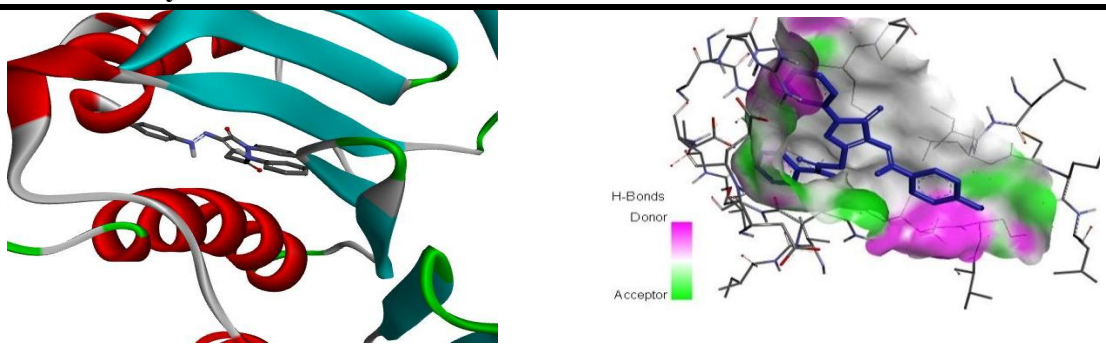
2a



2b



3b



4b

Figure 4. H-bonding and 3D structure of the synthesized compounds **2a**, **2b**, **3b,** and **4b** inside the active site of topoisomerase IV.

4. Conclusion:

A new set of pyrazoles and pyranopyrazole derivatives were designed and synthesized. All newly synthesized compounds were screened for their antibacterial activity in comparison with chloramphenicol as a reference. The biological data showed that **2a,b, 4a, 6b**,**d,** and **2a, 3b, 5b, 6b** displayed a high degree of inhibition against *Bacillus subtilis* and *Staphylococcus aureus*, respectively. Compounds **2b, 4b, 2a, 5b, 6b** were potent inhibitors against *Escherichia coli* and *Pseudomonas aeruginosa,* respectively. Docking studies were performed for all synthesized compounds and the results indicated that the synthesized compounds were well fit into the binding site of topoisomerase IV. The resulting complexes were found to be stable, and the binding energies were in the range of -5.21 to -10.87 kcal/mol. The negative binding energies indicated that the inhibition of topoisomerase IV by those compounds was thermodynamically favorable. It could be conducted that pyrazoles and pyranopyrazoles constitute a basic nucleus in the discovery of new potent and safe antibacterial candidates. Furthermore, the data obtained from the theoretical calculations indicated that the synthesized compounds were promising candidates for further development of novel therapeutic and biomedical precursors.

Conflict of interest: no conflict of interest.

Declarations of interest: none.

Data availability statement: The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

4.REFERENCES:

Ali, M. I., & El-Morsy, M. M. S. 1979. Reactions with acetonedicarboxylic dianilides. I. Reactions at the Carbonyl Group. *Journal für Praktische Chemie, 321*(6), 1047-1052. doi:https://doi.org/10.1002/prac.19793210622

Amnerkar, N. D., & Bhusari, K. P. 2010. Synthesis, anticonvulsant activity and 3D-QSAR study of some prop-2-eneamido and 1-acetyl-pyrazolin

derivatives of aminobenzothiazole. *European Journal of Medicinal Chemistry, 45*(1), 149-159. doi:https://doi.org/10.1016/j.ejmech.2009.09.037

Bao, H., Zhang, Q., Zhu, Z., Xu, H., Ding, F., Wang, M., . . . Yan, Z. 2017. BHX, a novel pyrazoline derivative, inhibits breast cancer cell invasion by reversing the epithelial-mesenchymal transition and down-regulating Wnt/β-catenin signalling. *Scientific Reports, 7*(1), 9153. doi:10.1038/s41598-017-09655-7

Bekhit, A. A., Nasralla, S. N., El-Agroudy, E. J., Hamouda, N., El-Fattah, A. A., Bekhit, S. A., . . . Ibrahim, T. M. 2022. Investigation of the anti-inflammatory and analgesic activities of promising pyrazole derivative. *European Journal of Pharmaceutical Sciences, 168*, 106080. doi:https://doi.org/10.1016/j.ejps.2021.106080

Berghot, M. A., & Moawad, E. B. 2003. Convergent synthesis and antibacterial activity of pyrazole and pyrazoline derivatives of diazepam. *European Journal of Pharmaceutical Sciences, 20*(2), 173-179. doi:https://doi.org/10.1016/S0928-0987(03)00162-3

Biswas, S. K., & Das, D. 2022. One-pot synthesis of pyrano [2, 3-c] pyrazole derivatives via multicomponent reactions (MCRs) and their applications in medicinal chemistry. *Mini-Reviews in Organic Chemistry, 19*(5), 552-568. doi:https://doi.org/10.2174/1570193X19666211220141622

Chakraborty, S., Paul, B., De, U. C., Natarajan, R., & Majumdar, S. 2023. Water–SDS–[BMIm] Br composite system for one-pot multicomponent synthesis of pyrano [2, 3-c] pyrazole derivatives and their structural assessment by NMR, X-ray, and DFT studies. *RSC advances, 13*(10), 6747-6759. doi:10.1039/D3RA00137G

Ebenezer, O., Shapi, M., & Tuszynski, J. A. 2022. A Review of the Recent Development in the Synthesis and Biological Evaluations of Pyrazole Derivatives. *Biomedicines, 10*(5), 1124. doi:https://doi.org/10.3390/biomedicines10051124

Emtiazi, H., Sharif, S. A., & Ardestani, M. 2022. Synthesis, Biological Screening and Docking Study of Some Novel Pyrazolopyrano[2,3-B]quinolin Derivatives as Potent Antibacterial Agents. *Current Bioactive Compounds, 18*(5), 58-65. doi:http://dx.doi.org/10.2174/1573407218666220106122432

Ganta, R. K., Kerru, N., Maddila, S., & Jonnalagadda, S. B. 2021. Advances in Pyranopyrazole Scaffolds' Syntheses Using Sustainable Catalysts—A Review. *Molecules, 26*(11). doi:10.3390/molecules26113270

H**avrylyuk, D., Zimenkovsky, B., Vasylenko, O., Zaprutko, L., Gzella, A., & Lesyk, R. 2009.** Synthesis of novel thiazolone-based compounds containing pyrazoline moiety and evaluation of their anticancer activity. *European Journal of Medicinal Chemistry, 44*(4), 1396-1404. doi:https://doi.org/10.1016/j.ejmech.2008.09.032

Jeschke, P. 2016. Progress of modern agricultural chemistry and future prospects. *Pest Management Science, 72*(3), 433-455. doi:https://doi.org/10.1002/ps.4190

Joshi, S. D., Dixit, S. R., Kirankumar, M. N., Aminabhavi, T. M., Raju, K. V. S. N., Narayan, R., . . . Yang, K. S. 2016. Synthesis, antimycobacterial screening and ligand-based molecular docking studies on novel pyrrole derivatives bearing pyrazoline, isoxazole and phenyl thiourea moieties. *European Journal of Medicinal Chemistry, 107*, 133-152. doi:https://doi.org/10.1016/j.ejmech.2015.10.047

Kahriman, N., Haşimoğlu, Z., Serdaroğlu, V., Beriş, F. Ş., Barut, B., & Yaylı, N. 2017. Synthesis of Novel Pyrazolines, Their Boron–Fluorine Complexes, and Investigation of Antibacterial, Antioxidant, and Enzyme Inhibition Activities. *Archiv der Pharmazie, 350*(2), 1600285. doi:https://doi.org/10.1002/ardp.201600285

Karrouchi, K., Radi, S., Ramli, Y., Taoufik, J., Mabkhot, Y. N., Al-Aizari, F. A., & Ansar, M. h. 2018. Synthesis and pharmacological activities of pyrazole derivatives: A review. *Molecules, 23*(1), 134. doi:https://doi.org/10.3390/molecules23010134

Maddila, S., Jonnalagadda, B. S., Gangu, K. K., & Maddila, N. S. 2017. Recent Advances in the Synthesis of Pyrazole Derivatives Using Multicomponent Reactions. *Current Organic Synthesis, 14*(5), 634-653. doi:http://dx.doi.org/10.2174/1570179414666161208164731

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. 2009. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of computational chemistry, 30*(16), 2785-2791.

Musa, A., Ihmaid, S. K., Hughes, D. L., Said, M. A., Abulkhair, H. S., El-Ghorab, A. H., . . . El-Agrody, A. M. 2023. The anticancer and EGFR-TK/CDK-9 dual inhibitory potentials of new synthetic pyranopyrazole and pyrazolone derivatives: X-ray crystallography, in vitro, and in silico mechanistic investigations. *Journal of Biomolecular Structure and Dynamics*, 1-15. doi:10.1080/07391102.2023.2167000

Naim, M. J., Alam, O., Nawaz, F., Alam, M. J., & Alam, P. 2016. Current status of pyrazole and its biological activities. *Journal of pharmacy bioallied sciences, 8*(1), 2. doi: 10.4103/0975-7406.171694

Nassar, E. 2010. Synthesis,(in vitro) antitumor and antimicrobial activity of some pyrazoline, pyridine, and pyrimidine derivatives linked to indole moiety. *J. Am. Sci, 6*(8), 463-471.

Nguyen, H. T., Truong, M.-N. H., Le, T. V., Vo, N. T., Nguyen, H. D., & Tran, P. H. 2022. A New Pathway for the Preparation of Pyrano[2,3-c]pyrazoles and molecular Docking as Inhibitors of p38 MAP Kinase. *ACS Omega, 7*(20), 17432-17443. doi:10.1021/acsomega.2c01814

Olajuyigbe, O., & Ashafa, A. 2014. Chemical composition and antibacterial activity of essential oil of Cosmos bipinnatus Cav. leaves from South Africa. *Iranian journal of pharmaceutical research: IJPR, 13*(4), 1417-1423. doi:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4232809/

Sadashiva, R., Naral, D., Kudva, J., Shivalingegowda, N., Lokanath, N.

K., & Pampa, K. J. 2017. Synthesis, spectral, biological activity, and crystal structure evaluation of novel pyrazoline derivatives having sulfonamide moiety. *Medicinal Chemistry Research, 26*(6), 1213-1227. doi:10.1007/s00044-017-1838-5

Sadeghpour, M., & Olyaei, A. 2021. Recent advances in the synthesis of bis(pyrazolyl)methanes and their applications. *Research on Chemical Intermediates, 47*(11), 4399-4441. doi:10.1007/s11164-021-04592-7

Sarkar, A., Rakshit, S., & Bhattacharya, S. C. 2017. Interpreting the effect of confined cyclodextrin media on the FRET efficacy between Naproxen and a bio-active 3-pyrazolyl-2-pyrazoline derivative on the light of spectroscopic investigation appended by TD-DFT simulations and molecular docking analysis. *Journal of Photochemistry and Photobiology A: Chemistry, 343*, 77-84. doi:https://doi.org/10.1016/j.jphotochem.2017.04.024

Varghese, B., Al-Busafi, S. N., Suliman, F. O., & Al-Kindy, S. M. 2017. Unveiling a versatile heterocycle: pyrazoline–a review. *RSC advances, 7*(74), 46999-47016. doi:10.1039/C7RA08939B

Wei, Y., Charifson, P., & Letiran, A. 2010. Crystal Structure of E. coli Topoisomerase IV co-complexed with inhibitor, Structures from the PDB. 10.2210/pdb3FV5/pdb.

دراسة حول تخليق، توصيف، النشاط كمضادات بكتيريا، والنمذجة الجزيئية لبعض مشتقات البيرازول والبيرانوبيرازول

محمود محمد عبدالعاطى[1]، زينب رمضان فرج*[1]، أيمن محمد يوسف[1]، عبدالمنعم عبدالسلام مخلوف[1]
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مستخلص البحث

تم تطوير بروتوكول سهل لتحضير مركبات عضوية جديدة باستخدام طرق غير سامة ، بسيطة، وصديقة للبيئة. حيث تم تخليق مجموعة من المركبات التي تحتوي على نواة البيرازول و البيرانوبيرازول باستخدام 2- (5-أوكسو -4 ، 5-ثنائي هيدرو -1H-بيرازول-3-يل) -N- فينيل أسيتاميد (1 أ ، ب) كمادة أولية. تم اثبات تراكيب المواد المحضرة {البيرانوبيرازول 2 ومشتقات البيرازول 6-3 } باستخدام الطرق الطيفية المختلفة (مثل طيف الاشعة تحت الحمراء و الرنين النووى المغناطيسى والتحليل الطيفي الكتلي) بالإضافة إلى تحاليل العناصر. تم تقييم المركبات المحضرة فيما يتعلق بنشاطها كمضاد للبكتيريا في المختبر ضد سلالات بكتيرية مختلفة ممرضة للإنسان مثل الإشريكية القولونية ، المكورات العنقودية الذهبية ، الزائفة الزنجارية ، والبكتيريا العصوية الرقيقة مع الكلورامفينيكول كأحد المضادات البكتيرية التجارية كمرجع. أظهرت النتائج ان معظم المركبات المختبرة لها نشاطًا واعدًا (عالية الى معتدلة) كمضادًات للبكتيريا. تم إجراء النمذجة الجزيئية باستخدام برنامج Autodock-4 لتحديد الأنماط المختلفة للتفاعلات الترابطية والتي يمكن أن تحدث بين الربيطة المختارة ligand والبروتين المستهدف. من المفترض أن النتائج التي تم الحصول عليها يمكن أن توفر معلومات قيمة لتصميم وتطوير وتصنيع المركبات حلقية غير المتجانسة جديدة لها تطبيقات دوائية كمواد مضادة للبكتيريا.