Research Journal of Chemical Sciences ISSN 2231-606X
Vol. **12(2),** 21-24, June **(2022)** *Res. J. Chem. Sci.*

Synthesis, characterization, molecular docking and antibacterial evaluation of 3-aryl-1- phenyl-1*H*-pyrazole- 4-carbaldehyde-acylhydrazones

Sangeetha Rao
KLE's S. Nijalingappa College, Rajajinagar, Bengaluru-10, India
sgrao6591@gmail.com

Available online at: www.isca.in, www.isca.me
Received 12th March 2021, revised 19th November 2021, accepted 3rd April 2022

Abstract

In view of fostering research on efficacious antimicrobials, a series of new 3-aryl-1-phenyl-1H-pyrazole-4-carbaldehyde acylhydrazones were synthesized in three steps commencing with the reaction of substituted acetophenones with phenyl hydrazine to yield respective Schiff bases. The respective formyl pyrazoles were synthesized from the Schiff bases by subjecting them to Vilsmeier-Haack formylation. The recrystallized formyl pyrazoles were then treated with substituted benzoic acid hydrazides in ethanol medium with acetic acid as the catalyst to obtain 3-Aryl-1-phenyl-1H-pyrazole-4-carbaldehyde acylhydrazones (4a-4e). The pyrazole derivatives were assessed for their effective inhibitory properties against bacterial DNA Gyrase B enzymes by molecular docking studies which were then evaluated for their in vitro antibacterial activities.

Keywords: Schiff bases, synthesis, DNA Gyrase B, Molecular docking, Vilsmeier-Haack, Antibacterial.

Introduction

Pyrazole derivatives play an enunciated role in heterocyclic chemistry and serve as a pivotal moiety in the field of medicinal chemistry. Literature reports indicate the broad spectrum of biological activities of the compounds containing pyrazole nucleus such as anti-inflammatory, analgesic, antifungal and antibacterial properties[1-6]. Hence, these compounds have evoked the contemplation of synthetic chemists on account of their strikingly interesting biological activities. Drugs containing 1,3-diaryl pyrazole moieties have been occupying a prominent position in the list of best selling pharmaceutical agents. This adroit nature of pyrazole makes it as a best option for its incorporation in the compound, thus targeting effective drug design and development[7].

The variably substituted hydrazones have drawn attention of synthetic chemists due to their wide range of applications in biological, clinical and analytical areas[8-10]. Apart from being a versatile intermediate in organic synthesis, the azomethine functional group present in the molecule imparts properties responsible for exhibiting biological activities by these compounds[11].

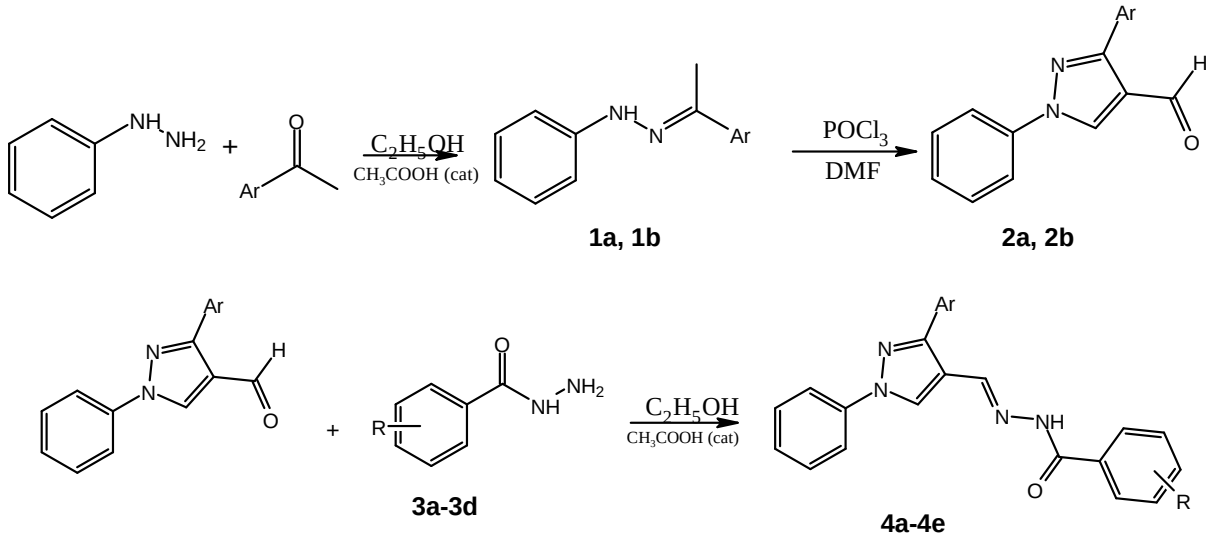
Materials and methods

The analytical grade chemicals were purchased from Sigma-Aldrich India. IR spectrum was recorded on Shimadzu FT-IR Infrared spectrometer and ^{1}H-NMR (400 MHz) spectrum was recorded at 400MHz. Mass spectrum was recorded using Shimadzu LCMS-8030 mass spectrometer and VARIO-EL-III Elemental Analysensysteme GmBH was used to obtain elemental analysis data. Molecular modelling studies were carried out using Schrődinger Suite 2015-2.

Synthesis of 3- aryl- 1- phenyl- 1*H*- pyrazole - 4-carbaldehyde- acylhydrazones Synthesis of substituted phenyl hydrazones (1a, 1b): Phenyl hydrazine and substituted aryl ketones (0.01 mol each) [4-acetyl biphenyl/2-acetyl naphthalene] were refluxed for 4 hours in ethanol using a few drops of acetic acid which serves as the catalyst. The solid was collected by filtration, dried and recrystallized in a mixture of ethanol and dimethyl formamide.

Synthesis of 3-Aryl-1-phenyl-1*H*-pyrazole-4-carbaldehydes (2a, 2b): $POCl_3$ solution was added drop wise to DMF at 0^0C to prepare the Vilsmeier-Haack reagent. The solution of phenyl hydrazine in DMF was added portion wise to the above reagent with constant stirring. The reaction was carried out under reflux and stirring conditions with the temperature maintained at 80^0C. The completion of the reaction was checked using thin layer chromatography, after which the mixture was cooled and plunged into crushed ice to obtain the solid product. This was followed by neutralization by saturated sodium bicarbonate solution, which yielded the solid product after filtration.

Synthesis of 3-Aryl-1-phenyl-1*H*-pyrazole-4-carbaldehyde-acylhydrazones (4a-4e): Equimolar mixture of 3-Aryl-1-phenyl-1*H*-pyrazole-4-carbaldehydes and substituted benzoic acid hydrazides were refluxed in ethanol for 4-5 hours using acetic acid as the catalyst. The solid separated was filtered and dried to obtain the crude products which were then recrystallized using suitable solvents (Scheme-1).



Scheme-1: Synthetic scheme for the preparation of 3-Aryl-1-phenyl-1*H*-pyrazole-4-carbaldehyde-acylhydrazones.

Compound	R	Ar	Compound	R	Ar
1a, 2a	-	Biphen-4-yl	4a	3-Br	Biphen-4-yl
1b, 2b	-	Naphthalen-2-yl	4b	4-Br	Biphen-4-yl
3a	t-But	-	4c	2,4-di-Cl	Biphen-4-yl
3b	4-F	-	4d	2,4-di-Cl	Naphthalen-2-yl
3c	2-Br	-	4e	2-Br	Naphthalen-2-yl
3d	2,4-di-Cl	-			

Results and discussion

Characterization of the synthesized compounds: The IR Spectrum exhibited distinctive absorption due to N-H group at 3050cm^{-1}. The spectrum also displayed characteristic absorption bands corresponding to the carbonyl and azomethine groups in the molecule. The ^{1}H-NMR data displayed the presence of a sharp singlet at downfield region (10.11ppm) due to the highly deshielded N-H proton. The pyrazole ring proton resonated in the downfield region of about 8.5ppm. These two peaks represented the characteristic functional groups in the molecule. The aromatic protons constituting the naphthyl/biphenyl ring, phenyl and substituted phenyl ring resonated in the range 7.3-7.8ppm which represents the aromatic region. The azomethine proton resonated within the aromatic region.

4a: Yield:78%, Bright yellow solid, Melting point: 210-212^{0}C, IR (ν, cm^{-1}): 3051 (N-H), 2923 (CH-aryl), 1707 (C=O), 1606 (C=N); ^{1}H-NMR ($CDCl_3$) (400 MHz) δ ppm: 7.38-7.94 (m, 18H, Ar-H); 7.82 (HC=N-); 8.55 (Pyrazole-H), 10.11 (N-H); m/z: 520.1 (M^+) and 522.1 (M^{++}); Anal. Calc. (Found) for $C_{29}H_{21}N_4BrO$: C, 66.79 (66.72); H, 4.03 (4.05); N, 10.74 (10.71).

4b: Yield:73%, Orange yellow solid, Melting point: 280-282^{0}C, IR (ν, cm^{-1}): 3045 (N-H), 2908 (CH-aryl), 1707 (C=O), 1606 (C=N); ^{1}H-NMR ($CDCl_3$) (400 MHz) δ ppm: 7.38-7.94 (m, 18H, Ar-H); 7.82 (HC=N-); 8.56 (Pyrazole-H), 10.11 (N-H); m/z:520.1 (M^+) and 522.1 (M^{++}) ;Anal. Calc. (Found) for $C_{29}H_{21}N_4BrO$: C, 66.79 (66.74); H, 4.03 (4.01); N, 10.74 (10.75).

4c: Yield:81%, Pale yellow solid, Melting point: 226-228^{0}C, IR (ν, cm^{-1}): 3040 (N-H), 2919 (CH-aryl), 1702 (C=O), 1617 (C=N); ^{1}H-NMR ($CDCl_3$) (400 MHz) δ ppm: 7.25-7.93 (m, 17H, Ar-H); 7.83 (HC=N-); 8.56 (Pyrazole-H), 10.11 (N-H); m/z: 511.3 (M^+) and 513.5 (M^{++}) ;Anal. Calc. (Found) for $C_{29}H_{21}N_4Cl_2O$: C, 68.10 (68.11); H, 3.91 (3.93); N, 10.95 (10.93).

4d: Yield:78%, Yellow solid, Melting point: 224-226^{0}C, IR (ν, cm^{-1}): 3045 (N-H), 2923 (CH-aryl), 1702 (C=O), 1606 (C=N); ^{1}H-NMR ($CDCl_3$) (400 MHz) δ ppm: 7.25-8.30 (m, 17H, Ar-H); 7.91 (HC=N-); 8.76 (Pyrazole-H), 9.76 (N-H); m/z: 485.3 (M^+) and 487.3 (M^{++}) ;Anal. Calc. (Found) for $C_{29}H_{18}N_4Cl_2O$: C, 66.80 (66.81); H, 3.71 (3.73); N, 11.54 (11.57).

4e: Yield:71%, Pale orange, Melting point: 232-234^{0}C, IR (ν, cm^{-1}): 3045 (N-H), 2929 (CH-aryl), 1702 (C=O), 1612 (C=N); ^{1}H-NMR ($CDCl_3$) (400 MHz) δ ppm: 7.27-8.34 (m, 17H, Ar-H); 7.80 (HC=N-); 8.73 (Pyrazole-H), 9.92 (N-H); m/z: 495.2 (M^+) and 495.2 (M^{++}) ;Anal. Calc. (Found) for $C_{29}H_{19}N_4BrO$: C, 65.45 (65.42); H, 3.83 (3.84); N, 11.31 (11.30).

In view of developing better antimicrobial agents, the synthesized compounds were subjected to docking into the

active pocket of 2 bacterial enzymes: 24kDa domain of *Escherichia coli* DNA Gyrase B (PDB ID: 4DUH) and 24kDa domain of *Staphylococcus aureus* DNA Gyrase B (PDB ID: 4URM). In addition to the high binding energy, the docking simulations exhibited distinct ligand-enzyme non-covalent interactions such as hydrogen bonding, π-π stacking and π-cation interactions between the compounds and the amino acid residues in the active site of the enzyme. The docking scores (in kcal/mol) of the compounds are depicted in Table-1.

Table-1: Results of docking of the compounds 4a-4e with the DNA Gyrase B enzyme of *E. coli* (PDB ID: 4DUH).

Compound	Docking Score (kcal/mol)
4e	-7.288
4c	-5.653
4b	-5.372
4a	-5.068
4d	-4.621

Compound 4e with the best docking score exhibited 1 hydrogen bond and 1 π-cation interaction with Val 120 and Lys 103 residues in the active pocket. Figure-1 depicts the 2D interaction diagram of this compound withthe enzyme.

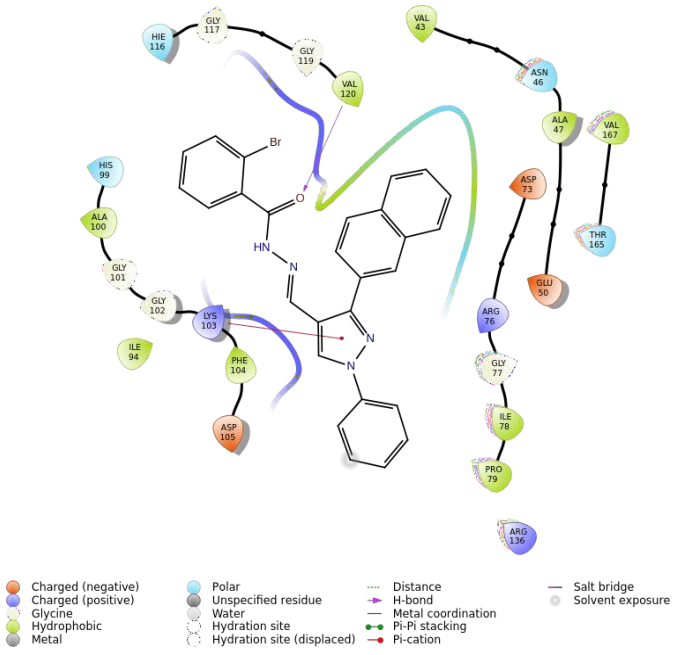


Figure 1: 2D interaction diagram depicting interactions between compound **4e** with the active pocket of DNA Gyrase B enzyme of *Escherichia coli* (PDB ID: 4DUH)

The compounds were also docked into the active pocket of *Staphylococcus aureus* DNA Gyrase B enzyme (PDB ID: 4URM). The prominent ligand-protein interactions observed were π- cation, π-π stacking and hydrogen bonds. The docking scores of the compounds are represented in Table-2.

Table-2: Results of docking of the compounds **4a-4e** with the DNA Gyrase B enzyme of *Staphylococcus aureus* (PDB ID: 4URM).

Compound	Docking Score
4a	-7.891
4e	-7.717
4c	-6.642
4d	-6.380
4b	-5.333

In view of validating the results of the docking studies, the antibacterial evaluation was carried out against the selected bacterial strains: *Staphylococcus aureus* MTCC-7433, *Bacillus subtilis* MTCC-441, *Pseudomonas aeruginosa* MTCC-424 and *Klebsiella pneumoniae* MTCC-139. The compounds exhibited remarkable antibacterial properties, the results of which are depicted in Table-3.

Table-3: Results depicting antibacterial activity of the synthesized compounds 4a-4e with respect to Tetracycline expressed in terms of zone of inhibition (mm)

Compound	*S. aureus* MTCC-7433	*B. subtilis* MTCC-441	*K. pneumoniae* MTCC-139	*P. aeruginosa* MTCC-424
4a	18.0	21.0	19.5	18.5
4b	22.0	20.5	19.5	19.0
4c	16.0	17.5	20.0	21.0
4d	23.0	16.5	18.5	19.0
4e	20.0	17.5	19.5	18.0
Tetracycline	26.0	22.0	25.0	23.0

Conclusion

Pyrazole tethered heterocyclic compounds were synthesized with the view of developing new antimicrobials. The compounds were characterized by IR, Mass and ^{1}H-NMR spectroscopic techniques. The molecular docking studies revealed distinct non-covalent interactions with the target proteins which included hydrogen bond, π-cation and π-π stacking interactions. The docking results were validated with

the *in vitro* antibacterial analysis. The results of the analysis illustrated augmented antibacterial activity of the tested compounds.

Acknowledgement

The author acknowledges KLE's S. Nijalingappa College for the research facilities.

References

1. Rahimizadeh, M., Pordel, M., Bakavoli, M., Rezaeian, S., Sadeghian, A. (2009). Synthesis and antibacterial activity of some new derivatives of pyrazole. *World J. Microbiol. Biotechnol.*, 26, 317-321. https://doi.org/10.1007/s11274-009-0178-0

2. Desai, N. C., Rajpara, K. M. and Joshi, V. V. (2013). Synthesis of pyrazole encompassing 2-pyridone derivatives as antibacterial agents. *Bioorg. Med. Chem. Lett.* 23 (9), 2714-2717. https://doi.org/10.1016/j.bmcl. 2013.02.077

3. Zhang, C., Liu, X., Wang, B., Wang, S. and Li, Z. (2010). Synthesis and antifungal activities of new pyrazole derivatives via 1,3-dipolar cycloaddition reaction. *Chem. Biol. Drug Des.*, 75(5), 489-493. https://doi.org/10.1111/j.1747-0285.2010.00948.x

4. Taher, A. T., Sarg, M. T. M., El-Sayed Ali, N. R., Elnagdi, N. H. (2019). Design, synthesis, modelling studies and biological screening of novel pyrazole derivatives as potential analgesic and anti-inflammatory agents. *Bioorg. Chem.* 89, 103023. https://doi.org/10.1016/j.bioorg.2019.103023

5. Bekhit, A. A. and Abdel-Aziem. T. (2004). Design, synthesis and biological evaluation of some pyrazole derivatives as anti-inflammatory-antimicrobial agents. *Bioorg. Med. Chem.* 12 (8), 1935-1945. https://doi.org/10.1016/j.bmc.2004.01.037

6. Bekhit, A. A., Ashour, H. M. A., Ghany, Y. S. A., Bekhit, A. E. A. and Baraka, A. (2008). Synthesis and biological evaluation of some thiazolyl and thiadiazolyl derivatives of 1*H*-pyrazole as anti-inflammatory antimicrobial agents. *Eur. J. Med. Chem.,* 43(3), 456-463. https://doi.org/10.1016/j.ejmech.2007.03.030

7. Da Costa, L., Scheers, E., Coluccia, A., Casulli, A., Roche, M., Di Giorgio, C., Neyts, J., Terme, T., Cirilli, R., La Regina, G., Silvestri, R., Mirabelli and C., Vanelle, P. (2018). Structure-Based drug design of potent pyrazole derivatives against Rhinovirus replication. *J. Med. Chem.* 61 (18), 8402-8416. https://doi.org/10.1021/acs.jmedchem. 8b00931

8. Vicina, P., Zani, F., Cozzini, P. and Doytchinova, I. (2002). Hydrazones of 1,2-benzisothiazole hydrazides: synthesis, antimicrobial activity and QSAR investigations. *Eur. J. Med. Chem.* 37 (7), 553-564. https://doi.org/10.1016/S0223-5234(02)01378-8

9. Pieczonka, A. M., Strzelczyk, A., Sadowska, B., Mloston, G., Staczek, P. (2013). Synthesis and evaluation of antimicrobial activity of hydrazones derived from 3-oxido-1H-imidazole-4-carbohydrazides. *Eur. J. Med. Chem.* 64, 389-395. https://doi.org/10.1016/j.ejmech.2013.04.023

10. Metwally, K. A., Abdel-Aziz, L. M., Lashine, E. S., Husseiny, M. I. and Badawy, R. H. (2006). Hydrazones of 2-aryl-quinoline-4-carboxylic acid hydrazides: Synthesis and preliminary evaluation as antimicrobial agents. *Bioorg. Med. Chem*., 14(24), 8675-8682. https://doi.org/10.1016/j.bmc.2006.08.022

11. Odabasoglu, M., Albayrak, C., Ozkanca, R., Aykan, F. Z. and Lonecke, P. (2007). Some polyhydroxy azo-azomethine derivatives of salicylaldehyde: Synthesis, characterization, spectroscopic, molecular structure and antimicrobial activity studies. 840(1-3), 71-89. https://doi.org/10.1016/j.molstruc.2006.11.025