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SYNTHESIS AND CHARACTERIZATION OF PHENOLPHTHALEIN HYDRAZIDES WITH ANTIMICROBIAL ACTIVITIES

Shazia Kousar[*1], Aneela Ghafoor[2], Muhammad Ahmad Mudassir[*3], Syed Adnan Ali Shah[*4], Muhammad Shahid[5], Madiha Irfan[6], Ayesha Monawar[7]

[*1,2,6,7]*Institute of Chemistry Khwaja Fareed University of Engineering and Information Technology (KFUEIT), Rahim Yar Khan 64200, Pakistan*
[*3]*Chemistry Department, University of Management and Technology (UMT), Sialkot Campus Sialkot, Pakistan*
[*4]*Faculty of Pharmacy, Universiti Teknologi MARA Selangor, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia*
[5]*Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan*

[*1]shazia.kousar@kfueit.edu.pk, [*3]ma.mudassir@kfueit.edu.pk, [*4]syedadnan@uitm.edu.my

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Corresponding Author: *
Shazia Kousar,
Muhammad Ahmad Mudassir,
Syed Adnan Ali Shah

Abstract

The synthesis of innovative organic compounds has gained significant attention due to their diverse biological activities. Hydrazides have emerged as promising candidates due to their pharmacological relevance, biological potential, and commercial applicability. Previously, various hydrazide derivatives have been synthesized using simple hydrazines. To our knowledge, none of the earlier research used phenyl hydrazines to synthesize hydrazide analogs. Leveraging this knowledge gap, novel phenolphthalein hydrazides were synthesized via reactions of substituted phenyl hydrazine derivatives in an ethanol solvent. The successful formation of all analogs was monitored by thin-layer chromatography and confirmed through UV, IR, and NMR spectroscopic analyses. The synthesized derivatives exhibited antibacterial properties, especially against Staphylococcus aureus (Gram-positive) bacteria. The application test was designed as a prototype investigation to provide preliminary results, underscoring the great potential of these phenolphthalein hydrazides as anticancer, anti-inflammatory, antioxidant, and antidiabetic agents, bioimaging probes, pH-sensitive drug carriers, enzyme inhibitors, and metal chelators for therapeutic applications.

INTRODUCTION

Although the advancements in science and technology significantly benefit humanity, they are also associated with severe environmental challenges due to rapid industrialization driven by increasing populations and energy demands (Mudassir *et al.*, 2021; Mudassir *et al.*, 2023). Rapid environmental pollution affecting land, air, and water poses severe consequences for the sustainable development of the earth. The ecological imbalance adversely affects society's health, mainly contributing to the spread of infectious diseases worldwide (Mudassir *et al.*, 2021). Contemporary healthcare requirements have urged the discovery of new biologically active agents. Various cutting-edge materials, including polymers, metal/metal oxide nanoparticles, graphene analogs, and nanocomposites, have been developed to explore

their potential biomedical applications (Mudassir *et al.*, 2017; Mudassir *et al.*, 2018; Mudassir *et al.*, 2019; Akhter *et al.*, 2024; Batool *et al.*, 2024). However, unforeseen complications, such as antibiotic resistance, have necessitated the development of novel pharmaceuticals and compounds with improved biological functions (Batool *et al.*, 2024; Kousar *et al.*, 2025). The research community expressed great interest in synthesizing such molecules to test potential biological actions (Khan *et al.*, 2017).

The development of novel triazole, benzopyran, coumarin, indole-cyanoacetic acid, phenoxazine, and sulfonamide derivatives with antifungal, antibacterial, antiviral, antihaemolytic, anticancer, antioxidant, anti-inflammatory, and antitubercular activities has garnered substantial interest and medicinal value (Khilkovets *et al.*, 2022; Alani *et al.*, 2024; Citarella *et al.*, 2024; Nazeri *et al.*, 2024; Salma *et al.*, 2024; Yaman *et al.*, 2024). Likewise, the 2′,7′-dichlorofluorescein, thioamidoalkyl fluorescein, and fluorescein hydrazide analogs have attracted considerable attention for their applications in oxidative stress measurement, cellular imaging, fluorescent labeling, medical diagnostics, and bioanalytical applications (Kousar *et al.*, 2022; Joshi *et al.*, 2024; Kousar *et al.*, 2024; Kousar *et al.*, 2025).

Additionally, acid hydrazides and their derivatives have been recognized as beneficial for various pharmacological, biological, and commercial applications. Furthermore, phenolphthalein derivatives have emerged as viable options for developing therapeutic compounds exhibiting cytotoxicity potential.[19] Considering the bioactive potential of phenolphthalein and acid hydrazides, we aimed to synthesize phenolphthalein hydrazides to explore their biological performance as antimicrobial agents.

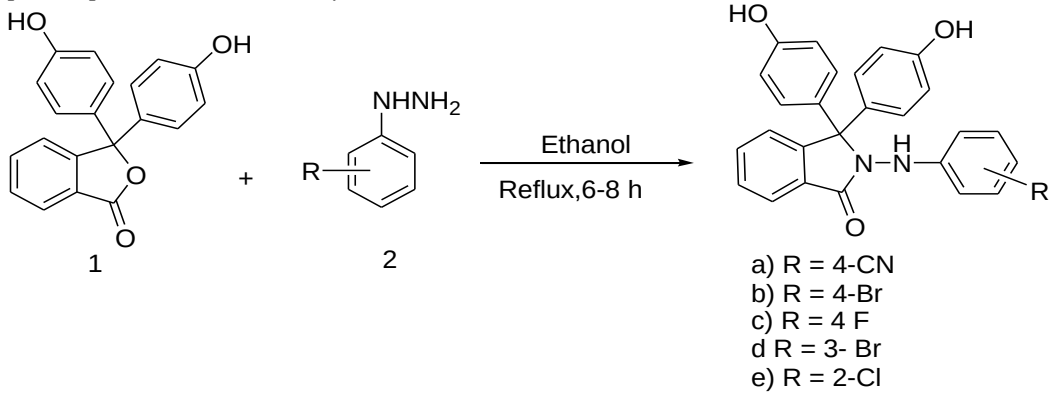
2. EXPERIMENTAL

2.1 Instruments and Chemicals

All the chemicals required to synthesize the desired compounds were of analytical grade and used without further purification. Phenolphthalein was obtained from Merck (Germany), whereas various phenyl hydrazine substituted compounds and solvents such as ethanol, methanol, n-hexane, and dichloromethane were purchased from Sigma Aldrich (USA). TLC was performed on silica-coated TLC plates to monitor reaction progress using DCM and n-hexane (4:6) mobile phase. TLC plates were then visualized under an ultraviolet lamp at 254 and 366 nm. The successful synthesis of all the compounds was verified by an Ultraviolet-visible spectrophotometer (UV–vis, Shimadzu UV-1800). The Fourier transform infrared (FTIR) spectra were recorded by (FTIR, Agilent 630 series) spectrometer for functional group identification. The ^{1}HNMR and ^{13}CNMR spectra were obtained with the help of a Bruker Avance DPX FT-NMR spectrometer.

2.2. Synthesis

In a round-bottom flask, 2 millimoles of phenolphthalein were refluxed with different substituted phenyl hydrazine at 75-85 °C using ethanol as a solvent for 6-8 hours, as illustrated in **Scheme 1**. The reaction mixture was filtered off and washed with n-hexane, and dried to produce targeted substances.



Scheme 1. The synthesis of phenolphthalein hydrazides by its reactions with phenyl hydrazines.

3 RESULTS AND DISCUSSION

3.1. Characterization

3.1.1. UV/Visible Spectra

In UV–vis spectra, all compounds exhibited λ_{max} values ranging from 239 to 242 nm due to π-π^* transitions, indicating the presence of an aromatic benzene ring acting as a chromophore. Additionally, they demonstrated n-π^* transitions at 280 nm and 280-290 nm, highlighting the presence of secondary amine and C=O groups, respectively. Substituents that act as auxochromes, such as CN, Br, and Cl, accounted for the transitions observed at 224 nm, 211 nm, and 209.5 nm, respectively.

3.1.2. IR Spectra

The IR spectra of all the compounds were examined to recognize their functional groups. However, we are discussing the results of only two compounds herein. For AN-P1, a strong peak at 1628 cm^{-1} confirmed the presence of C=O stretching of amide. The distinctive stretching of C=C at 1493 cm^{-1}, C-N at 1340 cm^{-1}, C-H at 2820 cm^{-1}, N-H at 3340 cm^{-1}, O-H at 3224 cm^{-1}, and C≡N at 2250 cm^{-1} validated its synthesis. For AN-P5, a prominent peak at 1670 cm^{-1}, C-Cl stretching at 1110 cm^{-1}, and C-N stretching at 1175 cm^{-1} confirmed its formation.

3.1.3. NMR Spectra

^{1}HNMR of AN-P1 confirmed its formation with a broad signal at 9.7 ppm, reflecting the presence of the NH proton. Due to para substitutions, aromatic protons (8H) on phenyl groups with OH emerged as doublets at 6.5-8 ppm. Their appearance in deshielded areas may be mainly associated with the ring current effect of the aromatic rings. A multiplet was observed at 7-8 ppm due to protons (4H) on the isoindolinone molecule, resulting from ortho and meta coupling. In contrast, the protons on benzonitrile appeared as doublets due to para coupling. Since the CN group, an electron-withdrawing entity, deshields the proton, these peaks were observed in the deshielded region at 7-8 ppm.

4-{[1,1-bis(4-hydroxyphenyl)-3-oxo-2,3-dihydro-1H-isoindol-2-yl]amino}benzonitrile (AN-P1):

Yellowish brown precipitates; Yield 82%; R_f 0.63; Yield 82%; mp > 300°C; UV/Vis (nm) 244, 280, 284, 224; IR (KBr) (v, cm^{-1}) 3224, 3340, 2250, 1612, 1516, 1490; ^{1}HNMR (500 MHz, DMSO-d_6) δ 9.7 (s, 1H), 6.7-7 (dd, 8H, J = 7.3 Hz) 7.69-7.9 (m, 3H, J_{ortho}= 7.7Hz, J_{meta} = 2.5Hz), 9.6 (s, 1H), 4.3 (s, 1H) ^{13}CNMR (125 MHz, DMSO-d_6) 115.1, 128.1, 129.4, 131.1, 157.4, 152.5, 133.4, 134.7, 169.1.

2-[(4-bromophenyl)amino]-3,3-bis(4-hydroxyphenyl)-2,3-dihydro-1H-isoindol-1-one (AN-P2):

Brown precipitates; Yield 83%; R_f 0.61; mp > 300°C; UV/Vis (nm) 242, 280, 286, 210; IR (KBr) (v, cm^{-1}) 3248, 1608, 1512, 1489, 1210, 659; ^{1}HNMR (500 MHz, DMSO-d_6) δ 9.67 (s, 2H, broad), 6.75-6.78 (dd, 8H, J = 7.6Hz), 7.6-7.9 (m, 3H, J_{ortho} = 7.9Hz, J_{meta} = 2.3 Hz), 9.6(s, 1H, broad), 4.29 (s, 1H); ^{13}CNMR (125 MHz, DMSO-d_6) 115.4, 125.2, 128, 131.1, 134.7, 152,157.4,169.

2-[(4-fluorophenyl)amino]-3,3-bis(4-hydroxyphenyl)-2,3-dihydro-1H-isoindol-1-one (AN-P3):

Brownish-yellow precipitates; Yield 86%; R_f 0.63; mp > 300°C; UV/Vis (nm) 241, 280, 284; IR (KBr) (v, cm^{-1}) 3212, 1618, 1542, 1492, 1320; ^{1}HNMR (500 MHz, DMSO-d_6) δ 2.5 (s, solvent peak), 6.75-7 (m, 8H, J = 7.3Hz), 7-7.1 (dd, 4H, J = 7.8), 7.6-7.8 (m, 4H, $J_{H.H(ortho)}$ = 7.1Hz, $J_{H.H(meta)}$ = 2.1Hz), 4.3 (s, 1H), 3.2-3.4 (s, 1H), 9.6 (s, 1H); ^{13}CNMR (125 MHz, DMSO-d_6) 116.1, 129.7, 131.4, 137.6, 158.6. 153.3, 159.2, 168.1.

2-[(3-bromophenyl)amino]-3,3-bis(4-hydroxyphenyl)-2,3-dihydro-1H-isoindol-1-one (AN-P4):

Brown precipitates; Yield 81%; R_f 0.62; mp > 300°C; UV/Vis (nm) 242, 280, 283, 210; IR (KBr) (v, cm^{-1}) 3261, 1602, 1518, 1491, 1216, 659; ^{1}HNMR (500 MHz, DMSO-d_6) δ 9.67 (s, 2H, broad), 6.75-6.78 (dd, 8H, J = 7.7Hz), 7.85-7.9 (m, 3H J_{ortho} = 7.4Hz, J_{meta} = 2.4Hz), 9.6 (s, 1H), 4.09 (s, 1H); ^{13}CNMR (125 MHz, DMSO-d_6) 116.4, 115.9, 126.4, 128, 157.4, 123.4, 137.6, 134.4, 156,131.1, 112.2, 129.5, 169.

2-[(2-chlorophenyl)amino]-3,3-bis(4-hydroxyphenyl)-2,3-dihydro-1H-isoindol-1-one (AN-P5):

Fade yellow precipitates; Yield 79%; R_f 0.62; mp > 300°C; UV/Vis (nm) 244, 280, 287, 209; IR (KBr) (v, cm^{-1}) 3232, 1710, 1537, 1490, 1350, 775; ^{1}HNMR (500 MHz, DMSO-d_6) δ 9.68 (s, 2H, broad), 6.75-6.8 (dd, 8H, J = 7.8Hz), 7.60-7.65 (m, 3H, J = 8.3Hz), 9.6 (s, 1H), 3.5 (s, 1H); ^{13}CNMR (125 MHz, DMSO-d_6) 115.1, 125.2, 129.4, 131.1, 157.4, 152.5, 128, 169.

3.2. Biological Activities

The biological activities of the obtained products have been examined for their antibacterial properties.

3.2.1. Antibacterial Activity

The disc diffusion methodology has been employed to estimate the antibacterial properties of all compounds. Although the compounds were biologically active, they demonstrated less activity than positive control against *Staphylococcus aureus* (Gram-positive) and *Proteus mirabilis* (Gram-negative). Also, they were found to be comparatively more potent against *Staphylococcus aureus* than *Proteus mirabilis*. Amongst them, AN-P1 and AN-P5, hydrazides with 4-cyano and 2-chloro substitutions, respectively, exhibited reasonable activity against *Staphylococcus aureus* strains, with 17 mm inhibition. Likewise, AN-P2 and AN-P3, with 4-bromo and 2-chloro derivatives, respectively, showed better activity against *Proteus mirabilis* with 7 mm inhibition, as given in **Figure 1**.

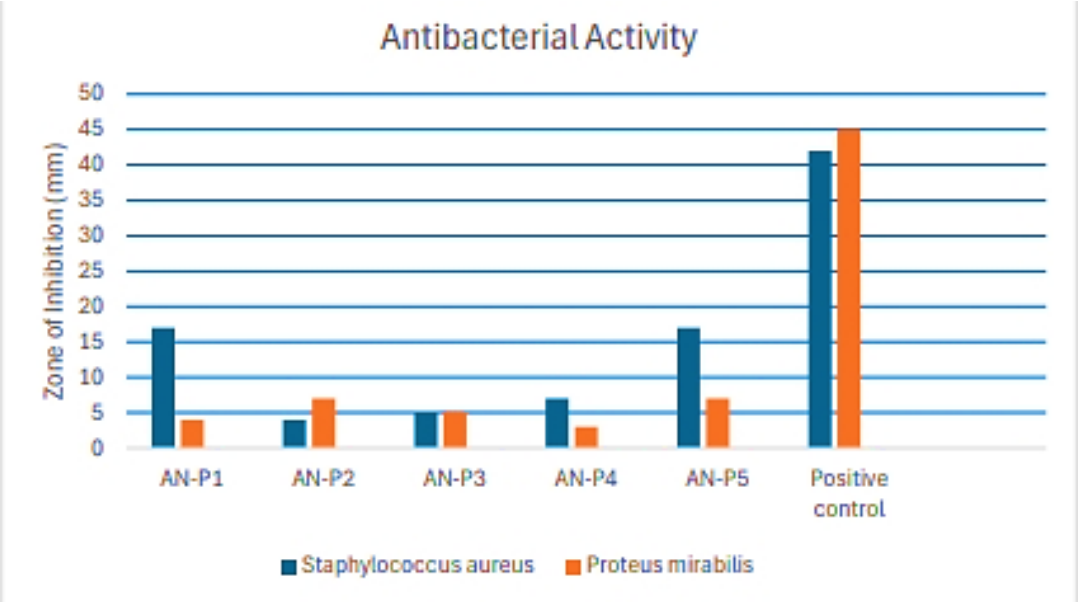


Figure 1. Antibacterial activities of phenolphthalein hydrazide derivatives.

5.CONCLUSION

The hydrazides of phenolphthalein were synthesized using various phenyl hydrazine derivatives. Spectroscopic techniques confirmed the presence of the desired product. They were found to be biologically active, demonstrating antibacterial potential, especially against Gram-positive bacteria. Therefore, these compounds may be utilized as antimicrobial agents. Furthermore, this method can facilitate the preparation of more valuable novel compounds.

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