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Synthesis, Characterization, and Biological Activity Study of New Heterocyclic Compounds †

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Abstract: The synthesis of novel heterocyclic compounds is achieved through a multi-step process involving azo dye (**S1**), ester (**S2**), and hydrazide (**S3**). Initially, azo dye (**S1**) is synthesized through the reaction between resorcinol and p-aminobenzoic acid. Subsequently, ester (**S2**) is formed by reacting azo dye (**S1**) with concentrated sulfuric acid. Hydrazide (**S3**) is then synthesized by reacting ester (**S2**) with 80% hydrazine hydrate. Further reactions of hydrazide (**S3**) with various anhydrides (maleic anhydride, phthalic anhydride, 3-nitrophthalic anhydride, and succinic anhydride) result in cyclization facilitated by acetic acid, yielding six-membered heterocyclic compounds. Additionally, compound **S3** undergoes cyclization with acetyl acetone, ethyl acetoacetate, methyl acetoacetate, and diethyl malonate to produce five-membered heterocyclic compounds. The biological activity of these synthesized compounds is also investigated. Characterization of the prepared compounds is performed using techniques such as Fourier-Transform Infrared Spectroscopy (FT-IR), Proton Nuclear Magnetic Resonance (^{1}HNMR), Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR), and Elemental Analysis (CHNS).

Keywords: resorcinol; azo dyes; pyridazine; phthalazine; pyrazole; biological activity

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1. Introduction

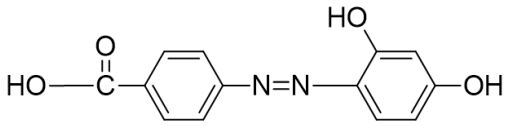
Azo dyes serve multiple biological functions, encompassing roles as antineoplastic, antidiabetic, cleansing, and anti-inflammatory agents, among other chemotherapeutic applications. These compounds are not only highly colored, making them useful as dyes and pigments, but also exhibit significant environmental resilience, electrical, and optical properties [1–3]. Heterocyclic compounds, particularly those with multiple ring members, have gained prominence in both pharmacological and industrial applications. For instance, 1,3,4-oxadiazoles are versatile heterocyclic compounds employed in the synthesis of medicines, polymers, and pigments [4–6]. Hydrazide–hydrazone derivatives have garnered considerable attention due to their promising biological properties, such as antibacterial and anticancer activities [7–10]. These derivatives play a pivotal role in the fields of organic and medicinal chemistry. Similarly, pyridazine derivatives exhibit a broad spectrum of biological activities, including anti-inflammatory, antibacterial, anticancer, cardiovascular, and agrochemical properties [11–15]. Compounds such as pyrazole, pyrazolidine, and pyrazoline, along with their derivatives, have been the subject of extensive research due to their diverse potential biological activities. Literature reviews indicate that these derivatives possess a wide array of pharmacological effects, making them versatile molecules with numerous applications and biological features. For example, pyrazole and some of its derivatives have been identified as UV stabilizers or emitters [16].

2. Materials and Methods

2.1. Chemicals

All chemicals were sourced from CDH and Merck Company, ensuring a purity level exceeding 99%.

2.2. Synthesis of Compound **S1**



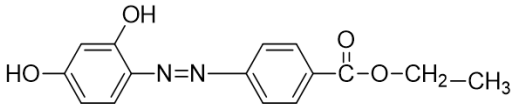
P-amino benzoic acid (0.01 mole, 1.37 g) was dissolved in 17 mL of distilled water and 3 mL of HCl, maintaining a temperature range of 0–5 °C. To this solution, sodium nitrite ($NaNO_2$, 0.01 mole, 0.69 g) dissolved in 10 mL of distilled water was added dropwise over a period of 15 min. The resulting diazonium salt solution was then added dropwise to a coupling component solution, prepared by dissolving resorcinol (0.01 mole, 1.10 g) and sodium hydroxide (1 g) in 10 mL of distilled water. The precipitate was subsequently filtered and rinsed with water [17].

IR (v, cm^{-1}): O-H (3267), C-O (1240), C-H Ar (3101), C=O carboxylic acid (1683), N=N (1423).

^{1}H-NMR (δ, ppm): (3H, Ar-H): 7.63–8.08, (2H, Ar-OH): 6.33–6.53, (H, COOH): 10.

^{13}C-NMR (δ, ppm): (39–40) for C-N, (151–161) for aromatic ring.

2.3. Synthesis of Compound **S2**



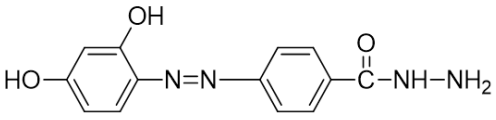
Compound **S1** (0.01 mole, 2.58 g) was dissolved in absolute ethanol. Concentrated sulfuric acid (25 mL) was then added dropwise to the solution. The mixture was refluxed overnight. Subsequently, the reaction mixture was poured into ice water, filtered, and rinsed with water [18].

IR (v, cm^{-1}): O-H (3410), C-H Ar (2982), C-H aliphatic (2904, 2937), C=O Ester (1716), C=C Ar (1516), N=N (1473).

^{1}H-NMR (δ, ppm): DMSO-d6 (solvent): (H, CH_2, H, CH_3): 1.32–1.36, (H, Ar-H): 7.67–8.09, (H, Ar-OH): 4.8–4.35.

^{13}C-NMR (δ, ppm): (14.16, 14.44) for C aliphatic, 39 for C-N, 60 for C-O, 103–132 for C=C, 153–165 for aromatic ring.

2.4. Synthesis of Compound **S3**



Compound **S2** (0.01 mole, 2.86 g) was dissolved in absolute ethanol. To this solution, 80% hydrazine hydrate (0.01 mole, 0.48 mL) was added dropwise. The mixture was re-

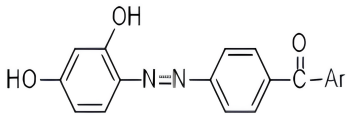
fluxed overnight and then poured into ice water to facilitate solvent evaporation [18].

IR (v, cm^{-1}): NH_2NH_2 (3180, 3223, 3346), C=O amide (1685), N=N (1599), O-H (3423), C=C Ar (1516).

^{1}H-NMR (δ, ppm): DMSO-d6 (solvent): (H, NH): 5.96, (H, NH_2): 7.65.

^{13}C-NMR (δ, ppm): 59 for C-N, 103–131 for C=C, 153–165 for aromatic ring.

2.5. Synthesis of Compounds **S4**–**S7**



Ar =(maleic anhydride , phthalic anhydride ,3-nitrophthalic anhydride ,succinic anhydride.)

Compound **S3** (0.01 mole, 2.72 g) was combined with 0.01 mole of maleic anhydride (0.98 g), phthalic anhydride (1.48 g), 3-nitrophthalic anhydride (1.93 g), and succinic anhydride (1 g), respectively, in acetic acid (0.57 mL). The mixtures were refluxed for 7 h, subsequently cooled, and then poured onto crushed ice. The resulting products were filtered and rinsed with water [4].

(**S4**) IR (cm^{-1}): NH (3309), C-H aliphatic (2980), C-H Ar (2929), C=O (1716), C=C (1521).

^{1}H-NMR (δ, ppm): (H, Ar-H): 7.52–8.13, (H, Ar-OH): 6.15–6.98, (H, NH): 7.25.

^{13}C-NMR (δ, ppm): 103–136 for aromatic ring, 155–172 for amide.

(**S5**) IR (cm^{-1}): NH (3317), C-H Ar (3082), C=O (1784), C=C (1510).

^{1}H-NMR (δ, ppm): (H, Ar-H): 7.14–8.13, (H, Ar-OH): 4.03–4.38, (H, NH): 6.49.

^{13}C-NMR (δ, ppm): 39.16–39.66 for C-N, 103, 108 for C=C, 123–136 for aromatic ring, 153–172 for amide.

(**S6**) IR (cm^{-1}): NH (3090), C-H aliphatic (2902), C-H Ar (2982), C=O (1786), C=C (1541).

^{1}H-NMR (δ, ppm): (H, Ar-H): 7.59–8.37, (H, Ar-OH): 4.014–4.38, (H, NH): 6.50.

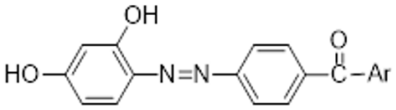
^{13}C-NMR (δ, ppm): 39.34–40.59 for C-N, 102, 106 for C=C, 118–145 for aromatic ring, 160–265 for amide.

(**S7**) IR (cm^{-1}): NH (3329), C-H aliphatic (2980), C-H Ar (3076), C=O (1707), C=C (1521).

^{1}H-NMR (δ, ppm): (H, Ar-H): 7.57–7.99, (H, Ar-OH): 4.28–4.31, (H, NH): 7.03.

^{13}C-NMR (δ, ppm): 26–40 for C-N, 103–136 for C=C, 153–162 for aromatic ring, 172, 174 for amide.

2.6. Synthesis of Compounds **S8**–**S11**



Ar = (acetyl acetone, ethyl acetoacetate, methyl acetoacetate ,diethylmalonate)

Mixtures of hydrazide (**S3**) (0.01 mole, 2.72 g) and 0.01 mole of acetyl acetone (1.025 mL), ethyl acetoacetate (1.26 mL), methyl acetoacetate (1.078 mL), and diethylmalonate (1.51 mL), respectively, in absolute ethanol were heated under reflux for 13 h. The reaction mixtures were then cooled, and the formed precipitates were filtered to yield the products [4].

(**S8**) IR (cm^{-1}): O-H (3350), C-H Ar (3024), C=O (1705), C=N (1602), C=C (1519).

^{1}H-NMR (δ, ppm): (H, Ar-H): 7.02–8.15, (H, Ar-OH): 5.16–7, (H, CH_3): 3.97–4.36, (H, aliphatic cyclic): 0.86–1.37.

^{13}C-NMR (δ, ppm): 18 for C aliphatic, 39–42 for C-N, 103, 108 for C=C, 117–130 for aromatic ring, 156–170 for amide.

(**S9**) IR (cm^{-1}): O-H (3348), C-H Ar (3057), C=O (1699), C=N (1602), C=C (1521).

^{1}H-NMR (δ, ppm): (H, Ar-H): 7.05–8.11, (H, Ar-OH): 5.99–6.59, (2H, CH_3): 4.10–4.34.

^{13}C-NMR (δ, ppm): 14 for C aliphatic, 39–40 for C-N, 103, 108 for C=C, 110–132 for aromatic ring, 143–165 for amide.

(**S10**) IR (cm^{-1}): O-H (3367), C-H Ar (3064), C=O (1701), C=N (1602), C=C (1518).

^{1}H-NMR (δ, ppm): (H, Ar-H): 7.66–8.10, (H, Ar-OH): 5.99, 6.59, (H, CH_3): 3.57–4.35, (H, aliphatic cyclic): 1.28, 1.35.

^{13}C-NMR (δ, ppm): 14 for C aliphatic, 39–40 for C-N, 103, 108 for C=C, 124–133 for aromatic ring, 148–165 for amide.

(**S11**) IR (cm^{-1}): O-H (3348), C-H Ar (3061), C=O (1714), C=N (1602), C=C (1518).

^{1}H-NMR (δ, ppm): (H, Ar-H): 7.62–8.12, (H, Ar-OH): 6.32–7.05, (H, CH_3): 3.98–4.35.

^{13}C-NMR (δ, ppm): 12–18 for C aliphatic, 34–39 for C-N, 103–108 for C=C, 118–132 for aromatic ring, 144–165 for amide.

Figure 1 shows reparation of compounds **S1**–**S11**.

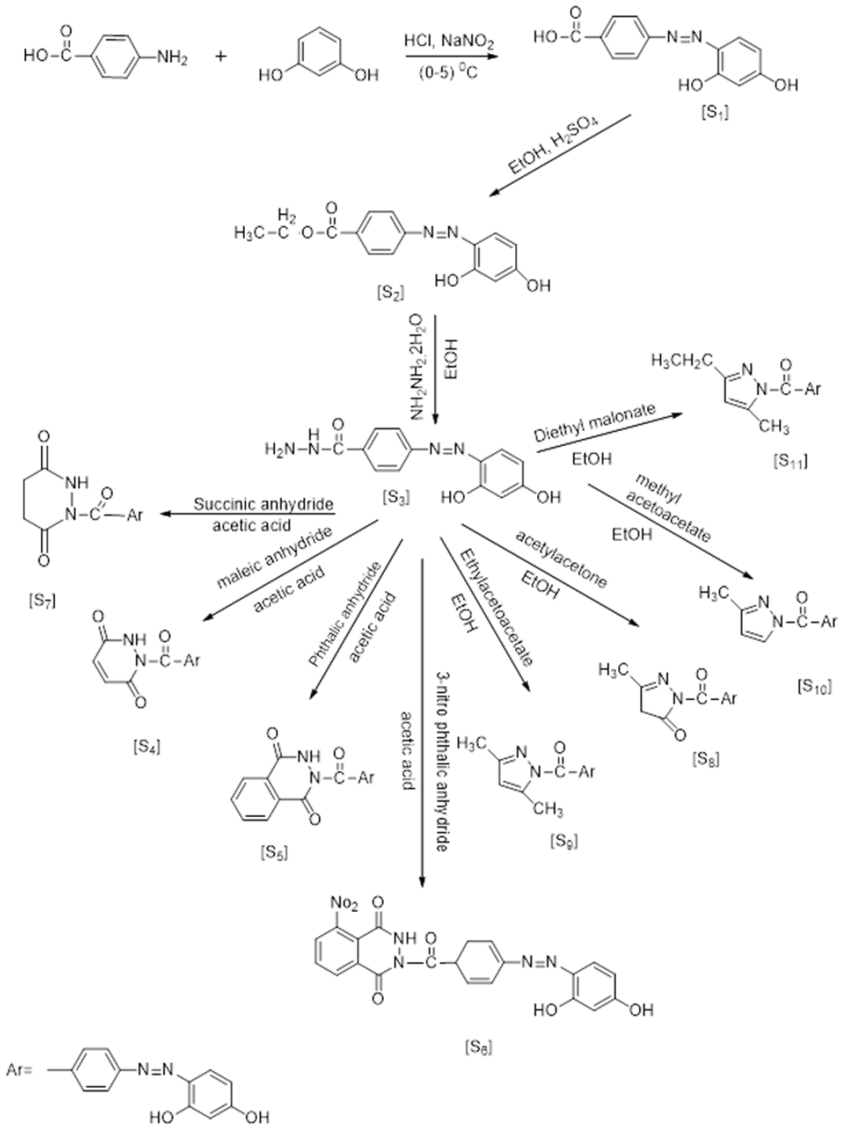


Figure 1. Preparation of compounds **S1**–**S11**.

3. Results and Discussion

P-amino benzoic acid reacted with resorcinol in the presence of HCl, $NaNO_2$, and NaOH at 0–5 °C to produce azo dye (**S1**). This azo dye subsequently reacted with concentrated H_2SO_4 to yield ester (**S2**), which further reacted with hydrazine hydrate to form hydrazide (**S3**).

The hydrazide derivatives were then reacted with maleic anhydride and phthalic anhydride, respectively, in acetic acid to produce pyridazin-3,6-dione and phthalazin-3,8-dione derivatives. Additionally, the hydrazide derivatives were reacted with acetyl acetone, ethyl acetoacetate, methyl acetoacetate, and diethylmalonate in absolute ethanol to produce pyrazole derivatives (**S8**–**S11**). The chemical structures of the synthesized compounds were characterized using various techniques, such as FT-IR, ^{1}H-NMR, ^{13}C-NMR, and CHNS analysis. The data from these measurements are presented in Tables 1 and 2.

Table 1. Physical properties of the synthesized compounds **S1**–**S11**.

Compound No.	MP (°C)	Yield (%)	Color	W (g/mol)	Molecular Formula
S1	195–200	93	Orange	258	$C_{13}H_{10}N_2O_4$
S2	184–189	93	Orange	286	$C_{15}H_{14}N_2O_4$
S3	75–80	85	Black	272	$C_{13}H_{12}N_4O_3$
S4	112–118	58	Light Brown	352	$C_{17}H_{12}N_4O_5$
S5	91–97	88	Dark Brown	402	$C_{21}H_{14}N_4O_5$
S6	120–124	81	Light Brown	449	$C_{21}H_{15}N_5O_7$
S7	138–142	36	Brown	354	$C_{17}H_{14}N_4O_5$
S8	120–125	16	Light Brown	338	$C_{17}H_{14}N_4O_4$
S9	107–110	30	Dark Brown	336	$C_{18}H_{16}N_4O_3$
S11	111–116	14	Dark Brown	350	$C_{19}H_{18}N_4O_3$

Table 2. C.H.N.S data of the prepared compounds **S1**–**S11**.

Compound No.	C (%)		H (%)		N (%)	
	Calculated	Measured	Calculated	Measured	Calculated	Measured
S1	54.545	53.22	3.496	3.94	21.70	9.23
S2	62.937	62.34	4.895	4.01	19.58	9.13
S3	57.352	57.81	4.411	4.82	20.588	20.11
S4	57.954	57.13	3.409	3.03	19.88	19.46
S5	62.682	61.48	3.482	3.94	17.412	17.01
S6	56.124	56.87	3.340	2.98	21.82	21.46
S7	57.627	56.93	3.954	4.22	19.77	19.33
S8	60.355	60.77	4.142	4.67	16.56	16.93
S9	64.285	63.88	4.761	4.70	16.666	16.54
S10	61.445	61.92	4.347	4.84	17.391	16.99

- **S1**: The FTIR spectrum exhibited a new absorption band at 1423 cm^{-1} corresponding to N=N and a disappearing absorption band at 3458–3360 cm^{-1} for NH_2. The ^{1}H-NMR spectrum showed signals for OH protons at 6.53.
- **S2**: The FTIR spectrum showed the disappearance of the C=O carboxylic acid band at 1683 cm^{-1} and the appearance of the C=O ester band at 1716 cm^{-1}. The ^{1}H-NMR spectrum displayed signals for -CH_2- protons at 1.36 and for -CH_3 protons at 1.32.
- **S3**: The FTIR spectrum indicated the absence of the absorption band for C=O ester at 1716 cm^{-1} and the appearance of a new absorption band for C=O amide at 1685 cm^{-1}. The ^{1}H-NMR spectrum showed signals at 7.65 for protons of -NH_2- and NH proton signals at 6.15.
- **S4**–**S7**: As shown in Figure 1, the FTIR spectrum revealed the disappearance of the two bands of the NH–NH_2 group in the region 3223–3423 cm^{-1} and the appearance of a band due to the N-H group in the range 3309–3317 cm^{-1}. Two carbonyl groups of compounds (**S4** and **S5**) appeared at 1716 and 1784 cm^{-1} for the amide carbonyl. The ^{1}H-NMR spectrum displayed signals for the -NH- proton.
- **S8**–**S11**: As depicted in Figure 1, the FTIR spectra showed the disappearance of NH_2 and NH bands in the region 3233–3346 cm^{-1} and the appearance of amide C=O bands at 1705 and 1699 cm^{-1}, respectively.

Additionally, C-H aliphatic appeared at 2928 cm^{-1} and C=C aromatic at 1519 cm^{-1}. The ^{1}H-NMR spectrum displayed signals for the -CH_3- proton in **S8**–**S11** and the -CH_2- proton in **S11**. The antibacterial activity of the synthesized compounds **S3**–**S11** is summarized in Table 3. The activity was assessed using the agar disc-diffusion method against both Gram-positive bacteria (Staphylococcus epidermidis and Staphylococcus aureus) and Gram-negative bacteria (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *E. coli*). Dimethyl sulfoxide (DMSO) served as the solvent control, and the concentration of the tested compounds was 10^{-3} M. The study revealed that compounds **S3** and **S8** exhibited

significant antibacterial activity [4]. This activity is attributed to the structural features of these compounds, which interact with the cell walls of the selected bacteria, resulting in high levels of inhibition.

Table 3. Antibacterial activity of compounds **S3**–**S11**.

Compound No.	*Pseudomonas*	*Klebsiella pneumoniae*	*E. coli*	*Staphylococcus aureus*	*Staphylococcus epidermidis*
S3	30	13	30	26	26
S4	20	10	20	14	25
S5	13	0	20	12	18
S6	15	9	13	13	25
S7	20	9	15	13	20
S8	27	12	26	11	0
S9	20	12	25	12	23
S10	18	11	13	15	13
S11	20	0	23	13	0

4. Conclusions

A robust and efficient method for the synthesis of azo dyes was established, offering several advantages including high yield rates, eco-friendly water-based solvents, reduced reaction times, and low-temperature conditions. This study also led to the successful synthesis of a series of novel heterocyclic compounds derived from hydrazide, which were characterized with high yield percentages. Comprehensive analytical techniques, such as FTIR, ^{1}HNMR, ^{13}CNMR, and CHNS Elemental Analysis, were employed to confirm the structure and composition of these compounds. The physical properties, including melting points and molecular weights, were systematically cataloged, providing valuable data for future research and applications. Notably, biological activity assays revealed that the synthesized five- and six-membered heterocyclic compounds exhibited significant antibacterial properties, particularly against pathogenic bacteria, such as *Pseudomonas* and *E. coli*. These findings not only contribute to the field of synthetic chemistry but also hold promise for applications in antibacterial treatments.

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Conflicts of Interest: The authors declare no conflicts of interest.

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