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Synthesis of new derivatives of hydrochlorothiazide containing diazonium groups and study of their biological activity

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azo compounds; diazonium salt; pyrazole; biological activity; physical characteristics

ABSTRACT

Diazonium salts are highly reactive compounds that serve as intermediates in numerous chemical reactions. In this study, they were synthesized and subsequently allowed to react with phenolic drugs, ethyl acetoacetate, and acetylacetone in order to yield compounds M1–M5. Following further processing in order to produce compounds M6 and M7, compounds M4 and M5 interacted with hydrazine hydrate, resulting in an 80% conversion. The synthesized compounds (M1–M7) were characterized through an analysis of their physical properties, nuclear magnetic resonance spectra, elemental composition, and infrared spectra. The biological activities of the same compounds were also evaluated against various bacterial strains (i.e., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Serratia marcescens*, *Morganella*, *Acinetobacter*, *Salmonella*, and *Streptococcus pneumoniae*) and a yeast species (*Candida albicans*), using the agar well diffusion method.

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1. Introduction

Hydrochlorothiazide is a widely prescribed thiazide diuretic for managing hypertension[1]. Its diuretic efficacy is mediated through the modulation of electrolyte reabsorption in the distal renal tubules, resulting in a nearly equivalent increase in sodium and chloride excretion. While the exact mechanism of action remains unclear, redistribution and elimination of systemic sodium may contribute to its therapeutic effect. Aromatic diazonium salts, typically represented as Ar–$N_2^+X^-$, serve as versatile intermediates in the synthesis of diverse or-

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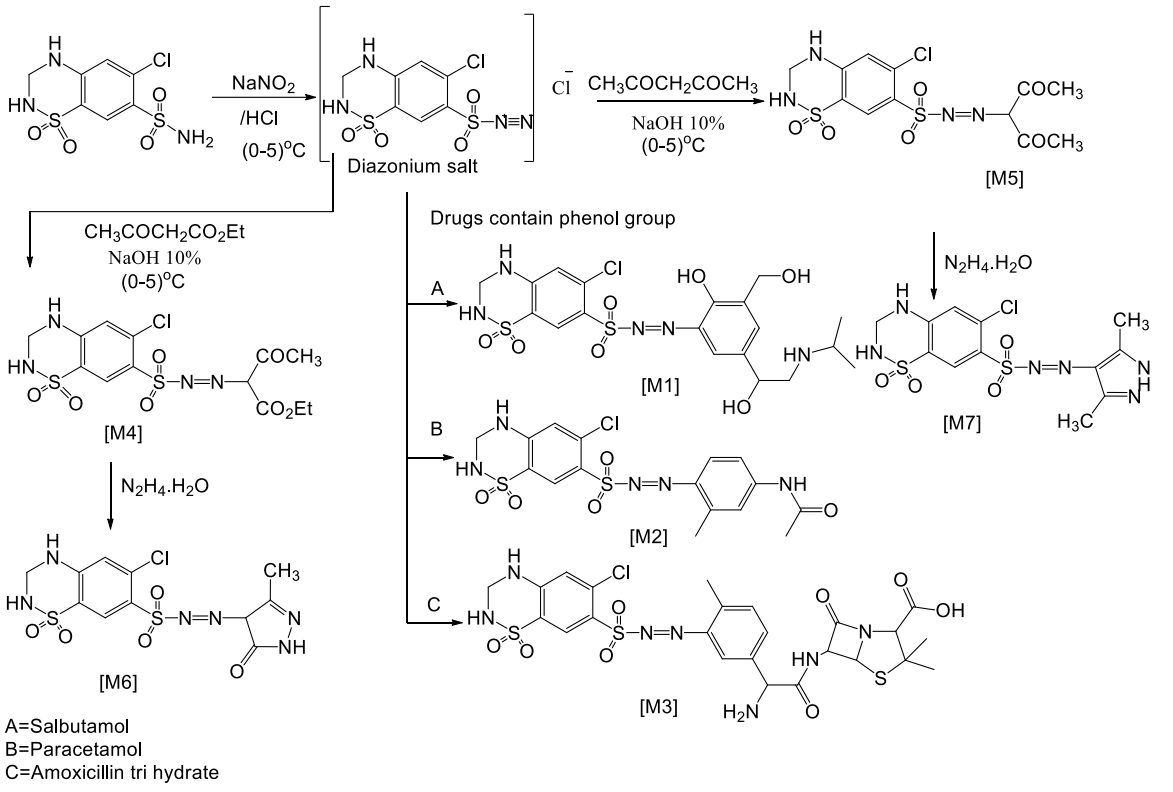

Figure 1. *Pathway of the synthesis of compounds M1–M7.*

ganic aromatic compounds[2]. These salts may incorporate various inorganic and organic anions; due to the low hydrolytic tendency of aryl diazonium ions, strong acid salts dissociate fully in neutral aqueous media, thereby enabling the biological activity observed in certain derivatives. Azo compounds are pharmacologically significant and have been employed for their anti-diabetic, antimicrobial, antibacterial, anticancer, antiseptic, antioxidant, antifungal, anti-HIV, and electroconductive properties. Pyrazole, a five-membered heteroaromatic ring with two nitrogen atoms, is a fundamental scaffold for many drugs exhibiting a broad spectrum of therapeutic effects (including antifungal, anticancer, antigenic, and antimicrobial activity)[3]. This study describes the preparation of novel heterocyclic derivatives and the evaluation of their antibacterial properties. Structural elucidation of the synthesized compounds was performed by using Fourier-transform infrared spectroscopy (FT-IR) and proton nuclear magnetic resonance (^{1}H-NMR).

2. Methodology

2.1. Chemicals

Reagents were procured from Thomas Baker, Merck, BDH, GCC, and Scharlau, and were used without further purification. Melting points were determined using a Gallenkamp capillary melting point apparatus. FT-IR spectra were obtained using a Shimadzu spectrometer (Japan), while the ^{1}H-NMR spectra of the products were recorded on a Bruker DMX-500 spectrophotometer.

2.2. Synthesis of azo compounds M1–M5 (Figure 1)

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As detailed previously[4,5], the aromatic primary amine (1 g; 0.003 mol) was dissolved in 2.5 mL concentrated HCl and 7 mL H_2O under heating (60°C, 10 min) until complete dissolution and salt formation. Upon cooling (0°C–5°C), diazotization was carried out using 0.2 g sodium nitrite in 2 mL H_2O, added dropwise with vigorous stirring. The temperature was maintained at 0°C–5°C for 30 min. The resulting diazonium salt was added to aqueous solutions of phenolic drugs, ethyl acetoacetate, and acetylacetone (0.003 mol each) at 0°C–5°C and pH adjusted to 8–9 using 10% NaOH. After stirring for 60 min, the precipitate was filtered and repeatedly washed with 1:1 ethanol to water ratio. The process produced the following compounds: (i) compound M1 (light orange precipitate; yield: 85%; MP: 217°C–219°C; formula: $C_{13}H_{15}ClN_4O_7S_2$; MW: 438.86 mg/mol; elemental analysis (%) calculated / found: C = 65.50/65.55, H = 3.76/3.72, N = 22.23/22.26), (ii) compound M2 (yellow precipitate; yield: 66%; MP: 220°C–222°C; formula: $C_{16}H_{16}ClN_5O_5S_2$; MW: 457.91 mg/mol; elemental analysis (%) calculated / found: C = 57.3/57.9, H = 3.76/3.72, N = 17.07/17.25), (iii) compound M3 (white precipitate; yield: 54%; MP: 218°C–220°C; formula: $C_{19}H_{24}ClN_5O_7S_2$; MW: 457.91 mg/mol; elemental analysis (%) calculated / found: C = 65.50/65.55, H = 3.76/3.72, N = 13.82/13.90), (iv) compound M4 (light yellow precipitate; yield: 62%; MP: 208°C–210°C; formula: $C_{24}H_{26}ClN_7O_8S_3$; MW: 672.15 mg/mol; elemental analysis (%) calculated / found: C = 60.39/60.45, H = 4.12/4.23, N = 25.92/25.35), and (v) compound M5 (yellow precipitate; yield: 75%; MP: 185°C–187°C; formula: $C_{12}H_{13}ClN_4O_6S_2$; MW: 408.84 mg/mol; elemental analysis (%) calculated / found: C = 66.70/66.78, H = 5.08/5.15, N = 18.33/18.46).

2.3. Synthesis of pyrazole compounds M6 and M7 (Figure 1)

Following prior methodologies[4], compounds M4 and M5 (0.001 mol each) were dissolved in 7 mL absolute methanol. After stirring for 10 min at room temperature, 0.001 mol of 80% hydrazine hydrate was added gradually. The mixture was refluxed for 7–8 h, monitored *via* thin layer chromatography, and the resulting product was filtered, dried, and purified using methanol. The process produced the following compounds: (i) compound M6 (orange precipitate; yield: 50%; MP: 175°C–177°C; formula: $C_{11}H_{11}ClN_6O_5S_2$; MW: 406.83 mg/mol; elemental analysis (%) calculated / found: C = 65.54/65.65, H = 5.58/5.26, N = 13.57/13.67) and (ii) compound M7 (dark yellow precipitate; yield: 77%; MP: 185°C–187°C; formula: $C_{12}H_{13}ClN_6O_4S_2$; MW: 404.85 mg/mol; elemental analysis (%) calculated / found: C = 58.66/58.70, H = 4.45/4.22, N = 15.11/15.24).

2.4. Biological activity testing

Compounds M1–M7 were assessed for antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Serratia marcescens*, *Morganella*, *Acinetobacter*, *Salmonella*, *Streptococcus pneumoniae*, and *Candida albicans* at a concentration of 100 mg/mL, using the agar well diffusion method[6]. Nutrient agar was prepared with beef extract (3 g), agar (15 g), peptone (5 g), NaCl (5 g), and distilled water (up to 1,000 mL). All glassware, culture tubes, pipettes, and broth media were autoclaved and sterilized prior to incubation at 25°C ± 2°C for 72 h.

3. Results and Discussion

Hydrochlorothiazide, an aromatic primary amine, reacts with freshly generated nitrous acid (HONO) – formed by sodium nitrite in the presence of excess mineral acid – to produce a diazonium salt; a well-characterized intermediate in organic synthesis. Due to its instability, the diazonium salt must be prepared in cold solution. The synthesis of azo dyes involves two main steps: (i) the formation of the diazonium salt and (ii) the subsequent coupling with a phenolic drug, which acts as a highly reactive nucleophile because of its strong electron-donating hydroxyl group. Further coupling of the diazonium salt with ethyl acetoacetate and acetylacetone under alkaline conditions has yielded azo compounds M4 and M5.

The compounds were characterized using physical property evaluation, elemental analysis, FT-IR

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spectroscopy, and ^{1}H-NMR spectroscopy. The FT-IR spectra of compounds M1–M5 revealed the disappearance of NH_2 stretching vibrations (3453–3341 cm^{-1}), consistent with diazotization, and the appearance of N=N stretches at 1458, 1450, 1462, 1404, and 1455 cm^{-1}, respectively. In compound M4, ester and carbonyl bands appeared at 1766 and 1710 cm^{-1}, while compound M5 exhibited a ketonic carbonyl stretch at 1735 cm^{-1}. The ^{1}H-NMR spectrum of compound M3 showed characteristic signals at δ 1.19 (NH–amine), δ 1.80 (s, $2CH_3$), δ 2.42 (s, CH–methine), δ 2.49 (s, CH–methane), δ 2.95 (d, CH–propiolactam), δ 3.31 (s, CH_2–methylene), δ 4.26 (s, NH_2–amine), δ 4.67 (d, CH–propiolactam), δ 4.83 (s, NH–aromatic), δ 5.39 (s, OH–phenol), δ 6.88–8.40 (m, aromatic protons), δ 8.88 (s, NH–secondary amide), and δ 12.50 (s, OH–carboxylic)[7].

Reaction of compound M3 with 80% hydrazine hydrate produced compound M6. FT-IR analysis showed loss of the ester and carbonyl bands of compound M4 (1766 and 1710 cm^{-1}), and emergence of amide-related bands at 1689 cm^{-1} (C=O) and 3232 cm^{-1} (NH stretch). The ^{1}H-NMR spectrum of compound M6 showed signals at δ 1.19 (NH–amine), δ 2.40 (s, 3H–CH_3), δ 3.12 (s, CH–methylene), δ 4.67 (s, NH–aromatic), δ 6.93–7.67 (m, aromatic protons), and δ 8.21 (s, NH–pyrazole).

Similarly, the treatment of compound M5 with 80% hydrazine hydrate yielded compound M7. The FT-IR spectrum of M7 displayed the disappearance of the 1735 cm^{-1} ketonic carbonyl band, and new absorptions at 3381 cm^{-1} (NH stretch) and 1639 cm^{-1} (C=N stretch). The ^{1}H-NMR spectrum of compound M7 revealed signals at δ 2.18 (s, NH–S), δ 2.33 (s, 6H–CH_3), δ 4.66 (s, CH–methylene), δ 5.31 (s, NH–aromatic), δ 7.04–7.97 (m, aromatic protons), and δ 10.98 (s, NH–pyrazole)[8,9].

These data collectively confirm the successful transformation of hydrochlorothiazide into the desired azo and pyrazole derivatives. The synthesized compounds exhibited promising antimicrobial activity when compared to ciprofloxacin, particularly against *Escherichia coli*, *Morganella*, *Salmonella*, and *Candida albicans* (results not shown).

4. Conclusion

This study successfully synthesized and characterized a series of novel pyrazole and azo derivatives. Structures were confirmed *via* FT-IR, elemental analysis, and ^{1}H-NMR spectroscopy. These compounds hold considerable relevance in medicinal and pharmaceutical contexts, and several of them have demonstrated moderate antibacterial activity.

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Conflicts of interest

None exist.

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