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Synthesis, characterization, immunological, and biological evaluation of several heterocyclic rings obtained from antipyrine derivatives

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ABSTRACT

The structures of newly synthesized heterocyclic compounds incorporating 4-aminoantipyrine (a key intermediate for the development of diverse derivatives) were elucidated through melting point analysis, Fourier-transform infrared spectroscopy (FTIR), and evaluation of physical properties. These compounds were subsequently tested for antibacterial activity against both Gram-positive and Gram-negative bacterial strains, specifically: *Staphylococcus aureus* and *Streptococcus mutans* (Gram-positive); *Shigella flexneri* and *Pseudomonas aeruginosa* (Gram-negative). Among heterocycles bearing three nitrogen atoms, antipyrine-based derivatives represent a widely studied structural class with significant relevance in academic research and medical applications. Although not naturally occurring, these compounds exhibit versatile utility across organic synthesis, chemical biology, polymer chemistry, supramolecular chemistry, and pharmaceutical development. Accordingly, the establishment of a straightforward and efficient synthetic route for antipyrine derivatives remains a priority. All tested compounds demonstrated notable antibacterial efficacy when benchmarked against conventional antibiotics. Moreover, *in vivo* studies revealed their capacity to induce pro-inflammatory cytokines such as interferon-gamma (IFN-γ), secreted by $CD4^+$ T helper 1 (Th1) cells, thereby suggesting an immunomodulatory role.

PHARMAKEFTIKI, 37, 2S, 2025 | 313-316

1. Introduction

Antipyrine and its derivatives have garnered significant attention due to their roles as analgesic, antipyretic, and anti-inflammatory agents. These compounds exhibit a broad spectrum of biological activities, including anti-inflammatory, analgesic, immunomodulatory, anthelmintic, and insecticidal properties[1,2]. Notable derivatives include β-lactams and imidazolidines, which have been evaluated for their antioxidant potential. Additionally, antipyrine derivatives serve as valuable precursors in the synthesis of biologically active Schiff bases and metal complexes[3], as well as macrocyclic compounds and corrosion inhibitors for copper in acidic media.

4-Aminoantipyrine, a key intermediate, was herein employed in the synthesis of Schiff bases and polyfunctionally substituted heterocyclic rings with antibacterial and immunomodulatory properties[1,3]. In this study, novel drug candidates were synthesized and their antibacterial efficacy was assessed alongside their ability to elicit a cellular immune response in an experimental rabbit model.

2. Methodology

2.1. Synthesis of compound I

Compound I, (E)-1,5-dimethyl-4-((4-nitrobenzylidene)amino)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one, was synthesized by dissolving 0.03 mol of 4-aminoantipyrine in 25 mL of ethanol, followed by the addition of 0.03 mol of 2-nitrobenzaldehyde. A few drops of glacial acetic acid were added, and the mixture was stirred continuously for 20–25 min using a magnetic stirrer. Subsequently, 0.02 mol of compound I were gradually introduced, and the reaction was completed over 8 h at 60°C–65°C. The product was isolated with an 85% yield, a melting point of 134°C–137°C, and Rf of 0.80. The dry residue was recrystallized from absolute ethanol.

2.2. Synthesis of compound II

Compound II, (2-chloro-4-(4-nitrophenyl)-3-oxoazetidin-1-yl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one, was obtained by dissolving 0.03 mol of compound I in 0.01 mol of chloroacetyl chloride and 1 mL of triethylamine. The reaction was carried out in 10 mL of dioxane at 0°C–5°C for 10 h. The mixture was evaporated and dried, and the product was recrystallized from ethanol. Thin-layer chromatography confirmed purity: Rf: 0.75; melting point: 187°C–193°C; red solid; yield: 75%.

2.3. Synthesis of compound III

Compound III, 1,5-dimethyl-4-(2-(4-nitrophenyl)-5-oxoimidazolidin-1-yl)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one, was synthesized by combining 0.03 mol of compound I with 25 mL of tetrahydrofuran, followed by continuous shaking. Then, 0.03 mol of aminoacetic acid were added gradually, and the reaction was completed over 14 h at 50°C. The product was dried and recrystallized from absolute ethanol: Rf: 0.61; melting point: 147°C–150°C; brown solid; yield: 87%.

2.4. Antibacterial and immunological evaluation

The antibacterial activity of compounds I–III was assessed *in vitro* using the agar-well diffusion method against two Gram-positive bacterial strains (*Staphylococcus aureus* and *Streptococcus mutans*) and two Gram-negative strains (*Shigella flexneri* and *Pseudomonas aeruginosa*). Compounds were dissolved in dimethyl sulfoxide (DMSO) at concentrations of 10, 20, and 30 μg/mL[4].

Eighteen male rabbits (*Oryctolagus cuniculus*) were immunized with 1 mL of each compound at the aforementioned concentrations. Two rabbits received sterile normal saline as controls. A multisite injection protocol was used (0.25 mL per site). Cellular immune responses were evaluated by measuring systemic levels of interferon-gamma (IFN-γ) using an enzyme-linked immunosorbent assay (ELISA) kit (Beckman Coulter), with results compared to the control group[5].

3. Results and Discussion

Compound I was characterized by melting point and

PHARMAKEFTIKI, 37, 2S, 2025 | 313-316

Table 1. Inhibition zone diameters (in mm) of the synthesized chemical compounds against bacterial growth at varying concentrations.

Chemical compound	**Concentration** (μg/mL)	**Mean of inhibition zone** (mm)			
		Gram-positive bacteria		**Gram-negative bacteria**	
		S. aureus	*S. mutans*	*S. flexneri*	*P. aeruginosa*
I	10	9	12	10	11
	20	13	15	17	20
	30	19	18	20	23
II	10	9	13	10	11
	20	16	16	15	18
	30	23	24	28	30
III	10	11	12	12	14
	20	15	18	25	23
	30	27	29	34	35

FTIR spectroscopy. The FTIR spectrum showed a new absorption band at 1649 cm^{-1}, corresponding to the azomethine (C=N) group, and the disappearance of bands associated with NH_2 and C=O groups of the starting materials. A peak at 1595 cm^{-1} was attributed to C=C stretching in the pyrazole ring, while a band at 1718 cm^{-1} corresponded to the ring's C=O group. A broad band near 3392 cm^{-1} indicated intermolecular hydrogen bonding *via* the hydroxyl (O–H) group.

Compound II exhibited a characteristic absorption band at 1651–1660 cm^{-1}, attributed to the C=O stretch of the azetidine ring. Compound III showed NH symmetric and asymmetric stretching bands in the range 3226–3294 cm^{-1}, along with a tautomeric O–H band and a strong carbonyl stretch at 1710 cm^{-1}, indicative of the imidazolidinone ring.

All compounds demonstrated antibacterial activity, with compounds [II] and [III] showing the most potent effects across all tested bacterial strains (Table 1). This activity is likely due to disruption of bacterial cell wall permeability, protein denaturation, and interference with metabolic pathways, ultimately leading to cell death[6].

Finally, a statistically significant increase (p<0.05) in IFN-γ levels was observed, suggesting activation of Th1 cells and induction of cell-mediated immunity.

4. Conclusion

Our *in vivo* experiments have confirmed that the herein assessed novel antipyrine derivatives can stimulate cell-mediated immunity. These compounds have also exhibit marked antibacterial activity against the tested microorganisms.

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Conflicts of interest

None exist.

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PHARMAKEFTIKI, 37, 2S, 2025 | 313-316

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