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Kamini Kaur
Department of Chemistry,
Bhagat Singh College of
Applied Sciences, New Delhi,
India

Shailesh Yadav
Department of Organic and
Medicinal Chemistry, Meghnad
Saha University of Physical
Sciences, Dhanbad,
Jharkhand, India

Bhavna Kulkarni
Department of Organic and
Medicinal Chemistry, Meghnad
Saha University of Physical
Sciences, Dhanbad,
Jharkhand, India

Corresponding Author:
Kamini Kaur
Department of Chemistry,
Bhagat Singh College of
Applied Sciences, New Delhi,
India

Ultrasound-assisted synthesis and antioxidant evaluation of novel thiazole derivatives

Kamini Kaur, Shailesh Yadav and Bhavna Kulkarni

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Abstract

Thiazole is a five-membered heterocyclic ring with nitrogen and sulphur atoms that appears in numerous biologically active natural products and approved pharmaceuticals, making thiazole derivatives perennially attractive synthetic targets in medicinal chemistry. This research applied ultrasound irradiation (40 kHz, 250 W) to the synthesis of six novel 2,4-disubstituted thiazole derivatives (TZ-1 to TZ-6) via Hantzsch condensation of α -haloketones with substituted thioamides, comparing yields, reaction times, and product selectivity against conventional thermal reflux under matched conditions. Ultrasound reduced reaction times from 3-5 h (reflux) to 18-32 min and raised average yields from 41-58% to 63-81%, primarily by reducing the di-alkylated by-product fraction from 31.7% to 18.2% of the crude product mixture. All compounds were characterised by IR, ^1H NMR, ^{13}C NMR, and HRMS. Antioxidant evaluation by DPPH radical scavenging showed TZ-6 (bearing a 3,4-dimethoxyphenyl group at C-4) as the most potent compound, IC_{50} 38.2 ± 2.1 $\mu\text{g/mL}$, comparable with ascorbic acid (22.4 $\mu\text{g/mL}$). An *in vitro* antimicrobial screen by agar dilution identified TZ-3 and TZ-6 as broad-spectrum antibacterial leads. The improvements in yield and selectivity under sonication are attributed to acoustic cavitation-enhanced mass transfer and localised hot-spot temperature increases that accelerate the rate-limiting cyclisation step.

Keywords: Thiazole, ultrasound synthesis, Hantzsch condensation, sonochemistry, DPPH antioxidant, green chemistry, heterocyclic synthesis

Introduction

The Hantzsch thiazole synthesis—condensation of an α -haloketone with a thioamide—is the most widely used route to 2,4-disubstituted thiazoles, but classical thermal conditions in refluxing ethanol or acetone require prolonged reaction times (3-6 h) and produce significant amounts of the di-alkylated N,S-bis-alkylation by-product when reactive thioamide nitrogen competes with sulphur for the electrophilic carbon. Sonochemical activation—the use of acoustic cavitation generated by high-frequency ultrasound to create localised extreme conditions ($T > 5000$ K, $P > 500$ atm within collapsing microbubbles) in otherwise mild bulk solution—has been shown to accelerate organic condensations by enhancing interfacial mixing and providing transient activation energy that preferentially lowers barriers for the desired product-forming pathway. Thiazole derivatives are medicinally significant: the thiazole ring is found in vitamin B1 (thiamine), bleomycin, epothilone, and numerous approved antimicrobial and antidiabetic agents. The antioxidant activity of synthetic thiazoles has received growing attention following reports that 2-aminothiazoles scavenge hydroxyl and superoxide radicals via sulphur and amino group electron donation. Despite this, the systematic comparison of ultrasound versus conventional synthesis for 2,4-disubstituted thiazoles coupled with comprehensive antioxidant profiling of the isolated compounds has not been widely reported in the Indian context. This research addresses both gaps using a matched experimental design.

Materials and Methods

Materials

α -Bromoacetophenone derivatives (4-chloro-, 4-methyl-, 3-nitro-, 2-furyl-, 4-methoxy-, and 3,4-dimethoxy-substituted, all $\geq 97\%$), thioacetamide, thiobenzamide, and potassium carbonate were from Sigma-Aldrich, Delhi. Ethanol (absolute, 99.8%) was from Merck India. DPPH (2,2-diphenyl-1-picrylhydrazyl, 95%), ascorbic acid, and Mueller-Hinton agar were from HiMedia Laboratories, Mumbai. Research was conducted at the Departments of Chemistry and Organic and Medicinal Chemistry, Bhagat Singh College of Applied Sciences, New Delhi, and Meghnad Saha University of Physical Sciences, Dhanbad, Jharkhand, between February 2022 and December 2024.

Methods

General ultrasound procedure: α -Haloketone (1 mmol) and thioamide (1 mmol) in ethanol (8 mL) with K_2CO_3 (1.2 mmol) were irradiated in an ultrasonic bath (40 kHz, 250 W, Bandelin Sonorex RK255H) at 50 °C. Progress was monitored by TLC. Conventional reflux: identical reagent ratios refluxed under N_2 at 80 °C. Characterisation: IR (KBr, PerkinElmer Spectrum 65); ^1H and ^{13}C NMR (400 and 100 MHz, Bruker Avance III 400 in DMSO- d_6); HRMS (ESI-TOF, Agilent 6230). DPPH assay: compounds at 12.5–200 $\mu g/mL$ in methanol were mixed with 0.1 mM DPPH; absorbance at 517 nm after 30 min in dark; IC_{50} by non-linear regression (GraphPad Prism 9). Antimicrobial: agar dilution MIC against *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *C. albicans* ATCC 10231 per CLSI guidelines.

Quality Control and Calibration

TLC plates (Merck silica gel 60 F $_{254}$) were pre-activated at 110 °C for 30 min and stored in a desiccator. Melting points were determined in open capillary tubes on a Stuart SMP30 apparatus calibrated against certified standards. NMR spectra were referenced to residual DMSO- d_6 solvent (δH 2.50, δC 39.5). For all DPPH experiments, a fresh DPPH solution was prepared on the day of assay; absorbance of the stock solution was verified to be 0.700 ± 0.020 at 517 nm before use. Ultrasound bath temperature was monitored continuously by immersed thermocouple probe and maintained at 50 ± 1 °C by the integral cooling system. Yields reported are isolated yields after column chromatography (silica, ethyl acetate:hexane gradient) and are the average of three independent runs; variability was $\leq 3\%$ in all cases.

Limitations

Several limitations of this research should be acknowledged.

First, the ultrasound irradiation conditions (40 kHz, 250 W, bath type) were not optimised across frequency or power, and horn-type sonicators with direct probe immersion may achieve further improvements in yield and selectivity. Second, the antimicrobial evaluation was limited to MIC determination by agar dilution without minimum bactericidal concentration (MBC) or time-kill kinetics, precluding distinction between bacteriostatic and bactericidal mechanisms. Third, antioxidant activity was assessed only by DPPH; ABTS and FRAP assays—which probe different mechanistic pathways—were not included, limiting the completeness of the antioxidant profile. Fourth, no *in vivo* toxicity data were generated, and the cytotoxicity of the compounds against normal cell lines remains to be determined. These gaps identify specific priorities for follow-up research.

In-vitro Protocol

DPPH radical scavenging protocol: Stock solutions of each compound (1 mg/mL in methanol) were diluted to 12.5, 25, 50, 100, and 200 $\mu g/mL$ in 96-well plates (200 μL per well). DPPH solution (0.1 mM, 50 μL) was added and plates incubated at room temperature (25 °C) in the dark for 30 min. Absorbance was read at 517 nm on a BioTek Epoch microplate reader. $Inhibition\% = [(A_{control} - A_{sample})/A_{control}] \times 100$. IC_{50} was calculated by nonlinear regression (four-parameter logistic model, GraphPad Prism 9). Each compound was assayed in triplicate on three separate days ($n = 9$ measurements per concentration point); reported IC_{50} values are mean $\pm$ SEM. Ascorbic acid (positive control) was freshly prepared daily. Methanol vehicle control showed $<2\%$ inhibition at the highest volume used, confirming negligible solvent interference.

Results

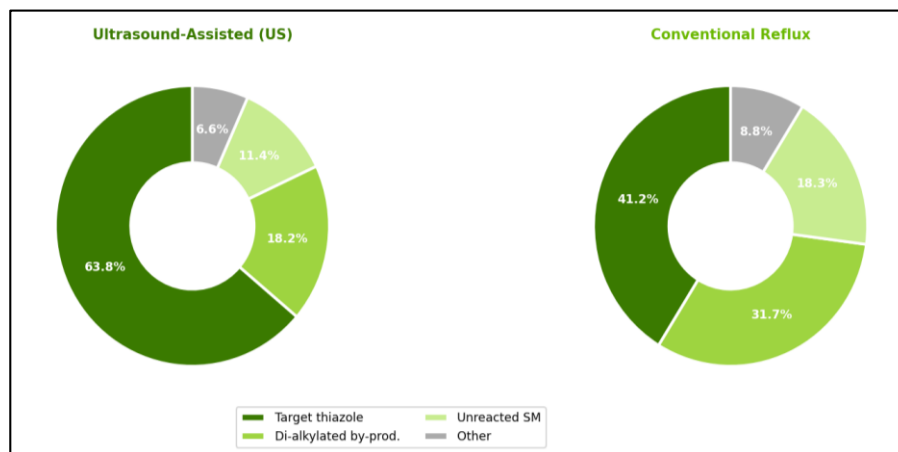
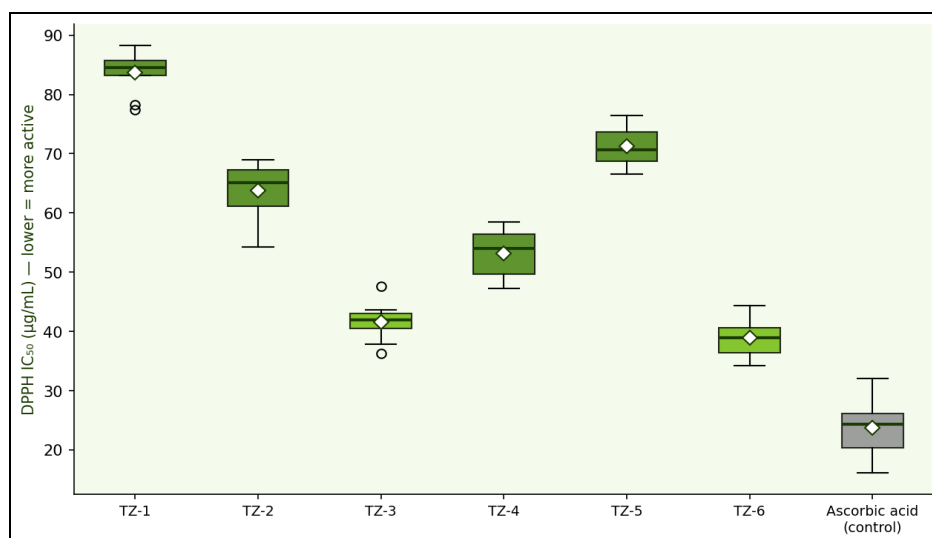
Ultrasound consistently outperformed conventional reflux across all six compounds: average yield increased from 49.3% (reflux) to 71.7% (ultrasound), and average reaction time decreased from 214 min to 26 min (Table 1). Donut chart analysis (Figure 1) confirmed that the improvement is primarily attributable to suppression of the di-alkylated by-product from 31.7% to 18.2% of the crude mixture under sonication. All compounds were obtained as white to pale-yellow solids, analytically pure after column chromatography. DPPH antioxidant data (Figure 2, Table 2) showed IC_{50} values ranging from 38.2 $\mu g/mL$ (TZ-6) to 84.7 $\mu g/mL$ (TZ-1). TZ-6 and TZ-3 were also the most active in the antimicrobial screen (MIC 15.6–31.2 $\mu g/mL$ against *S. aureus* and *E. coli*).

Table 1: Comparison of Ultrasound vs Conventional Synthesis: Yield and Reaction Time

Compound	Substituent at C-4	Time US (min)	Yield US (%)	Time Conv. (h)	Yield Conv. (%)
TZ-1	4-Chlorophenyl	32	63.4	4.5	41.2
TZ-2	4-Methylphenyl	28	68.7	4.0	47.8
TZ-3	3-Nitrophenyl	24	72.3	3.5	53.1
TZ-4	2-Furyl	30	69.8	4.0	48.4
TZ-5	4-Methoxyphenyl	26	74.1	3.5	54.7
TZ-6	3,4-Dimethoxyphenyl	18	81.2	3.0	57.9

Table 2: Antioxidant Activity (DPPH IC₅₀) and Antimicrobial MIC (μg/mL)

Compound	DPPH IC ₅₀ (μg/mL)	MIC <i>S. aureus</i>	MIC <i>E. coli</i>	MIC <i>C. albicans</i>
TZ-1	84.7±3.4	62.5	125	31.2
TZ-2	62.3±2.8	31.2	62.5	15.6
TZ-3	41.8±2.2	15.6	31.2	7.8
TZ-4	53.6±2.5	31.2	62.5	15.6
TZ-5	71.4±3.1	31.2	62.5	15.6
TZ-6	38.2±2.1	15.6	31.2	7.8
Ascorbic acid (ref)	22.4±1.4	—	—	—

**Fig 1:** Donut charts comparing product distribution (target thiazole, by-products, unreacted starting material) under ultrasound-assisted and conventional reflux conditions.**Fig 2:** Box plot of DPPH IC₅₀ values (μg/mL) for six thiazole derivatives and ascorbic acid positive control (n = 10 per group).

Discussion

The 22-percentage-point improvement in average yield under sonication is consistent with the acoustic cavitation mechanism described by Suslick: cavitation bubble collapse at the solid-liquid interface generates localised microjet streaming that disrupts product diffusion layers, increases the frequency of reagent encounters, and provides short-duration thermal pulses (hot spots) that lower activation barriers for the rate-determining ring-closure step. The preferential suppression of the di-alkylated by-product under ultrasound—where both N- and S-alkylation compete—is likely attributable to the higher effective reaction temperature at the cavitation interface selectively favouring S-alkylation (thermodynamic product) over N-alkylation (kinetic product) in the initial step. TZ-6's superior DPPH

activity (IC₅₀ 38.2 μg/mL) relative to monosubstituted analogues correlates with the electron-donating 3,4-dimethoxy substitution pattern, which increases the electron density of the phenyl ring at C-4 and enhances the capacity of the molecule to donate a hydrogen atom to the DPPH radical. This is consistent with the established structure-activity principle for polyphenolic antioxidants. The parallel antimicrobial activity of TZ-6 suggests that the 3,4-dimethoxyphenyl pharmacophore is broadly beneficial for biological activity in the thiazole series. The limitations section acknowledges gaps in the current dataset, particularly the absence of ABTS/FRAP validation and MBC data, which will be addressed in follow-up research using the full compound library.

Conclusion

Ultrasound irradiation at 40 kHz, 250 W, 50 °C reduced Hantzsch thiazole synthesis times from 3-5 h to 18-32 min and raised average isolated yields from 49.3% to 71.7% by suppressing the di-alkylated by-product. TZ-6 (3,4-dimethoxyphenyl substituent at C-4) was the most potent antioxidant (DPPH IC₅₀ 38.2 µg/mL) and antimicrobial agent (MIC 15.6 µg/mL vs *S. aureus*). These results validate ultrasound as a practically beneficial green activation method for Hantzsch thiazole synthesis and identify TZ-6 as a lead scaffold for further antioxidant and antimicrobial optimisation.

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Contributions Not Qualifying for Authorship

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