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Microwave- and ultrasound-promoted greener synthesis of thiazolyl-pyrazoline derivatives and investigation of their biological activities

Arif MERMER[1*]

Department of Biotechnology, Hamidiye Institute of Health Sciences, University of Health Sciences, İstanbul, Turkey.

Abstract: Six thiazolyl-pyrazoline derivatives were synthesized starting from corresponding chalcone compounds for their antioxidant capacity and antiurease inhibitory activities. In addition to the conventional method, ultrasonic sonication and microwave irradiation methods which are environmental methods were used in the synthetic stage. Compound 2-(5-(3-bromophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-4-phenyl thiazole (5a) exhibited the most potent antiurease activity with IC_{50} of 2,28±0,02, which was comparable to the positive control.

Keywords: Thiazole; Ultrasound Sonication; Microwave Irradiation; Antioxidant Capacity; Antiurease Inhibiton; ADME prediction.

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Corresponding Author. E-mail: arfmermer@hotmail.com, Tel:+90506 462 2979.

INTRODUCTION

Thiazoles and their derivatives have taken continuing attention over the years because of their various biological activities such as anti-hypertensive, anti-inflammatory, analgesic, antimicrobial, anti-HIV, and herbicidal activity (1-5). Commercially available drugs such as blenoxane, bleomycine, and tiazofurin have a thiazole ring in their structure (6). In many studies, it has been seen that thiazole derivative compounds have tuberculostatic, antibacterial, antifungal, antiurease, and antioxidant activities (7-9). One of the important heterocyclic structure, pyrazoles have attracted the attention of organic chemists in recent years due to their wide biological activity properties. Compounds containing pyrazole nucleus are known to have analgesic, anti-inflammatory, antipyretic, antiarrhythmic, muscle relaxant, psychoanaleptic, anticonvulsant, hypotensive, antidiabetic and antibacterial activities (10-16). The yield, which is one of the problems experienced in the field of synthetic organic chemistry, has an important place in modern drugs discovery. In organic reactions, the compounds should be quick and easy to synthesize, and the obtained compounds should be pure and easy to separate. From this perspective, organic chemists and researchers from many other scientific fields show a great deal of interest in the application of new methods and synthetic strategies. Compared to conventional method, microwave-assisted and ultrasonic sonicated synthesis have applied as advantageous methods for achieving these objectives (17-27).

Urease (urea amidohydrolase; E.C.3.5.1.5), a nickel-containing metalloenzyme, catalyzes the hydrolysis of urea to ammonia and carbon dioxide (28). Urease activity has been an important virulence factor in the pathogenesis of

many clinical conditions that are detrimental to human and animal health and agriculture. At the same time, urease is the main cause of the occurrence of pathogens induced by Helicobacter pylori and thus the colonization of the bacteria survives even at the low pH of the stomach. Therefore, these bacteria can cause gastric and peptic ulcers. (29). Many strategies based on urease inhibition are used for the treatment of infections caused by urease-producing bacteria. In this paper, we reported here the synthesis and biological activity screening studies of a series of thiazolyl-pyrazoline derivatives by using green chemistry techniques (Microwave irradiation and ultrasound sonication) and conventional method.

EXPERIMENTAL

All chemicals used in synthetic phase and biological activity assays were purchased from FlukaChemie AG Buchs (Switzerland). The Büchi B-540 apparatus was used to determine the melting points of the obtained compounds. The progress of reactions were followed by thin-layer chromatography (TLC) on silica gel $60F_{254}$ aluminum sheets. Ethyl acetate:diethyl ether (1:1) and ethyl acetate:chloroform (2:1) were used as mobile phase and detection was performed using UV light. FT-IR spectra were recorded using a Perkin Elmer 1600 series FTIR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were registered in DMSO-d_6 on a Bruker Avance II 400 MHz NMR Spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C). The chemical shifts are given in ppm relative to Me_4Si as an internal reference, J values are given in Hz. Microwave and ultrasound mediated syntheses were carried out using monomode CEM-Discover microwave apparatus and Bandelin Sonorex Super RK102H ultrasonic bath, respectively. The Mass spectra were obtained on a Quattro LC-MS (70 eV) Instrument.

General method for the synthesis of compounds 3a,b

Method 1. Substituted benzaldehyde compounds (10 mmol) and acetophenone (10 mmol) were dissolved in 30 mL of ethanol. The mixture was stirred for several minutes at 10 °C until dissolved. Then 1 mL of a 40% aqueous potassium hydroxide solution was added slowly to the reaction flask with a self-equalizing addition funnel. The reaction solution was allowed to stir at room temperature for approximately 4-5 h. After completion of the reaction, a solid precipitate formed and filtered. The corresponding product was crystallized from ethanol.

Method 2. The solution of corresponding benzaldehyde (10 mmol) and acetophenone (10 mmol) in ethanol (5 mL) was irradiated 30 °C, 50 W for 35-40 min in closed vessel in the presence of 1 mL of a 40% aqueous potassium hydroxide solution. After completion of the reaction (the progress of the reaction was followed by TLC), a solid precipitate formed and filtered. The corresponding product was crystallized from ethanol.

Method 3. The solution of corresponding benzaldehyde (10 mmol) and acetophenone (10 mmol) in ethanol (5 mL) was sonicated in the presence of 1 mL of a 40% aqueous potassium hydroxide solution for 30-35 min. After completion of the reaction (the progress of the reaction was followed by TLC), a solid precipitate formed and filtered. The corresponding product was crystallizated from ethanol.

(E)-3-(3-bromophenyl)-1-phenylprop-2-en-1-one (3a)

FT-IR(υ_{max}, cm^{-1}): 3063, 1656, 1604, 1215. ^{1}H NMR (DMSO-d_6, δppm): 7.42 (t, 1H, J=16.0 Hz, arH), 7.58 (t, 2H, J=16.0 Hz, arH), 7.64 (d, 1H, J=8.0 Hz, arH), 7.68 (d, 1H, J=8.0 Hz, arH), 7.88 (d, 1H, J=4.0 Hz, CH), 8.03 (d, 1H, J=4.0 Hz,CH), 8.18 (s, 1H, arH), 8.21 (d, 2H, J=8.0 Hz, arH). ^{13}C NMR (DMSO-d_6, δppm): arC: (126.86 (C), 128.74 (CH), 129.13 (2CH), 129.28 (2CH), 131.33 (CH), 133.81 (CH), 137.65 (C), 137.80 (C)), 124.02 (CH), 142.69 (CH), 189.53 (C=O). EI MS m/z (%):287.17 ($(M)^+$, 100), 289.19 ($(M+2)^+$, 44).

(E)-3-(2-chloro-6-fluorophenyl)-1-phenylprop-2-en-1-one (3b)

FT-IR(υ_{max}, cm^{-1}): 3090, 1664, 1598, 1270. ^{1}H NMR (DMSO-d_6, δppm): 7.42 (t, 1H, J=20.0 Hz, arH), 7.49-7.57 (m, 2H, arH), 7.60 (t, 2H, J=16.0 Hz, arH), 7.71 (t, 1H, J=16.0 Hz, arH), 7.83 (s, 1H, CH), 7.86 (s, 1H, CH), 8.06 (d, 2H, J=4.0 Hz, arH). ^{13}C NMR (DMSO-d_6, δppm): arC: (116.05 (d, J=20.0 Hz, CH), 121.80 (d, J=15.0 Hz, C), 128.95 (2CH), 129.25 (d, J=12.0 Hz, CH), 129.49 (2CH), 132.68 (d, J=10.0 Hz, CH), 133.72 (CH), 134.04 (CH), 137.49 (C), 135.51 (C), 161.73 (d_{C-F}, J=253.0 Hz, C)), 126.82 (d, J=3.0 Hz, CH), 142.69 (CH), 189.78 (C=O). EI MS m/z (%):261.70 ($(M+1)^+$, 100).

General method for the synthesis of compounds 4a,b

Method 1. A mixture of chalcone derivatives (10 mmol), thiosemicarbazide (15 mmol), and KOH (10 mmol) was refluxed in ethanol (30 mL) for 6-7 h. After cooling, the solution was poured into ice-water and stirred for a few minutes. The

precipitate was filtered and crystallized from ethanol.

Method 2. A mixture of chalcone derivatives (10 mmol), thiosemicarbazide (15 mmol), and KOH (10 mmol) in ethanol (10 mL) was irradiated in a closed vessel at 100 W, 100 °C for 40-50 min. After cooling, the solution was poured into ice-water and stirred for a few minutes. The precipitate was filtered and crystallized from ethanol.

Method 3. A mixture of chalcone derivatives (10 mmol), thiosemicarbazide (15 mmol), and KOH (10 mmol) in ethanol (10 mL) was sonicated at 50 °C for 35 min. After cooling, the solution was poured into ice-water and stirred for a few minutes. The precipitate was filtered and crystallized from ethanol.

5-(3-bromophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (4a)

FT-IR(υ_{max}, cm^{-1}): 3482, 3343, 3063, 1564, 1470, 1341. ^{1}H NMR (DMSO-d_6, δppm): 3.20 (q, 1H, J=20.0 Hz), 3.91 (q, 1H, J=32.0 Hz), 5.94 (q, 1H, J=12.0 Hz, CH), 7.14 (d, 1H, J=8.0 Hz, arH), 7.30 (d, 2H, J=4.0 Hz, arH), 7.43-7.47 (m, 4H, arH), 7.89 (d, 2H, J=8.0 Hz, arH), 8.12 (bs, 2H, NH_2). ^{13}C NMR (DMSO-d_6, δppm): 42.69 (CH_2), 62.82 (CH), arC: (122.14 (C), 124.88 (CH), 127.65 (2CH), 128.57 (CH), 129.16 (2CH), 130.29 (CH), 131.12 (CH), 131.22 (C), 131.33 (CH), 146.14 (C)), 155.46 (C=N), 176.14 (C=S). EI MS m/z (%): 300.22 (42), 361.29 ($(M+1)^+$, 100).

5-(2-chloro-6-fluorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (4b)

FT-IR(υ_{max}, cm^{-1}): 3255, 3084, 1583, 1473. ^{1}H NMR (DMSO-d_6, δppm): 3.25 (q, 1H, J=20.0 Hz), 3.99 (q, 1H, J=28.0 Hz), 6.17 (q, 1H, J=16.0 Hz, CH), 7.14-7.20 (m, 1H, arH), 7.31 (s, 2H, arH), 7.48 (s, 3H, arH), 7.88 (s, 2H, arH), 8.03 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6, δppm): 43.20 (CH_2), 58.37 (CH), arC: (115.68 (CH), 125.78 (CH), 127.48 (2CH), 128.51 (d, J=6.0 Hz, C), 129.14 (2CH), 129.81 (d, J=10.0 Hz, CH), 130.90 (CH), 131.29 (C), 133.98 (d, J=7.0 Hz, C), 161.32 (d_{C-F}, J=248.0 Hz, C)), 154.76 (C=N), 175.99 (C=S). EI MS m/z (%): 334.83 (60), 356.88 ($(M+Na)^+$, 100).

General method for the synthesis of compounds 5a-f

Method 1. The mixture of corresponding compound (10 mmol) and substituted phenacyl halides (10 mmol) in ethanol (25 mL) was refluxed in 80 °C for 3-4 h. After completion of reaction, the solvent was evaporated under reduced pressure and a solid appeared. The precipitate was filtered by washing cold water and crystallized from methylene chloride/ethanol (1:1).

Method 2. The mixture of corresponding compound (10 mmol) and substituted phenacyl halides (10 mmol) in ethanol (10 mL) was irradiated in monomode microwave reactor in closed vessel at 100 °C, 100 W for 20 min. After completion of reaction, the solvent was evaporated under reduced pressure and a solid appeared. The precipitate was filtered by washing cold water and crystallized from methylene chloride/ethanol (1:1).

Method 3. The mixture of corresponding compound (10 mmol) and substituted phenacyl halides (10 mmol) in ethanol (10 mL) was sonicated at 50°C for 15 min. After completion of reaction, the solvent was evaporated under reduced pressure and a solid was appeared. The precipitate was filtered by washing cold water and crystallized from methylene chloride/ethanol (1:1).

2-(5-(3-bromophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-4-phenylthiazole (5a)

FT-IR(υ_{max}, cm^{-1}): 3107, 2916, 1617, 1532, 1318. ^{1}H NMR (DMSO-d_6, δppm): 3.46 (q, 1H, J=24.0 Hz), 4.06 (q, 1H, J=32.0 Hz), 5.67 (q, 1H, J=20.0 Hz, CH), 7.34 (t, 1H, J=16.0 Hz, arH), 7.41 (s, 1H, arH), 7.43 (s, 3H, arH), 7.49 (d, 5H, J=4.0 Hz,arH), 7.69 (s, 1H, CH), 7.74 (d, 2H, J=8.0 Hz, arH), 7.80 (d, 2H, J=8.0 Hz, arH). ^{13}C NMR (DMSO-d_6, δppm): 42.69 (CH_2), 62.82 (CH), 105.87 (CH), arC: (122.07 (C), 126.21 (2CH), 126.98 (2CH), 127.65 (2CH), 128.99 (2CH), 129.31 (2CH), 130.58 (CH), 130.70 (CH), 130.96 (CH), 131.26 (C), 131.30 (CH), 132.48 (C), 133.72 (C), 144.63 (C)), 149.62 (C), 153.74 (C=N), 164.95 (thiazole C2). EI MS m/z (%): 304.39 (35), 384.46 (48), 461.41 ($(M+1)^+$, 100).

2-(5-(3-bromophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-4-(4-chlorophenyl)thiazole (5b)

FT-IR(υ_{max}, cm^{-1}): 3061, 2917, 1609, 1532, 1306. ^{1}H NMR (DMSO-d_6, δppm): 3.38 (q, 1H, J=16.0 Hz), 4.03 (q, 1H, J=28.0 Hz), 5.67 (q, 1H, J=20.0 Hz, CH), 6.91 (d, 2H, J=8.0 Hz, arH), 7.31 (d, 2H, J=8.0 Hz, arH), 7.45 (s, 1H, CH), 7.49 (d, 3H, J=8.0 Hz,arH), 7.64 (s, 2H, arH), 7.75 (d, 1H, J=8.0 Hz, arH), 7.81 (d, 3H, J=8.0 Hz, arH). ^{13}C NMR (DMSO-d_6, δppm): 43.25 (CH_2), 64.27 (CH), 105.89 (CH), arC: (122.92 (C), 126.21 (CH), 126.98 (2CH), 127.66 (2CH),

128.99 (2CH), 129.30 (2CH), 130.58 (CH), 130.70 (CH), 130.95 (CH), 131.27 (C), 131.30 (CH), 132.47 (C), 133.73 (C), 144.63 (C)), 149.61 (C), 153.75 (C=N), 164.95 (thiazole C2). EI MS m/z (%): 246.76 (35), 332.45 (58), 495.88 ($(M+1)^+$, 100).

2-(5-(3-bromophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-4-(2,4-dichlorophenyl) thiazole (5c)

FT-IR (υ_{max}, cm^{-1}): 3069, 2919, 1625, 1518, 1358. ^1H NMR (DMSO-d_6, δppm): 3.45 (q, 1H, J=24.0 Hz), 4.06 (q, 1H, J=28.0 Hz), 5.67 (q, 1H, J=20.0 Hz, CH), 7.28 (d, 1H, J=8.0 Hz, arH), 7.33-7.38 (m, 4H, arH), 7.50 (d, 4H, J=8.0 Hz, arH), 7.71 (d, 1H, J=4.0 Hz, arH), 7.74 (s, 1H, CH), 7.81 (d, 2H, J=8.0 Hz, arH). ^{13}C NMR (DMSO-d_6, δppm): 43.23 (CH_2), 64.35 (CH), 105.05 (CH), arC: (122.05 (C), 125.98 (CH), 126.22 (CH), 126.96 (CH), 128.05 (CH), 128.15 (C), 128.98 (2CH), 129.31 (2CH), 130.54 (CH), 130.75 (CH), 130.92 (CH), 131.29 (CH), 131.32 (C), 134.86 (C), 136.45 (C), 144.76 (C)), 150.87 (C), 153.55 (C=N), 164.83 (thiazole C2). EI MS m/z (%): 316.41 (30), 461.29 (64), 530.31 ($(M+1)^+$, 100).

2-(5-(2-chloro-6-fluorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-4-phenylthiazole (5d)

FT-IR (υ_{max}, cm^{-1}): 3055, 2921, 1605, 1542, 1340. ^1H NMR (DMSO-d_6, δppm): 3.51 (q, 1H, J=8.0 Hz), 4.05 (q, 1H, J=16.0 Hz), 6.18 (q, 1H, J=20.0 Hz, CH), 7.26 (d, 1H, J=8.0 Hz, arH), 7.33 (d, 4H, J=8.0 Hz, arH), 7.50 (d, 5H, J=8.0 Hz, arH), 7.70 (s, 1H, CH), 7.80 (d, 4H, J=8.0 Hz, arH). ^{13}C NMR (DMSO-d_6, δppm): 43.76 (CH_2), 60.21 (CH), 104.60 (CH), arC: (125.87 (2CH), 126.78 (2CH), 127.97 (2CH), 128.92 (2CH), 129.32 (2CH), 130.39 (CH), 130.84 (d, J=9.0 Hz, CH), 131.37 (2C), 134.88 (C), 136.39 (C), 161.88 (d_{C-F}, J=248.0 Hz, C)), 150.73 (C), 153.05 (C=N), 164.08 (thiazole C2). EI MS m/z (%): 434.94 ($(M+1)^+$, 100), 456.95 ($(M+Na)^+$, 57).

2-(5-(2-chloro-6-fluorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-4-(4-chlorophenyl)thiazole (5e)

FT-IR (υ_{max}, cm^{-1}): 3111, 2972, 1612, 1543, 1320. ^1H NMR (DMSO-d_6, δppm): 3.52 (q, 1H, J=24.0 Hz), 4.06 (q, 1H, J=28.0 Hz), 6.17 (q, 1H, J=20.0 Hz, CH), 7.39 (d, 4H, J=8.0 Hz, arH), 7.42 (s, 1H, CH), 7.49 (s, 3H, arH), 7.74 (s, 2H, arH), 7.80 (d, 3H, J=4.0 Hz, arH). ^{13}C NMR (DMSO-d_6, δppm): 42.82 (CH_2), 60.02 (CH), 105.02 (CH), arC: (126.80 (2CH), 127.53 (2CH), 128.95 (2CH), 129.33 (2CH), 129.75 (C), 130.45 (2CH), 130.84 (CH), 130.94 (CH), 131.32 (C), 132.42 (C), 133.75 (C), 136.54 (C), 161.88 (d_{C-F}, J=249.0 Hz, C)), 149.47 (C), 153.27 (C=N), 164.16 (thiazole C2). EI MS m/z (%): 286.56 (37), 358.46 (58), 469.40 ($(M+1)^+$, 100).

2-(5-(2-chloro-6-fluorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-4-(2,4-dichlorophenyl)thiazole (5f)

FT-IR (υ_{max}, cm^{-1}): 3084, 2918, 1621, 1557, 1306. ^1H NMR (DMSO-d_6, δppm): 3.38 (q, 1H, J=24.0 Hz), 4.03 (q, 1H, J=28.0 Hz), 5.65 (q, 1H, J=16.0 Hz, CH), 6.92 (d, 2H, J=8.0 Hz, arH), 7.34 (d, 2H, J=8.0 Hz, arH), 7.39 (s, 1H, CH), 7.43 (d, 2H, J=8.0 Hz, arH), 7.49 (d, 2H, J=8.0 Hz, arH), 7.76 (d, 1H, J=8.0 Hz, arH), 7.80 (d, 2H, J=8.0 Hz, arH). ^{13}C NMR (DMSO-d_6, δppm): 43.46 (CH_2), 64.15 (CH), 105.50 (CH), arC: (126.87 (2CH), 127.66 (2CH), 128.45 (C), 128.49 (2CH), 129.00 (2CH), 129.31 (2CH), 130.45 (CH), 131.47(C), 132.39 (C), 133.84 (C), 134.10 (C), 136.75 (C), 160.34 (d_{C-F}, J=243.0 Hz, C)), 149.69 (C), 153.44 (C=N), 164.87 (thiazole C2). EI MS m/z (%): 356.63 (36), 430.76 (51), 503.84 ($(M+1)^+$, 100).

Antioxidant activity

Antioxidant activity studies: In the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity method developed by Blois (33), freshly prepared 1mL methanolic DPPH solution and 100 µL dissolved compound in DMSO were mixed. After mixing, the reaction content was incubated for 30 min at room temperature in the dark and was then measured at 520 nm. By drawing the graph of absorbance readings, the SC_{50} value is calculated by determining the concentration of 50% of the total amount of DPPH radical.

FRAP (the ferric reducing ability of plasma) assay described by Benzie & Strain (34) with some modification was carried out to all synthesized compounds. According to this method, To 100 µL of each sample was added 2.9 mL newly prepared FRAP reagent containing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine) and 20 mmol/L $FeCl_3.6H_2O$ in proportions of 10:1:1 (v/v/v). The mixture was incubated for 30 min at 37 °C and measured at 593 nm. The values were expressed as µmol of Trolox/g. CUPRAC (cupric ion reducing antioxidant capacity) was measured following the procedure described by Apak et al. (35) with some modification. Briefly, 100 µL of each chemical solution was mixed with 900 mL bi-distilled water, 1 mL acetate buffer solution (1 mmol/L, pH: 7.0), 1 mL $CuCl_2$ (10 mmol/L) and 1 mL 7.5 mmol/L neocuproine to a final volume of 4 mL. The reaction mixture was then incubated in the dark for 30 min at room temperature, and

the absorbance of the reaction mixture was measured at 450 nm against a water blank. Trolox was used as the standard calibration curves, and the results were expressed as μmol Trolox equivalent per g.

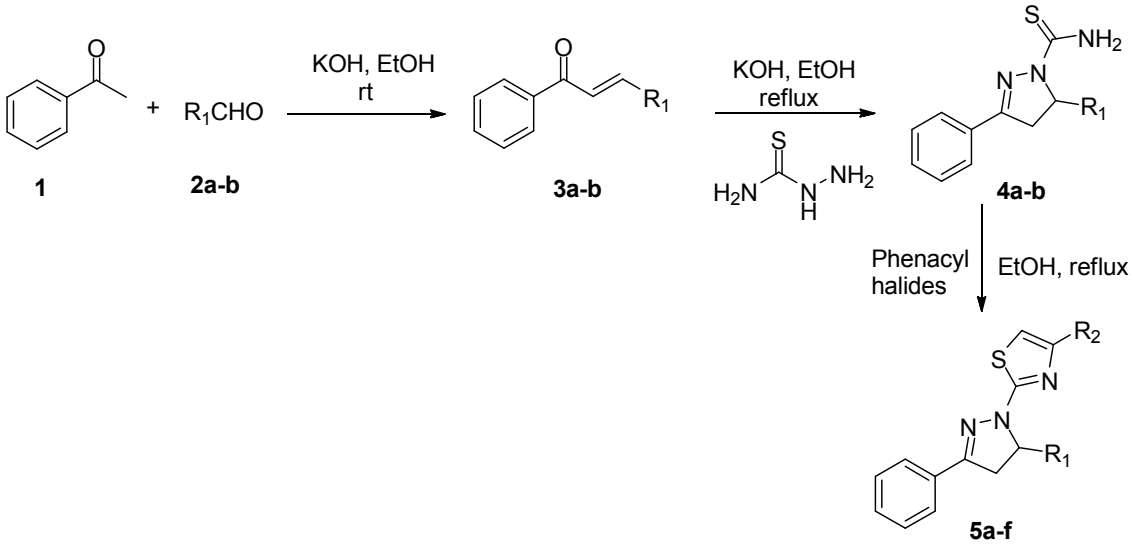
In vitro antiurease inhibition assay (36)

Reaction mixtures including 25 μL of Jack Bean urease, 55 μL of buffer (0.01 mol/L K_2HPO_4, 1 mmol/L EDTA and 0.01 mol/L LiCl, pH 8.2) and 10 mmol/L urea were incubated with 5 μL of the test compounds at room temperature for 15 min in microtiter plates. The production of ammonia was measured following the indophenol method and was used to determine the urease inhibitory activity. The phenol reagent (45 μL, 1% w/v phenol and 0.005% w/v sodium nitroprusside) and alkali reagent (70 μL, 0.5% w/v sodium hydroxide and 0.1% v/v NaOCl) were added to each well. This mixture was incubated for 15 minutes more at 35 °C and optical density was measured at 625 nm against a blank solution including distilled water instead of enzyme. For the determination of the IC_{50} value of the extracts, activity assays were conducted at five different extract concentration and dose-response curve was generated. Thiourea was used as standard inhibitor.

RESULTS AND DISCUSSION

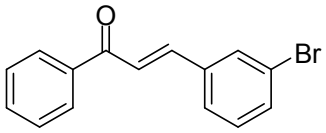
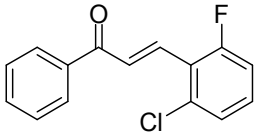
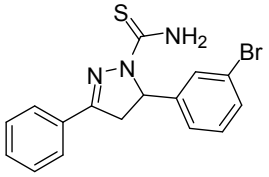
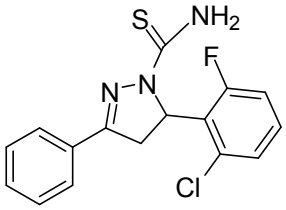
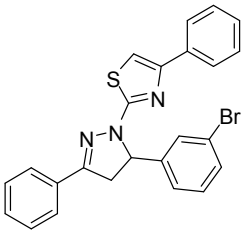
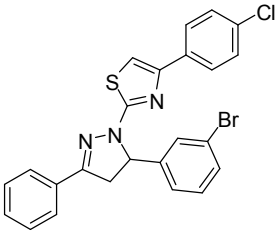
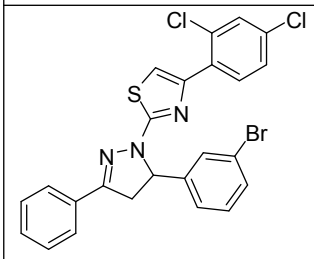
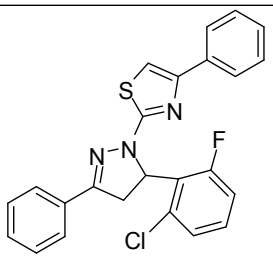
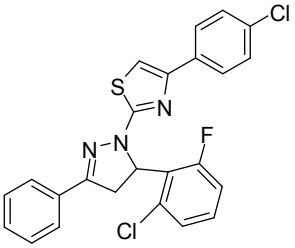
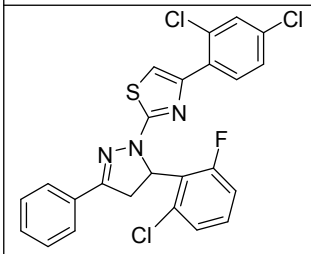
Chemistry

The synthesis of thiazole derivatives was demonstrated in Scheme 1. Acetophenone was treated with 3-bromobenzaldehyde and 2-chloro-6-fluorobenzaldehyde afforded substituted chalcones (**3a,b**) in excellent yields (92%, 88%), respectively. Pyrazoline derivatives (**4a,b**) were synthesized by the reaction between obtained chalcone compounds (**3a,b**) and thiosemicarbazide in basic medium via inter-molecular cyclization. The final compounds (**5a-f**) were obtained by condensing with pyrazolinyl substituted thioamides (**4a,b**) and α-halo ketones.



Scheme 1. Synthesis of thiazolyl-pyrazoline derivatives (**5a-f**).

Table 1. Molecular formula of all compounds.

 3a	 3b	 4a
 4b	 5a	 5b
 5c	 5d	 5e
 5f		

In the present study, all reactions were firstly performed by conventional method. Microwave irradiation and ultrasonic sonication methods which are greener techniques, were performed instead of conventional method due to lower yield, longer reaction times and too much solvent consumption. When applied the greener methods, MW and US, for synthesizing chalcone and thioamide derivatives (**3a,b** and **4a,b**), the reaction time decreased from 4-5 h to 35-40 min (for **3a,b**) and from 6-7 h to 35 min (for **4a,b**). Also, the reaction yields increased from 76-83% to 84-92% (Table 4). In order to obtain the final products, optimization studies were applied in microwave and ultrasound methods. To optimize reaction conditions in microwave irradiation method, 5a was selected and microwave (MW) irradiation was enforced at various power, temperature values and time (the progress of reaction was monitored by TLC) (Table 2). Microwave irradiation reduced the reaction time from 3 h to 20 min when compared to conventional method. Another advantage of the microwave-irradiated optimization study is the increase in reaction yield. When Table 2 is examined, the yields of 76% for have increased to 83%.

After observing the yield increase in the microwave irradiation method, we decided to perform this study with ultrasonic sonication method, which was used in previous studies and obtained very good reaction yields (30). For optimization conditions in ultrasound method, again 5a was chosen, and US was implemented at different temperature and time. When the results were investigated, the best reaction yield was obtained in 50 °C 15 min with 89% (Table 3). When the three methods were compared, the lowest reaction time, the best reaction yield and the minimum solvent consumption were obtained in the ultrasonic method, so the synthesis of the

remaining compounds was also performed according to this method.**Table 2.** Optimization conditions of the reaction in Microwave Irradiation.

All of the compounds represented in Table 1 were characterized by spectroscopic methods (FT-IR, ^{1}H NMR, ^{13}C NMR and EI-MS). In FT-IR spectra of compounds 3a and 3b, a sharp peak at 1656-1664 cm^{-1} verified the presence of carbonyl group. The methylene protons were observed at 7.83-8.03 ppm in ^{1}H NMR spectrum and another evidence that supports the formation of structures is the carbon peaks of the carbonyl group in ^{13}C NMR at 189.53-189.78 ppm. FT-IR spectrum of pyrazoline derivative compounds formed by intramolecular cyclization of chalcone and thiosemicarbazide, the carbonyl peak was lost and amino stretching vibrations were observed. The CH_2 and CH protons observed in the ^{1}H NMR spectrum showed that the pyrazoline ring was formed. Also, the absence of carbonyl carbon in the ^{13}C NMR spectrum and the resonance of the pyrazoline CH_2 and CH carbons at the relevant sites supported the formation of the structure. C=N stretching bands occurring at 1518-1625 cm^{-1} in the FT-IR spectrum of the thiazole derivatives (5a-f) and the disappearance of the amino group in the previous compounds proved the formation of structures. The CH protons in the thiazole ring resonated as a sharp singlet between 7-8 ppm in the ^{1}H NMR spectrum. Meanwhile, the vicinal protons in pyrazole ring appeared in the region of 3.38-4.06 and 5.65-6.18 ppm. In ^{13}C NMR spectra, carbon atom of CH from thiazole ring occurred in 164.08-164.95 ppm. The appearance of (M), (M+1), (M+2) and/or (M+Na) ion peaks at corresponding m/z values confirming their molecular masses for all compounds in EI-MS spectrum.

Biological Activity

Antioxidant Capacity: DPPH, FRAP, and CUPRAC methods which are important for determination of antioxidant capacity (AC, µmol TE/g) of synthesized compounds were applied. When the results of all assays were examined, the best activity among all compounds showed compounds 5a and 5e. (Table 5). For DPPH radical scavenging assay, trolox was used as the standard and the results are given as SC_{50} value. According to DPPH method, all compounds showed low activity except for 5a and 5e that exhibited good activity with 1,15±0.02 and 1.20±0.04 SC_{50} values, respectively. On the other hand, 3a (789.13±25.46 µmol TE/g) and 3b (864.68±16.30 µmol TE/g) for CUPRAC, 3b (5.87±0.03) for DPPH and 5f (428.62±7.42 µmol TE/g) had the lowest AC values among the synthesized compounds.

Table 2. Optimization conditions of the reaction in Microwave Irradiation.

Entry	Solvent	Power (W)	Temp. (°C)	Time (min)	Yield (%)
1	EtOH	100	50	5	76
2	EtOH	100	50	10	77
3	EtOH	100	100	10	81
4	EtOH	100	100	20	83
5	EtOH	100	100	30	80
6	EtOH	100	150	20	78

*Compound 5a was selected for optimization.

Table 3. Optimization conditions of the reaction in Ultrasound Sonication.

Entry	Solvent	Temp (°C)	Time (min)	Yield (%)
1	EtOH	25	10	72
2	EtOH	25	15	74
3	EtOH	40	10	80
4	EtOH	40	15	85
5	EtOH	50	15	89
6	EtOH	50	20	86

*Compound 5a was selected for optimization.

Table 4. Time and yield data of compounds 3-5 using CM, MW and US methods.

Compd.	Time (min)			Yield (%)			mp (°C)
	CM[a]	MW[b]	US[c]	CM[a]	MW[b]	US[c]	
3a	240	35	30	83	89	92	79-81
3b	300	40	35	79	85	88	84-86
4a	360	40	30	76	81	84	199-200
4b	420	50	45	78	82	85	178-179
5a	180	20	15	76	83	89	162-164
5b	180	20	15	78	82	85	155-156
5c	180	20	15	79	84	90	149-150
5d	210	20	15	80	83	88	157-158
5e	210	20	15	78	81	86	150-152
5f	240	20	15	75	79	84	145-147

[a]Conventional Method, [b]Microwave Irradiation Method, [c]Ultrasound Sonication Method.

Table 5. Antioxidant capacity (AC) values and antiurease activity of synthesized compounds.

Compd.	FRAP (μmol TE/g)	DPPH SC_{50}	CUPRAC (μmol TE/g)	Urease Inh. (IC_{50})
3a	465.42±6.12	5.16±0.02	789.13±25.46	13.55±0.02
3b	528.76±7.18	5.87±0.03	864.68±16.30	11.36±0.02
4a	496.37±6.21	5.36±0.04	986.42±12.66	9.89±0.02
4b	466.57±5.68	4.38±0.01	1001.64±6.96	8.74±0.02
5a	884.93±4.15	1.15±0.02	2274.28±18.54	2.28±0.02
5b	642.55±6.42	2.32±0.03	1286.63±8.23	7.12±0.02
5c	586.44±6.84	2.45±0.04	1558.74±5.10	6.56±0.02
5d	540.16±7.32	3.18±0.02	1440.14±10.56	6.48±0.02
5e	796.24±3.28	1.20±0.04	2145.45±15.43	3.36±0.02
5f	428.62±7.42	3.08±0.06	1232.28±16.12	6.82±0.02
Trolox		0.04±0.00		
Thiourea				12.02±0.06

Urease Inhibitory Activity: The obtained compounds were examined for their in vitro urease inhibitory activity against Jack bean urease. Among the synthesized compounds, the thiazolyl-pyrazoline derivatives 5a and 5e displayed excellent activity compared to the thiourea used as the standard drug. Other compounds showed good-moderate activity with different IC_{50} values ranging from 6.48±0.02 to 11.36±0.02. Compound 3a exhibited less activity than thiourea (Table 5).

ADME Prediction: The prediction of ADME properties was implemented by a computational study. In this context, we have calculated molecular volume (MV), molecular weight (MW), logarithm of partition coefficient (miLog P), number of hydrogen bond acceptors (n-ON), number of hydrogen bond donors (n-OHNH), topological polar surface area (TPSA), number of rotatable bonds (n-ROTB) and Lipinski's rule of five (31) using Molinspiration online property calculation toolkit (Molinspiration, 2015). % ABS was calculated by: %ABS=109-(0.345×TPSA) (32). It was monitored that all compounds displayed an excellent % absorption (%ABS) ranging from 94.64% to 100%. All results are indicated in Table 6.

CONCLUSION

In this study, thiazolyl-pyrazoline derivatives were synthesized in comparison with the conventional method, microwave irradiation and ultrasonic sonication methods. Due to the long reaction time and low reaction yield in the conventional studies, green chemical techniques MW and US were used for synthesis. It was observed that the US method was more effective when compared to each other. While the reaction yield in MW method was between 79-89%, it was in US method between 84-92%. When the results were examined in terms of reaction time, the reaction was carried out in the US method in 5 minutes less than the MW method. Also, antioxidant capacity and antiurease inhibition studies of all compounds were investigated. According to the antioxidant capacity results, all

compounds except for **5a** and **5e**, showed low activity. For DPPH, **5a** and **5e** displayed good-moderate activity with SC50 values 1.15±0.02 and 1.20±0.04, respectively when compared to used standard Trolox. Moreover, all compounds exhibited urease inhibition ranging from 1.15±0.02 to 13.55±0.02 when compared to standard drug, thiourea. And compounds 5a-f which may be regarded as new analogues of thiazolyl-pyrazoline derivatives showed good-moderate/excellent inhibitory effect with IC50 values. Especially 5a and 5e displayed excellent activity with the IC50 values 1.15±0.02 and 1.20±0.04, respectively. Based on the biological activity results, it is concluded that these newly synthesized compounds can be regarded as new drug candidates displaying antioxidant and antiurease activity.

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Table 6. Pharmacokinetic parameters for good oral bioavailability of synthesized compound 3-5.

Compd.	**% ABS**	**TPSA (A^2)**	**n-ROTB**	**MV**	**MW**	**miLog P**	**n-ON acceptors**	**n-OHNH donors**	**N violations**
	-	-	-	-	≤500	≤5	<10	<5	≤1
3a	103.11	17.07	3	219.74	287.16	4.60	1	0	0
3b	103.11	17.07	3	220.32	260.69	4.38	1	0	0
4a	94.64	41.62	3	271.32	360.28	4.16	3	2	0
4b	94.64	41.62	3	271.90	333.82	4.12	3	2	0
5a	99.17	28.49	4	361.54	460.40	6.77	3	0	1
5b	99.17	28.49	4	375.08	494.85	7.45	3	0	1
5c	99.17	28.49	4	388.61	529.29	8.05	3	0	2
5d	99.17	28.49	4	362.12	433.94	6.73	3	0	1
5e	99.17	28.49	4	375.66	468.38	7.41	3	0	1
5f	99.17	28.49	4	389.19	502.83	8.02	3	0	2

% ABS: percentage absorption, TPSA: topological polar surface area, n-ROTB: number of rotatable bonds, MV: molecular volume, MW: molecular weight, miLog P: logarithm of partition coefficient of compound between n-octanol and water, n-ON acceptors: number of hydrogen bond acceptors, n-OHNH donors: number of hydrogen bond donors.