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Scientific paper

Microwave Asisted Synthesis of Methylene Bisthiazolo Arylvinyl Pyrazoles as Potential Biological Agents

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Abstract

(2*E*,2'*E*)-1,1'-(5,5'-(5,5'-methylenebis(2-methoxy-5,1-phenylene))bis(3-(4-fluorophenyl)-6-phenyl-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*]thiazole-5,2-diyl))bis(3-phenylprop-2-en-1-ones) **5a–e** were synthesized from (5*Z*,5'*Z*)-2,2'-(5,5'-methylenebis(2-methoxy-5,1-phenylene))bis(5-(4-fluorobenzylidene)-3-phenylthiazolidin-4-ones) **4a–e** through cycloaddition reaction with cinnamic acid hydrazide and evaluated for their antibacterial, antifungal and antinematicidal activity. Among the tested compounds **5b** and **5d** containing chloro and nitro groups were shown to be the most effective against *Ditylenchus myceliophagus* and *Caenorhabditis elegans* with LD_{50} between 160 and 190 ppm, 190 and 210 ppm respectively. Compounds **5b** and **5e** showed good zone of inhibition against *Bacillus subtilis*, compounds **5c** and **5d** were active against *Staphylococcus aureus*, compounds **5a**, **5c** and **5e** were active against *Chromobacterium violaceum* and compounds **5b**, **5a** and **5d** were the most active against *Klebsiella aerogenes*, *Pseudomonas aeruginosa* and *Bacillus sphaericus*. Compounds **5b** and **5d** showed good inhibition towards *Candida albicans* at the concentration of 3.12 μg/mL which is less than the value for amphotericin B, used as the standard.

Keywords: Methylene bis Heterocycles, Thiazolo 2-arylvinylpyrazoles, Microwave irradiation, Biologically active molecules

1. Introduction

Hybrid heterocycles play very important role in drug discovery. Aza heterocyclic derivatives are widely used for the development of therapeutic molecules,[1–3] as they are active against different microorganisms. Following the COVID-19 pandemic, this approach received a lot of attention. Natural and synthetic five membered aza heterocycles exhibit different pharmacological activities, cytotoxic and COX inhibitory activity.[4–10] In recent years, pyrazole derivatives have been used to generate some FDA-approved and commercialized medications, including patented products.

Sulphur-bearing heterocycles create wide interest due to their diverse biological actions. Their derivatives have a variety of biological effects;[11–21] aza heterocyclic ring (pyrazole ring), when combined with other heterocycles, is an advantageous pharmacophore for the synthesis of novel lead compounds.[22–25] The pyrazole-tethered thiazole has attracted a lot of interest in recent years because of its amazing biological properties, which include antimicrobial,[26–30] antibiofilm,[22] as an apoptosis inducer,[31] anti-inflammatory,[29,32] anti tubercular,[33] and anti mycobacterial activity.[34] Pyrazoles bearing 2-arylvinyl (staryl) group at pyrazole nucleus exhibit powerful biological activity[35,36] as well as noteworthy physiochemical features.[37,38]

Microwave processing is an efficient green technology for the conversion of reactants into products.[39] Due to the simplicity, atom economy and better yields of multi component reactions[40] a lot of interest was devoted to this approach.

With this introduction of thiazoles, pyrazoles, 2-arylvinylpyrazoles, microwave processing, inspired by the biological profile of thiazoles, pyrazoles, 2-arylvinylpyrazoles, and part of routine work on development of new hybrid molecules,[41–46] we have synthesized a series of novel methylene bis(2-arylvinyl)thiazolo pyrazoles, and evaluated their antibacterial, antifungal and antinematicidal activities.

2. Experimental

All the reagents and solvents (of analytical grade) were purchased from Sigma Aldrich. Reactions were monitored and purity of the compounds was checked by thin-layer chromatography (TLC) on pre-coated Merck silica gel plates, and spots were observed by exposing them to UV light or immersing the plates in a 1% aqueous potassium permanganate solution. Separations were performed using silica gel chromatographic columns (60–120 mesh). Microwave reactions were carried out in a compact lab microwave catalytic reactor (ZZKD, WBFY-201), and reaction mixture temperatures were recorded using an immersed fiber optic sensor. All melting points are uncorrected and measured using Fisher John's apparatus. IR spectra were recorded using KBr disks on a Perkin–Elmer FT IR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were obtained using a Varian Gemini spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C). Chemical shifts are presented in δ (ppm) with TMS as the internal reference, while coupling constants (*J*) are reported in Hz units. Mass spectra were collected using a VG micro mass 7070H spectrometer. The Perkin–Elmer 240 CHN elemental analyzer was used to obtain elemental analyses (C, H, N) that were within ±0.4% of theoretical values.

General method for production of compounds 4a–e: A mixture of compound **3** (5 mmol), *para*-bromobenzaldehyde (10 mmol), and sodium acetate (5 mmol) in anhydrous glacial acetic acid (10 mL) was refluxed for 3 hours. The reaction mixture was concentrated and then poured into ice cold water. The solid thus separated was filtered, washed with water and the crude product obtained was purified by column chromatography on silica gel with hexane-ethyl acetate as eluent to yield pure product.

(5*Z*,5'*Z*)-2,2'-(5,5'-Methylenebis(2-methoxy-5,1-phenylene))bis(5-(4-bromobenzylidene)-3-phenylthiazolidin-4-one) (4a). M.p. 165–167 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (d, J = 6.2 Hz, 4H, Ar-H), 7.46–7.43 (m, Ar-H, 8H), 7.35 (s, 2H, =CH), 7.30 (d, J = 5.4 Hz, 2H, Ar-H), 7.37 (m, 4H, Ar-H), 7.26 (s, 2H, Ar-H), 6.93 (d, J = 5.2 Hz, 2H, Ar-H), 3.80 (s, 6H, 2×CH_3), 3.73 (s, 2H, CH_2). ^{13}C NMR (60 MHz, $CHCl_3$) δ 172.9, 154.6, 135.7,135.5, 133.9, 132.9, 131.9, 130.4, 129.3, 128.8, 126.2, 125.4, 125.0, 123.5, 114.1, 62.1, 56.7, 42.3. MS *m*/*z* 915 $[M+H]^+$. Anal. calcd. for $C_{47}H_{36}Br_2N_2O_4S_2$: C, 61.55; H, 3.93; N, 3.04. Found: C, 61.58; H, 3.96; N, 3.06.

(5*Z*,5'*Z*)-2,2'-(5,5'-Methylenebis(2-methoxy-5,1-phenylene))bis(5-(4-bromobenzylidene)-3-(4-chlorophenyl)thiazolidin-4-one) (4b). M.p. 212–214 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (d, J = 6.9 Hz, 4H, Ar-H), 7.38–7.42 (m, 12H, Ar-H and CH), 7.32 (s, 4H, =CH), 7.22 (s, 2H, Ar-H), 7.08 (d, J = 5.9 Hz, Ar-H), 6.91 (d, J = 6.8 Hz, 2H, Ar-H), 3.84 (s, 6H, 2×CH_3), 3.52 (s, 2H, CH_2). ^{13}C NMR (60 MHz, $CHCl_3$) δ 172.9, 154.66, 135.5, 133.9, 132.0, 131.9, 129.4, 125.4, 124.9, 123.5, 114.1, 62.1, 56.7, 42.3. MS *m*/*z* 983 $[M+H]^+$. Anal. calcd. for $C_{47}H_{34}Br_2Cl_2N_2O_4S_2$: C, 57.27; H, 3.48; N, 2.84. Found: C, 57.24; H, 3.45; N, 2.81.

(5*Z*,5'*Z*)-2,2'-(5,5'-Methylenebis(2-methoxy-5,1-phenylene))bis(5-(4-bromobenzylidene)-3-(4-bromophenyl)thiazolidin-4-one) (4c). M.p. 195–197 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (d, J = 6.2 Hz, 4H, Ar-H), 7.54 (d, J = 6.9 Hz, 4H, Ar-H), 7.38–7.42 (m, 10H, Ar-H and C-H), 7.32 (s, 2H, =CH), 7.22 (s, 2H, Ar-H), 7.07 (d, J = 6.9 Hz, 2H, Ar-H), 6.91 (d, J = 6.3 Hz, 2H, Ar-H), 3.85 (s, 6H, 2×CH_3), 3.52 (s, 2H, CH_2). ^{13}C NMR (60 MHz, $CHCl_3$) δ 172.9, 154.6, 135.5, 134.8, 133.9, 132.5, 132.1, 132.0, 131.9, 130.4, 128.8, 125.9, 125.4, 124.9, 123.5, 117.1, 114.1, 62.1, 56.7, 42.38. MS *m*/*z* 1071 $[M+H]^+$. Anal. calcd. for $C_{47}H_{34}Br_2N_2O_4S_2$: C, 52.53; H, 3.19; N, 2.61. Found: C, 52.51; H, 3.16; N, 2.59.

(5*Z*,5'*Z*)-2,2'-(5,5'-Methylenebis(2-methoxy-5,1-phenylene))bis(5-(4-bromobenzylidene)-3-(4-nitrophenyl) thiazolidin-4-one) (4d). M.p. 209–210 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (d, J = 5.9 Hz, 4H, Ar-H), 7.71 (d, J = 6.2 Hz, 4H, Ar-H), 7.60 (d, J = 5.9 Hz, 4H, Ar-H), 7.44–7.43 (m, 6H, Ar-H and CH), 7.39 (s, 2H, =CH), 7.18 (s, 2H, Ar-H), 7.10 (d, J = 7.2 Hz, 2H, Ar-H), 6.93 (d, J = 6.0 Hz, 2H, Ar-H), 3.87 (s, 6H, 2×CH_3), 3.60 (s, 2H, CH_2). ^{13}C NMR (60 MHz, $CHCl_3$) δ 172.9, 154.6, 144.3, 143.0, 135.5, 133.9, 132.1, 132.0, 131.9, 130.4, 128.8, 126.7, 125.4, 124.9, 123.5, 121.6, 114.1, 62.1, 56.7, 42.3. MS *m*/*z* 1005 $[M+H]^+$. Anal. calcd. for $C_{47}H_{34}Br_2N_4O_8S_2$: C, 56.07; H, 3.40; N, 5.57. Found: C, 56.05; H, 3.36; N, 5.49.

(5*Z*,5'*Z*)-2,2'-(5,5'-Methylenebis(2-methoxy-5,1-phenylene))bis(5-(4-bromobenzylidene)-3-*para*-tolylthiazolidin-4-one) (4e). M.p. 194–196 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (d, J = 5.9 Hz, 4H, Ar-H), 7.42–7.39 (m, 10H, Ar-H), 7.33 (s, 2H, =CH), 7.23 (d, J = 6.4 Hz, 4H, Ar-H), 7.17 (d, J = 6.1 Hz, 2H, Ar-H), 6.98 (d, J = 6.4 Hz, 2H, Ar-H), 6.71 (s, 2H, CH), 3.85 (s, 2H, CH_2), 3.75 (s, 6H, 2×CH_3), 2.34 (s, 6H, 2×CH_3). ^{13}C NMR (60 MHz, $CHCl_3$) δ 172.9, 154.6, 135.5, 134.7, 134.5, 132.1, 132.0, 131.9, 130.6, 130.4, 128.8, 125.4, 124.9, 123.5, 122.5, 114.1, 62.1, 56.7, 42.3, 21.1. MS *m*/*z* 943 $[M+H]^+$. Anal. calcd. for $C_{49}H_{40}Br_2N_4O_4S_2$: C, 62.29; H, 4.27; N, 2.97. Found: C, 62.27; H, 4.25; N, 2.96.

General procedure for synthesis of compounds 5a–e: A mixture of compound **4** (5 mmol), cinnamic acid hydrazide (10 mmol), and anhydrous sodium acetate (5 mmol) with a catalytic quantity of glacial acetic acid in water was heated in a microwave oven (280 W) up to 8 minutes at 100 °C. The reaction mixture was concentrated and cooled to room temperature, the separated solid was fil-

tered and thoroughly washed with water, and the crude product was purified using column chromatography on silica gel with hexane-ethyl acetate as eluent to get pure compounds.

(2*E*,2'*E*)-1,1'-(5,5'-(5,5'-Methylenebis(2-methoxy-5,1-phenylene))bis(3-(4-fluorophenyl)-6-phenyl-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*]thiazole-5,2-diyl))bis(3-phenylprop-2-en-1-one) (5a). M.p. 246–248 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51–7.49 (m, 6H, Ar-H and =CH), 7.41–7.39 (m, 6H, Ar-H), 7.32 (d, J = 7.0 Hz, 4H, Ar-H), 7.20–7.16 (m, 8H, Ar-H), 7.09 (m, 4H, Ar-H), 6.97 (d, J = 6.2 Hz, 2H, Ar-H), 6.78 (t, J = 7.4 Hz, 2H, Ar-H), 6.61 (d, J = 6.8 Hz, 4H, Ar-H), 6.53–6.52 (m, 4H, Ar-H), 5.61 (s, 2H, N-CH-S), 5.11 (d, J = 5.9 Hz, 2H, CH), 3.85 (s, 6H, 2×CH_3), 3.72 (s, 2H, CH_2). ^{13}C NMR (60 MHz, $CHCl_3$) δ 163.0, 161.8, 155.0, 146.6, 137.3, 135.8, 133.9, 131.8, 132.3, 132.0, 131.8, 129.5, 129.0, 128.0, 126.7, 126.6, 124.6, 120.2, 115.7, 114.3, 72.2, 66.6, 58.4, 56.7, 42.38. MS m/z 1083 $[M+H]^+$. Anal. calcd. for $C_{65}H_{52}F_2N_6O_4S_2$: C, 72.07; H, 4.84; N, 7.76. Found: C, 72.04; H, 4.80; N, 7.73.

(2*E*,2'*E*)-1,1'-(5,5'-(5,5'-Methylenebis(2-methoxy-5,1-phenylene))bis(6-(4-chlorophenyl)-3-(4-fluorophenyl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*]thiazole-5,2-diyl))bis(3-phenylprop-2-en-1-one) (5b). M.p. 271–273 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58–7.53 (m, 6H, Ar-H and =CH), 7.41–7.38 (m, 8H, Ar-H), 7.24–7.17 (m, 10H, Ar-H), 7.04 (m, 4H, Ar-H), 6.94 (d, J = 5.8 Hz, 2H, Ar-H), 6.67 (d, J = 6.8 Hz, 2H, =CH), 6.59 (d, J = 6.2 Hz, 4H, Ar-H), 6.49 (d, J = 7.2 Hz, 2H, CH), 5.61 (s, 2H, CH), 4.95 (d, J = 6.2 Hz, 2H, CH), 3.80 (s, 2H, CH_2), 3.76 (s, 6H, 2×CH_3). ^{13}C NMR (60 MHz, $CHCl_3$) δ 163.0, 161.8, 155.0, 146.6, 135.8, 135.6, 133.9, 132.3, 131.8, 131.6, 130.3, 129.6, 129.0, 128.0, 127.6, 124.6, 120.2, 115.7, 114.3, 72.2, 66.6, 58.4, 56.7, 42.3. MS m/z 1151 $[M+H]^+$. Anal. calcd. for $C_{65}H_{50}Cl_2F_2N_6O_4S_2$: C, 67.76; H, 4.37; N, 7.29. Found: C, 67.74;H, 4.35; N, 7.26.

(2*E*,2'*E*)-1,1'-(5,5'-(5,5'-Methylenebis(2-methoxy-5,1-phenylene))bis(6-(4-bromophenyl)-3-(4-fluorophenyl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*]thiazole-5,2-diyl))bis(3-phenylprop-2-en-1-one) (5c). M.p. 257–259 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (s, 4H, Ar-H), 7.39–7.34 (m, 12H, Ar-H and CH), 7.29 (d, J = 6.2 Hz, 4H, Ar-H), 7.23 (s, 2H, Ar-H), 7.12–7.06 (m, 8H, Ar-H), 6.53 (d, J = 6.2 Hz, 2H, =CH), 6.45 (d, J = 6.2 Hz, 4H, Ar-H), 6.29 (d, J = 7.4 Hz, 2H, CH), 5.61 (s, 2H, CH), 4.86 (d, J = 7.2 Hz, 2H, CH), 3.84 (s, 2H, CH_2), 3.74 (s, 6H, 2×CH_3). ^{13}C NMR (60 MHz, $CHCl_3$) δ 163.0, 161.1, 155.0, 146.6, 137.6, 135.8, 133.9, 133.2, 132.0, 132.3, 131.0, 129.4, 129.0, 128.8, 128.0, 126.6, 124.6, 120.2, 118.8, 115.7, 114.3, 72.2, 6.6, 58.4, 56.79, 42.38. MS m/z 1239 $[M+H]^+$. Anal. calcd. for $C_{65}H_{50}Br_2F_2N_6O_4S_2$: C, 62.91; H, 4.06; N, 6.77. Found: C, 62.89; H, 4.04; N, 6.72.

(2*E*,2'*E*)-1,1'-(5,5'-(5,5'-Methylenebis(2-methoxy-5,1-phenylene))bis(3-(4-fluorophenyl)-6-(4-nitrophenyl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*]thiazole-5,2-diyl))bis(3-phenylprop-2-en-1-one) (5d). M.p. 246–248 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, J = 6.9 Hz, 4H, Ar-H), 7.64–7.61 (m, 6H, Ar-H and =CH), 7.46 (d, J = 6.4 Hz, 4H, Ar-H), 7.40 (m, 2H, Ar-H), 7.25–7.21 (m, 6H, Ar-H), 7.11 (d, J = 5.6 Hz, 2H, Ar-H), 7.05 (m, 4H, Ar-H), 6.92–6.88 (m, 6H, Ar-H), 6.68 (d, J = 6.1 Hz, 2H, CH), 6.41 (d, J = 5.2 Hz, 2H, CH), 5.61 (s, 2H, CH), 4.80 (d, J = 6.1 Hz, 2H, CH), 3.83 (s, 6H, 2×CH_3), 3.57 (s, 2H, CH_2). ^{13}C NMR (60 MHz, $CHCl_3$) δ 163.0, 161.8, 155.0, 146.5, 143.9, 135.8, 133.9, 132.2, 132.0, 131.8, 129.0, 128.8, 128.0, 126.8, 126.2, 124.6, 120.2, 114.3, 115.7, 72.2, 66.6, 58.4, 56.7, 42.3. MS m/z 1173 $[M+H]^+$. Anal. calcd. for $C_{65}H_{50}F_2N_8O_8S_2$: C, 66.54; H, 4.30; N, 9.55. Found: C, 66.50; H, 4.28; N, 9.51.

(2*E*,2'*E*)-1,1'-(5,5'-(5,5'-Methylenebis(2-methoxy-5,1-phenylene))bis(3-(4-fluorophenyl)-6-*para*-tolyl-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*]thiazole-5,2-diyl))bis(3-phenylprop-2-en-1-one) (5e). M.p. 276–278 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (s, 4H, Ar-H), 7.40–7.37 (m, 8H, Ar-H and =CH), 7.31 (d, J = 6.4 Hz, 4H, Ar-H), 7.09–7.08 (m, 8H, Ar-H), 7.03–7.02 (m, 6H, Ar-H), 6.57 (m, 6H, Ar-H and =CH), 6.03 (d, J = 6.4 Hz, 2H, CH), 5.61 (s, 2H, CH), 4.78 (d, J = 6.4 Hz, 2H), 3.81 (s, 2H, CH_2), 3.76 (s, 6H, 2×OCH_3), 2.32 (s, 6H, 2×CH_3). ^{13}C NMR (60 MHz, $CHCl_3$) δ 163.3, 161. 8, 155.0, 146.6, 137.0, 136.5, 135.8, 133.9, 132.3, 132.0, 131.8, 130.7, 129.0, 128.6, 128.0, 127.1, 124.6, 120.2, 114.3, 115.7, 72.2, 66.6, 58.4, 56.7, 42.3, 21.1. MS m/z 1111 $[M+H]^+$. Anal. calcd. for $C_{67}H_{56}F_2N_6O_4S_2$: C, 72.41; H, 5.08; N, 7.56. Found: C, 72.39; H, 5.06; N, 7.53.

2. 1. Antibacterial Assay

Standard inoculums ($1–2·10^{-7}$ colony forming unit (c.f.u.)/mL 0.5 McFarland standards) were applied to the surface of sterile agar plates for the antibacterial assay, and a sterile glass spreader was employed to ensure equal distribution of the inoculums. The discs, measuring 6.26 mm in diameter, were made from Whatman no. 1 filter paper and sterilized with dry heat at 140 °C for 1 hour. The sterile discs had previously been soaked in a known concentration of the test substances and were put in nutritional agar medium. The plates were inverted and incubated for 24 hours at 37 °C. The inhibitory zones were measured and compared to standards. For determining MIC bacteria were cultured overnight in Luria–Bertani (LB) broth at 37 °C, centrifuged, and washed twice with sterile distilled water. Stock solutions for the series of chemicals were produced in DMSO. Each stock solution was diluted with standard method broth (Difco) to generate successive two-fold dilutions ranging from 50 to 0.8 mg/mL. Ten microtiters of broth containing about 105 CFU/mL of test bacteria were added to each well

of a 96-well microtiter plate. Culture plates were incubated at 37 °C for 24 hours, and growth was measured both visually and spectroscopically. The minimum inhibitory concentration (MIC, mg/mL) was discovered to be the lowest concentration required to stop bacterial growth compared to the criteria. To determine the minimum bacterial concentration (MBC), 0.1 mL of each tube was extracted and placed on agar plates. The number of CFUs was counted after 18–24 hours of incubation at 35 °C.

2. 2. Antifungal Assay

For antifungal assay Sabouraud's agar media was made by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 mL) and setting the pH to 5.7. To generate a suspension of fungal spores for lawns, normal saline was employed. A loopful of a certain fungal strain was added to 3 mL saline to create a suspension of the corresponding species. 20 mL of agar media were placed into each petri dish, the excess suspension was decanted, and the plates were dried in an incubator at 37 °C for 1 hour. Wells were produced with an agar punch and labeled individually. A control was likewise produced in triplicate and kept at 37 °C for 3–4 days. The MIC of compounds **5a**–e was measured using the broth dilution method.[38] *C. albicans* was cultured for 48 hours at 28 °C in YPD broth (1% yeast extract, 2% peptone, and 2% dextrose), collected by centrifugation, and washed twice with sterile distilled water. *Aspergillus fumigatus*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes* were plated on potato dextrose agar (PDA) (Difco) and cultured for two weeks at 28 °C. Spores were washed three times with sterile distilled water and resuspended in distilled water to yield an initial inoculum of 105 spores/mL. Each tested compound was dissolved in DMSO and diluted with potato dextrose broth (Difco) to produce serial two-fold dilutions ranging from 100 to 0.8 mg/mL. Each well received ten microtiters of the broth with approximately 103 (for yeast) and 104 (for filamentous fungus) cells/mL of test fungi. Culture plates were incubated at 28 °C for about 48–72 hours. To determine the minimum fungicidal concentration (MFC), 0.1 mL was extracted from each tube and put on agar plates. The number of c.f.u. was recorded after 48 hours of incubation at 35 °C.

3. Result and Discussion

The reaction of methylene bis(phenyl thiazolidenones) **3a**–**e**,[47] with 4-bromobenzaldehyde in acetic acid and sodium acetate for 3 hours produced arylidine thiazolidenones **4a**–**e**. The latter were converted to 2-arylvinylpyrazoles **5a**–**e** in dimethyl sulphoxide via microwave irradiation at 50 °C, yielding unusually high yields of thiazolo 2-arylvinylpyrazoles **5a**–**e**. Their structures were confirmed using FT-IR, mass spectrometry, ^{1}H and ^{13}C NMR spectroscopy techniques. The IR spectra of compounds **5a**–**e** showed the disappearance of the amide carbonyl absorption band at about 1700 cm^{-1}, which was present in compounds **4a**–**e**, confirming the involvement of the α,β-unsaturated carbonyl system. The bands around 1300–1337 cm^{-1}, characteristic for N–C–S bending vibration, provided confirmatory evidence for ring closure. In addition, the absorption bands corresponding to the C=N of the pyrazole moiety were detected at 1600 cm^{-1}. Further support was provided from the ^{1}H NMR spectra, which revealed that the N–CH–S protons of the thiazole ring appeared at δ 5.61 ppm as a singlet and the protons of the pyrazole ring at δ 6.59 ppm as a doublet. These signals indicated that the cyclisation step had occurred. In the ^{13}C NMR spectra, strong signals corresponding to the carbons of the thiazolo 2-arylvinylpyrazolo ring in all compounds are seen near δ 161.8, 146.6, 120.2, 72.2 and 66.6 ppm, providing further evidence of their structures. In conclusion, all of the synthesized compounds produced satisfactory spectral data compatible with their structures.

3. 1. Antinematicidal Activity

For antinematicidal activity[48] against *D. myceliophagus* and *C. elegans* by aqueous *in vitro* screening technique at various concentrations the compounds **5a**–**e** were screened. Median lethal dose at which 50% nematodes became immobile and the results was expressed in terms of LD_{50}. The screened data of the compound **5b** and **5d** reveal that they are the most effective against *D. myceliophagus* and *C. elegans* with LD_{50} values 160–190 ppm, and 190–210 ppm, respectively.

3. 2. Antibacterial Activity

The analysis of antibacterial screening[49–51] data reveals that practically all of compounds **5a**–**e** (Tables 2 and 3) are active and exhibit moderate to good antibacterial activity. Compounds **5b** and **5e** demonstrated good zone of inhibition against *B. subtilis*. Compounds **5c** and **5d** were effective against *S. aureus*. Compounds **5a**, **5c**, and **5e** were active against *C. violaceum*, while compounds **5b**, **5a**, and **5d** were most effective against *K. aerogenes*, *P. aeruginosa*, and *B. sphaericus* (Table 2). Compounds **5d** and **5e** effectively inhibited Gram-positive bacteria at a concentration of 6.12 μg/mL, as shown by their MIC and MBC values (Table 3). The majority of the compounds displayed good antibacterial activity almost similar compared to the standard. Few compounds have the same MBC as the MIC, but many have values that are two to four times greater than the corresponding MIC values.

Antifungal Activity

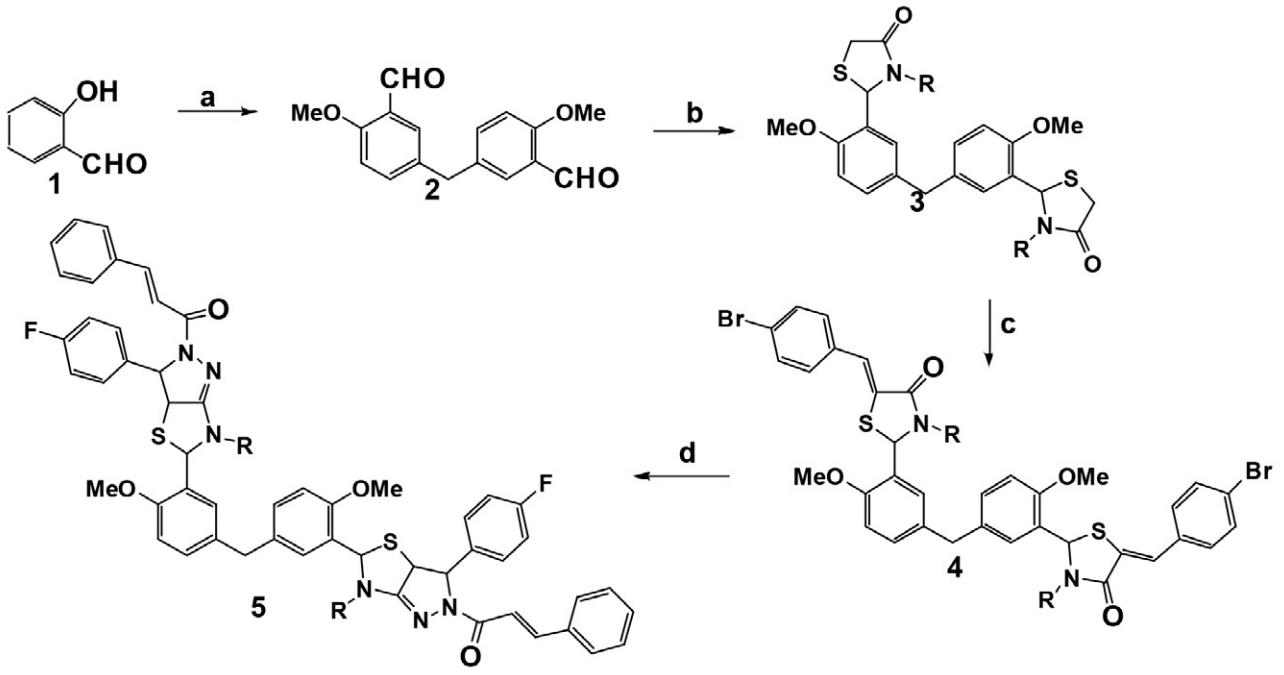
The compounds **5a**–**e** were further tested for antifungal activity against four fungal organisms: *C. albicans*

(10231), *A. fumigatus* (HIC 094), *T. rubrum* (IFO 9185), and *T. mentagrophytes* (IFO 40996) in dimethyl sulphoxide (DMSO) using the agar diffusion method.[52] Amphotericin B was employed as the reference medication, and the zones of fungal inhibition values are shown in Table 4. Table 5 also includes the MIC and MFC (minimum fungicidal concentration) values computed using the broth dilution method.[51] The antifungal screening data presented in Tables 4 and 5 show that the majority of the novel compounds are active and exhibit moderate to good antifungal activity. Among the screened compounds the compound **5e** in which thiazolo 2-arylvinylpyrazole moiety bearing *para*-methylphenyl group showed highest activity against all of the microorganisms employed. The activity of this compound is almost equal to the standard. Compounds **5b** and **5d** showed good inhibition towards *C. albicans* at the concentration of 3.12 μg/mL which is less than amphotericin B standard.

Reagents and conditions: (a) Trioxane, H_2SO_4, AcOH, reflux, 81%; (b) MeI, K_2CO_3, DMF, rt, 83%; (c) R-NH_2, $HSCH_2COOH$, $ZnCl_2$, MWI, 81–90%; (d) *para*-Br-C_6H_5CHO, AcOH, NaOAc, reflux, 80%; (e) 2-arylvinylhydrazide, AcOH, NaOAc, MWI, 80–85%.

Table 1. Nematicidal activity of compounds **5a**–**e**.

S. No	Compound	*D. myceliophagus*	*C. elegans*
1	**5a**	840	650
2	**5b**	160	190
3	**5c**	240	360
4	**5d**	190	210
5	**5e**	380	420
6	**Levamisole**	160	180



Scheme 1. R = H, Cl, Br, NO_2, CH_3

Table 2. Inhibitory zone (diameters, mm) of compounds **5a**–**e** against tested bacterial strain by disc diffusion method.

Compound	*B. subtilis*	*B. sphaericus*	*S. aureus*	*P. aeruginosa*	*K. aerogenes*	*C. violaceum*
5a	14	22	18	20	22	20
5b	24	24	17	21	24	23
5c	16	15	23	16	15	16
5d	18	20	21	19	21	21
5e	26	16	16	18	16	15
Streptomycin	30	20	41	15	25	20
Neomycin	25	28	40	25	30	25

Table 3. Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC)in μg/mL of compound **5a–e**

Compound	*B. subtilis*		*B. sphaericus*		*S. aureus*		*P. aeruginosa*		*K. aerogenes*		*C. violaceum*	
	MIC	MBC	MIC	MIC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
5a	12.5	25.0	25.0	50.0	12.5	12.5	12.5	50.0	12.5	50.0	25.0	25.0
5b	12.5	25.0	25.0	50.0	50.0	12.5	25.0	25.0	25.0	50.0	12.5	25.0
5c	12.5	50.0	12.5	25.0	25.0	12.5	12.5	25.0	12.5	25.0	12.5	50.0
5d	6.25	12.5	6.5	12.5	6.25	6.25	12.5	25.0	12.5	50.0	12.5	12.5
5e	6.25	12.5	6.5	50.0	6.25	25.0	12.5	50.0	6.25	12.5	6.25	6.25
Streptomycin	6.25	12.5	6.25	25.0	6.25	12.5	1.56	6.25	1.56	6.25	3.12	6.25
Penicillin	1.26	6.25	3.12	25.0	1.56	6.25	6,25	12.5	6.25	12.5	12.5	12.5

Table 4. Inhibitory zone diameters (mm) of compounds **5a–e** against tested fungal strains by disc diffusion method

Com pound	Mean zone inhibition (MZI) in mm			
	C. albi-cans	*A. fumi-gatus*	*T. rubrum*	*T. menta-grophytes*
5a	11	10	—	9
5b	22	16	15	16
5c	18	15	13	17
5d	21	17	14	16
5e	20	20	20	18
Amphotericin B	25	20	20	18

hibited *Candida albicans* at a concentration of 3.12 μg/mL, lower than the conventional amphotericin B.

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Conflict of Interest

Authors declared no conflict of interest.

Table 5. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) in μg/mL of compounds **5a–e**

Compound	*C. albicans*		*A. fumigatus*		*T. rubrum*		*T. mentagrophytes*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
5a	—	—	25.0	25.0	25.0	50.0	25.0	25.0
5b	3.12	6.25	25.0	25.0	12.5	25.0	25.0	25.0
5c	12.5	25.0	6.25	12.5	6.25	12.5	6.25	12.5
5d	3.12	6.25	6.25	12.5	6.25	12.5	12.5	25.0
5e	3.12	3.12	3.12	6.25	3.12	6.25	6.25	12.5
Amphotericin B	6.25	12.5	3.12	6.25	3.12	12.5	3.12	12.5

4. Conclusion

A series of new methylene bis 2-arylvinyl thiazolo pyrazoles were synthesized and tested for their antinematicidal, antibacterial, and antifungal properties. Among the studied compounds, **5b** and **5d** containing chloro and nitro substituents are the most efficient against *Ditylenchus myceliophagus* and *Caenorhabditis elegans*, with LD 50 between 160 and 190 ppm, 190 and 210 ppm, respectively. Compounds **5b** and **5e** inhibited *Bacillus subtilis* effectively, compounds **5c** and **5d** inhibited *Staphylococcus aureus*, compounds **5a**, **5c**, and **5e** inhibited *Chromobacterium violaceum*, and compounds **5b**, **5a**, and **5d** inhibited *Klebsiella aerogenes*, *Pseudomonas aeruginosa* and *Bacillus sphaericus* the most. Compounds **5b** and **5d** effectively in-

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Povzetek

S pomočjo cikloadicijske reakcije hidrazida cimetne kisline in (5*Z*,5'*Z*)-2,2'-(5,5'-metilenbis(2-metoksi-5,1-fenilen)) bis(5-(4-fluorobenziliden)-3-feniltiazolidin-4-onov) **4a**–**e** smo pripravili (2*E*,2'*E*)-1,1'-(5,5'-(5,5'-metilenbis(2-metoksi-5,1-fenilen))bis(3-(4-fluorofenil)-6-fenil-3,3a,5,6-tetrahidro-2*H*-pirazolo[3,4-*d*]tiazol-5,2-diil))bis(3-fenilprop-2-en-1-one) **5a**–**e**. Za pripravljene spojine smo raziskali delovanje proti bakterijam, glivam in nematodam. Izmed testiranih spojin sta se spojini **5b** and **5d**, ki vsebujeta kloro oz. nitro skupino, pokazali kot najbolj učinkoviti proti *Ditylenchus myceliophagus* in *Caenorhabditis elegans* z LD_{50} vrednostmi med 160 in 190 ppm, oz. 190 in 210 ppm. Spojini **5b** in **5e** sta izkazali dobro inhibicijo proti *Bacillus subtilis*, spojini **5c** in **5d** sta bili aktivni proti *Staphylococcus aureus*, spojine **5a**, **5c** in **5e** proti *Chromobacterium violaceum*, spojine **5b**, **5a** in **5d** pa so bile najbolj aktivne proti *Klebsiella aerogenes*, *Pseudomonas aeruginosa* in *Bacillus sphaericus*. Spojini **5b** in **5d** v koncentraciji 3.12 μg/mL dobro inhibirata *Candida albicans*; ta vrednost je manjša od vrednosti za amfotericin B, ki smo ga uporabili kot standard.