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SYNTHESIS AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF NOVEL SERIES OF 3,5-DIACETYLPYRIDINE COMPOUNDS

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Abstract: Bis diacetylpyridine derivative (**1**) was prepared and reacted with different halo-compounds, namely: epichlorohydrine and dichloroethyl ethyl ether to give **2a**,**b**, respectively, and reacted with morpholine and piperidine to afford Mannich products **3a**,**b**, successively. Compound **4** was synthesized by reaction of **1** with potassium thiocyanate. Reaction of **4** with 4-chlorobenzaldehyde, glucose and phthalic or maleic anhydrides produced **5**, **6** and **7a**,**b**. Compound **1** reacted with 4-chlorobenzaldehyde to give bisanylmethylene derivative **8**. Also some new compounds **9**-**11** were prepared from the reaction of compound **8** with nucleophiles, namely: hydrazine hydrate, thiosemicarbazide and hydroxylamine *via* Michael condensation reaction. On the other hand, compound **8** was reacted with cyclohexanone and cyclopentanone to give **12a**,**b**. The structures of newly synthesized products have been deduced on the basis of elemental analysis and spectral data. Some synthesized compounds were screened for their antimicrobial evaluation. Among the assayed compounds, derivatives **3b** and **12a** showed the highest antimicrobial activities.

Keywords: bis diacetylpyridines, aminothiazoles, pyrazoles, oxazoles, antimicrobial evaluation

1,4-Dihydropyridine is a six membered aromatic ring containing N atom at the 1st position and is saturated at the 1st and 4th positions. Literature survey exhibits that the pyridine derivatives possess wide spectrum of biological activities such as the calcium channel antagonistic effect (1), antianginal (2-4), antitumor (5), anti-inflammatory (6, 7), antitubercular (8), analgesic activity (9), antithrombotic (10, 11), vasolidation (12), anticonvalsant (13) and stress protective (14). Also, various pyridine derivatives have been synthesized as insecticides (15, 16), antifungal (17), antibacterial (18), herbicidal (19) and antimicrobial agents compared to oxytetracycline (20). Many studies have been devoted to the photochemistry and photooxidation of symmetrical dihydropyridine drugs such as lacidipine (21), nifedipine (22-26) and unsymmetrical dihydropyridine such as amlodipine (27), nisoldipine (28), nilvadipine (29) and nimodipine (30). As regards biological implications, thiosemicarbazide complexes have been intensively investigated for their antiviral, anticancer, antitumor, antimicrobial, antiamoebic and anti-inflammatory activities (31-41). This information encouraged us to synthesize new pyridine compounds to evaluate their antimicrobial activity against different strains of Gram positive, Gram negative bacteria and fungi.

EXPERIMENTAL

Chemistry

All melting points are uncorrected and were recorded in open glass capillary tubes using an Electrothermal IA 9100 digital melting point apparatus. Elemental microanalyses were carried out at Micro Analytical Unit, Central Service Lab (CSL), National Research Centre (NRS), using Vario Elementar apparatus and were found within ± 0.4% of the theoretical values. IR spectra were recorded on Jasco FT/IR, Fourier Transform, infrared spectrometer (Japan), while ^{1}H- and ^{13}C-NMR spectra were obtained using JEOL EX-270 and 500 using available solvent and TMS as internal standard. Mass spectra were recorded on Finnigan Mat SSQ-7000 mass spectrometer at CSL, NRS. TLC on silica gel-60, F254, aluminum sheets were also used.

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4-(4-Chloranylphenyl)-2,6-dimethyl-3,5-bis-(1-oxidanylideneethyl)-1,4-dihydropyridine (1)

A mixture of 4-chlorobenzaldehyde (0.01 mol), acetylacetone (0.02 mol) and 1 g ammonium acetate in 30 mL H_2O was refluxed for 6 h. The solid formed was filtered off and crystallized from diethyl ether.

Yield: 60%; m.p. 180-182°C. IR (KBr, cm^{-1}): 3153 (NH), 1700, 1703 (2C=O). ^1H NMR (DMSO-d_6, δ, ppm): 1.70 (s, 6H, $2CH_3$), 2.30 (s, 6H, $2CH_3$), 4.43 (s, 1H, pyridine-H), 7.00 (d, *J* = 9 Hz, 2H, Ar-H), 7.15 (d, *J* = 9 Hz, 2H, Ar-H), 9.71 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ, ppm): 8.70 ($2CH_3$), 23.10 ($2CH_3$), 26.40 (CH), 109.60 (2C=C), 128.30-135.80 (6 Ar-C), 140.40 (2C=C), 196.50 (2C=O). MS *m*/*z* (%): 303 (100), 192 (70). Analysis: calcd. for $C_{17}H_{18}ClNO_2$ (303.78): C, 67.21, H, 5.97, N, 4.61%; found: C, 67.00; H, 5.93, N, 4.65%.

General procedure for synthesis of compounds 2

A mixture of compound **1** (3.03 g, 0.01 mol) and sodium hydroxide (0.80 g, 0.02 mol) in ethanol (20 mL) was stirred at 60°C for 3 h. The reaction mixture was cooled and then epichlorohydrine or dichloroethyl ethyl ether (0.02 mol) was added. The reaction mixture was heated under reflux for 3 h, then evaporated. The residue was washed with H_2O, filtered off and recrystallized from ethanol.

4-(4-Chloranylphenyl)-2.6-dimethyl-3,5-bis(1-oxidanylideneethyl)-1-(oxiran-2-yl-methyl)-1,4-dihydropyridine (2a)

Yield: 52%; m.p. 162-164°C. IR (KBr, cm^{-1}): 1698, 1703 (2C=O). ^1H NMR (DMSO-d_6, δ, ppm): 1.71 (s, 6H, $2CH_3$, acetyl), 2.30 (s, 6H, $2CH_3$, acetyl), 2.51 (m, 2H, CH_2, oxiranyl ring), 2.77 (m, 1H, CH-oxiranyl ring), 2.80 (m, 2H, CH_2), 4.43 (s, 1H, pyridine-H), 7.00 (d, *J* = 9 Hz, 2H, Ar-H), 7.15 (d, *J* = 9 Hz, 2H, Ar-H). ^{13}C NMR (DMSO-d_6, δ, ppm): 16.50 ($2CH_3$), 23.00 ($2CH_3$), 26.70 (CH-pyroline), 44.61 (CH_2-oxiranyl ring), 50.30 (CH, oxiranyl ring), 52.11 (CH_2), 109.60 (2C=C), 128.80-135.80 (6 Ar-C), 140.40 (2C=C), 196.50 (2C=O). MS *m*/*z* (%): 359 (50), 303 (100). Analysis: calcd. for $C_{20}H_{22}ClNO_3$ (359.84): C, 66.75, H, 6.16, N, 3.89%; found: C, 66.80, H, 6.20, N, 4.10%.

1-(2-[(2-Chloranylethyl)oxidanyl]ethyl)-4-(4-chloranylphenyl)-2,6-dimethyl-3,5-bis(1-oxidanylideneethyl)-1,4-dihydropyridine (2b)

Yield: 51%; m.p. 126-128°C. IR (KBr, cm^{-1}): 1690, 1700 (2C=O). ^1H NMR (DMSO-d_6, δ, ppm): 1.71 (s, 6H, $2CH_3$), 2.30 (s, 6H, $2CH_3$), 2.8 (t, 2H, CH_2N), 3.51 (t, 2H, CH_2O), 3.55 (t, 2H, CH_2Cl), 3.61 (t, 2H, CH_2O), 4.43 (s, 1H, pyridine-H), 7.00 (d, *J* = 9 Hz, 2H, Ar-H), 7.15 (d, *J* = 9 Hz, 2H, Ar-H), 9.71 (s, 1H, NH, D_2O exchangeable). MS *m*/*z* (%): 410 (70), 303 (100). Analysis: calcd. for $C_{21}H_{25}Cl_2NO_3$ (410.33): C, 61.47, H, 6.14, N, 3.41%; found: C, 61.44, H, 6.14, N, 3.50%.

General procedure for synthesis of compounds 3

Formaldehyde (1 mL, 40%) was added to compound **1** (3.03 g, 0.01 mol) in dry ethanol (30 mL), and the reaction mixture was heated for 5 min, cooled, then secondary amine, morphine or piperidine (0.02 mol) was added and the reaction mixture was stirred overnight at room temp. The formed solid was filtered off, dried and recrystallized from methanol.

4-[(4-Chloranylphenyl)-2,6-dimethyl-3,5-bis(1-oxidanylideneethyl)pyridine-1-(4H)-yl)]-morpholine (3a)

Yield: 67%; m.p. 173-175°C. IR (KBr, cm^{-1}): 1698, 1703 (2C=O). ^1H NMR (DMSO-d_6, δ, ppm): 1.66 (s, 6H, $2CH_3$), 2.30 (s, 6H, $2CH_3$), 2.37 (t, 4H, morpholine-H), 3.67 (t, 4H, morpholine-H), 3.95 (s, 2H, N-CH_2-N), 4.43 (s, 1H, pyridine-H), 7.00 (d, *J* = 9 Hz 2H, Ar-H), 7.15 (d, *J* = 9 Hz, 2H, Ar-H). ^{13}C NMR (DMSO-d_6, δ, ppm): 16.20 ($2CH_3$), 23.00 ($2CH_3$), 26.70 (CH-pyridine), 54.70 (2C-morpholine), 69.70 (CH_2), 71.5 (2C-morpholine), 109.00 (2C=C), 128.80-135.60 (6 Ar-C), 140.40 (2C=C), 196.50 (2C=O). MS *m*/*z* (%): 402 (70), 303 (100). Analysis: calcd. for $C_{22}H_{27}ClN_2O_2$ (402.91): C, 65.58, H, 6.15, N, 6.92%; found: C, 65.66, H, 6.75, N, 7.00%.

4-[(4-Chloranylphenyl)-2,6-dimethyl-3,5-bis(1-oxidanylideneethyl)-1-(piperidin-1-yl-methyl)]-1,4-dihydropyridine (3b)

Yield: 65%; m.p. 121-123°C. IR (KBr, cm^{-1}): 1698, 1703 (2C=O). ^1H NMR (DMSO-d_6, δ, ppm): 1.17-1.49 (m, 6H, piperidine-H), 1.70 (s, 6H, $2CH_3$), 2.30 (s, 6H, $2CH_3$), 2.20-2.45 (m, 4H, piperidine-H), 3.72 (s, 2H, N-CH_2-N), 4.43 (s, 1H, pyridine-H), 7.00 (d, *J* = 9 Hz 2H, Ar-H), 7.15 (d, *J* = 9 Hz 2H, Ar-H). MS *m*/*z* (%): 400 (80), 303 (100). Analysis: calcd. for $C_{23}H_{29}ClN_2O_2$ (400.92): C, 68.90, H, 7.29, N, 6.99%; found: C, 68.00, H, 7.32, N, 6.98%.

3,5-Bis(2-amino-1,3-thiazol-5-yl)-4-(4-chloranylphenyl)-2,6-dimethyl-1,4-dihydropyridine (4)

A mixture of compound **1** (3.03 g, 0.01 mol) and potassium thiocyanate (1.94 g, 0.02 mol) was refluxed in glacial acetic acid containing 4 mL

bromine for 3 h. The reaction mixture was cooled and poured into ice water. The formed solid was filtered off, dried and crystallized from dioxane.

Yield: 60%; m.p. 202-204°C. IR (KBr, cm^{-1}): 3350 (NH_2), 3240 (NH). ^1H NMR (DMSO-d_6, δ, ppm): 1.71 (s, 6H, $2CH_3$), 4.00 (s, 4H, $2NH_2$, D_2O exchangeable), 4.43 (s, 1H, pyridine-H), 7.00 (d, *J* = 9 Hz, 2H, Ar-H), 7.15 (d, *J* = 9 Hz, 2H, Ar-H), 7.50 (s, 2H, thiazole-H), 9.8 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ, ppm): 18.80 ($2CH_3$), 43.40 (CH-pyridine), 107.80 (2C=C), 108.00 (2C=C), 128.80-130.60 (6Ar-C), 130.90 (2C=C), 139.00 (2CH), 172.00 (2C=N). MS *m*/*z* (%): 415 (85), 304 (100). Analysis: calcd. for $C_{19}H_{18}ClN_5S_2$ (415.96): C, 54.86, H, 4.36, N, 16.84%; found: C, 54.80, H, 4.40, N, 16.80%.

3,5-Bis(4-chloranylbenzylidene)amino-1,3-thiazole-5-yl)-4-(4-chloranylphenyl)-2,6-dimethyl-1,4-dihydropyridine (5)

A mixture of compound **4** (4.15 g, 0.01 mol) and 4-chlorobenzaldehyde (5.60 g, 0.02 mol) in acetic anhydride (30 mL) was refluxed for 11 h. The solution was cooled, poured into cold water and the precipitate formed was crystallized from glacial acetic acid.

Yield: 45%; m.p. 255-257°C. IR (KBr, cm^{-1}): 3230 (NH). ^1H NMR (DMSO-d_6, δ, ppm): 1.70 (s, 6H, $2CH_3$), 4.43 (s, 1H, pyridine-H), 6.92-7.61 (m, 12H, Ar-H), 8.00 (s, 2H, thiazole-H), 8.10 (s, 2H, Schiff's base), 9.8 (s, 1H, NH, D_2O exchangeable). Analysis: calcd. for $C_{33}H_{24}Cl_3N_5S_2$ (660.64): C, 63.74, H, 4.25, N, 10.93%; found: C, 64.00, H, 4.50, N, 10.90%.

3,5-Bis[(2,3,4,5,6-pentahydroxyhexylidine) amino-1,3-thiazol-5-yl]-4-(4-chloranylphenyl)-2,6-dimethyl-1,4-dihydropyridine (6)

Compound **4** (4.15 g, 0.01 mol) and glucose (7.20 g, 0.2 mol) in ethanol (30 mL) containing 1 mL glacial acetic acid was heated with continuous stirring at 80°C for 6 h. The formed precipitate was filtered off, dried and recrystallized from ethanol.

Yield: 63%; m.p. 188-190°C. IR (KBr, cm^{-1}): 3451-3219 (br, OH and NH), 1590 (CH=N). ^1H NMR (DMSO-d_6, δ, ppm): 1.71 (s, 6H, $2CH_3$), 3.32-3.92 (m, 12H glucose-H), 4.43 (s, 1H, pyridine-H), 4.19-5.00 (m, 10 H, OH, D_2O exchangeable), 6.94 (d, *J* = 9 Hz, 2H, Ar-H), 7.00 (d, *J* = 9 Hz, 2H, Ar-H), 7.40 (2H, CH=N), 8.00 (s, 2H, thiazole-H), 9.80 (1H, NH, D_2O exchangeable). Analysis: calcd. for $C_{31}H_{38}ClN_5O_{10}S_2$ (739.82): C, 53.39, H, 5.74; N, 9.73%; found: C, 53.52; H, 5.70, N, 9.70%.

General procedure for synthesis of compounds 7

To a solution of compound **4** (4.15 g, 0.01 mol) in acetic acid, phthalic anhydride or maleic anhydride (0.02 mol) was added. The mixture was refluxed for 8 h, then poured into ice water. The formed solid was filtered off, washed with water and recrystallized from dioxane.

4-(4-Chloranylphenyl)-3,5-bis[2-(2,7-dioxidanylidene-2,7-dihydroindolin-1-yl)-1,3-thiazol-5-yl]-2,6-dimethyl-1,4-dihydropyridine (7a)

Yield: 55%; m.p. > 300°C. IR (KBr, cm^{-1}): 3235 (NH), 1698 (2C=O), 1702 (2C=O). ^1H NMR (DMSO-d_6, δ, ppm): 1.70 (s, 6H, $2CH_3$), 4.43 (s, 1H, pyridine-H), 7.10-8.20 (m, 12H, Ar-H + 2H, pyrazole-H), 9.82 (s, 1H, NH, D_2O exchangeable). MS *m*/*z* (%): 676 (27). Analysis: calcd. for $C_{35}H_{22}ClN_5O_4S_2$ (676.17): C, 62.17, H, 3.28, N, 10.36%. found: C, 62.20, H, 3.30, N, 10.30%.

4-(4-Chloranylphenyl)-3,5-bis[2-(2,5-dioxidanylidene-2,5-dihydro-1H-pyrol-1-yl)-1,3-thiazol-5-yl]-2,6-dimethyl-1,4-dihydropyridine (7b)

Yield: 69%; m.p. 285-287°C. IR (KBr, cm^{-1}): 3230 (NH), 1701 (2C=O), 1705 (2C=O). ^1H NMR (DMSO-d_6, δ, ppm): 1.70 (s, 6H, $2CH_3$), 4.43 (s, 1H, pyridine-H), 6.12 (d, *J* =5.2 Hz, 2H, vinylic-H), 6.32 (d, *J* = 5.2 Hz, 2H, vinylic-H), 7.00 (d, *J* = 9 Hz 2H, Ar-H), 7.15 (d, *J* = 9 Hz, 2H, Ar-H), 7.50 (s, 2H, thiazole-H), 9.80 (s, 1H, NH, D_2O exchangeable). MS *m*/*z* (%): 576 (60). Analysis: calcd. for $C_{27}H_{18}ClN_5O_4S_2$ (576.48): C, 56.30, H, 3.15, N, 12.20%; found: C, 56.33, H, 3.13, N, 12.25%.

4-[(4-Chloranylphenyl)-2,6-dimethyl-3,5-bis(4-chloroanylphenyl)-1-oxidanylideneprop-2-enyl]-1,4-dihydropyridine (8)

A mixture of compound **1** (3.03 g, 0.01 mol) and 4-chlorobenzaldehyde (2.24 g, 0.02 mol) in ethanol containing 1 g sodium hydroxide was refluxed for 3 h, then cooled and poured into water. The precipitate formed was filtered off and recrystallized from dioxane.

Yield: 50%; m.p. 162-164°C. IR (KBr, cm^{-1}): 3250 (NH), 1698 (C=O). ^1H NMR (DMSO-d_6, δ, ppm): 1.71 (s, 6H, $2CH_3$), 4.45 (s, 1H, pyridine-H), 7.00-7.26 (m, 12H, Ar-H), 7.33 (d, *J* = 12.9 Hz, 2H, methylene), 7.96 (d, *J* = 12.9 Hz, 2H, methylene), 9.80 (s, 1H, NH, D_2O exchangeable). MS *m*/*z* (%): 548 (85), 437 (100). Analysis: calcd. for $C_{31}H_{24}Cl_3NO_2$ (548.88): C, 67.83, H, 4.41, N, 2.55%; found: C, 67.87, H, 4.38, N, 2.60%.

4-(4-Chloranylphenyl)-2,6-dimethyl-3,5-bis(chloranylphenyl)-1H-pyrazol-3-yl)-1,4-dihydropyridine (9)

A mixture of compound **8** (5.48 g, 0.01 mol) and hydrazine hydrate (1 mL, 0.03 mol) was refluxed in absolute ethanol (30 mL) for 4 h. The precipitated solid was filtered off, and crystallized from methanol.

Yield: 45%; m.p. 204-206°C. IR (KBr, cm^{-1}): 3160, 3217, 3220 (3NH). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.70 (s, 6H, $2CH_3$), 4.42 (s, 1H, pyridine-H), 6.50 (s, 2H, pyrazole-H), 7.00-7.42 (m, 12H, Ar-H), 9.80 (s, 1H, NH, D_2O exchangeable), 10.20 (s, 2H, NH-pyrazole, D_2O exchangeable). MS *m*/*z* (%): 572 (55), 461 (100). Analysis: calcd. for $C_{31}H_{24}Cl_3N_5$ (572.91): C, 64.99, H, 4.22, N, 12.22%; found: C, 65.20, H, 4.20, N, 22.40%.

3,5-Bis[1-(aminosulfanylidine)methyl-5-(4-chloranylphenyl)-1H-pyrazol-3-yl]-2,6-dimethyl-4-(4-chloranylphenyl)-1,4-dihydropyridine (10)

A mixture of compound **8** (5.48 g, 0.01 mol) and thiosemicarbazide (1.80 g, 0.02 mol) was refluxed in 30 mL ethanol containing sodium hydroxide (0.80 g, 0.02 mole) for 4 h. The reaction mixture was cooled, poured onto water and the formed solid was filtered off and recrystallized from dioxane.

Yield: 50%; m.p. 228-230°C. IR (KBr, cm^{-1}): 3150 (NH), 3360-3365 ($2NH_2$), 1228 (C=S). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.70 (s, 6H, $2CH_3$), 2.00 (s, 4H, $2NH_2$, D_2O exchangeable), 4.42 (s, 1H, pyridine-H), 6.50 (s, 2H, pyrazole-H), 7.00-7.62 (m, 12H, Ar-H), 9.80 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ, ppm): 18.80 ($2CH_3$), 43.40 (CH-pyridine), 104.00 (2CH, pyrazole), 107.80 (2C=C), 128.40-135.80 (18Ar-C), 130.70 (2C=C), 134.00 (2C=C), 150.00 (2C=C), 190 (2C=S). MS *m*/*z* (%): 691 (60), 580 (100). Analysis: calcd. for $C_{33}H_{26}Cl_3N_7S_2$ (691.09): C, 57.35, H, 3.79, N, 14.19%; found: C, 57.40, H, 3.80, N, 14.22%.

4-(4-Chloranylphenyl)-2,6-dimethyl-3,5-bis(4-chloranylphenylisoxazol-3-yl)-1,4-dihydropyridine (11)

A mixture of compound **8** (5.48 g, 0.01 mol) and hydroxyl amine hydrochloride (1.40 g, 0.02 mol) was refluxed in 30 mL pyridine for 3 h. The reaction mixture was cooled, poured into cold water and neutralized with dil. HCl. The formed solid was filtered off and recrystallized from acetic acid.

Yield: 45%; m.p. 150-152°C. IR (KBr, cm^{-1}): 3145 (NH). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.70 (s, 6H, $2CH_3$), 4.45 (s, 1H, pyridine-H), 6.55 (s, 2H, isoxazole-H), 7.00-7.45 (m, 12H, Ar-H), 9.80 (s, 1H, NH, D_2O exchangeable). MS *m*/*z* (%): 574 (30), 463 (100). Analysis: calcd. for $C_{31}H_{22}Cl_3N_3O_2$ (574.88): C, 64.77, H, 3.86, N, 7.31%; found: C, 64.77, H, 3.90, N, 7.35%.

General procedure for synthesis of compounds 12

A mixture of compound **8** (5.48 g, 0.01 mol), cyclohexanone or cyclopentanone (0.04 mol) was stirred in 30 mL ethanol containing sodium hydroxide (0.06 mol) for 12 h at room temp. The mixture was extracted with ethyl acetate (20 mL) and dried over sodium sulfate anhydrous. After removing off the solvent *in vacuo*, the collected gummy product was precipitated in CCl_4/hexane (3 : 1) and crystallized from dioxane.

[4-(4-Chloranylphenyl)-2,6-dimethyl-3,5-bis(4-chloranylphenyl)-1-oxidanylidene-prop-2-enyl]-1,4-dihydropyridine]cyclohexanone (12a)

Yield: 45%; m.p. 107-109°C. IR (KBr, cm^{-1}): 1695, 1698 (2C=O), 1707-1710 (2C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.70 (s, 6H, $2CH_3$), 4.43 (s, 1H, pyridine-H), 1.80-2.59 (m, 18H, cyclohexanone), 3.17-3.20 (m, 2H, propyl-H), 3.25 (dd, *J* = 11.66, 2.60 Hz, 2H, propyl-H), 3.40 (dd, *J* = 12.90, 3.37 Hz, 2H, propyl-H,), 7.00-7.19 (m, 12H, Ar-H), 9.80 (s, 1H, NH, D_2O exchangeable). MS *m*/*z* (%): 745 (70). Analysis: calcd. for $C_{43}H_{44}Cl_3NO_4$ (745.17): C, 69.31, H, 5.95, N, 1.88%; found: C, 69.40, H, 5.92, N, 2.00%.

[4-(4-Chloranylphenyl)-2,6-dimethyl-3,5-bis(4-chloranylphenyl)-1-oxidanylideneprop-2-enyl]-1,4-dihydropyridine]cyclopentanone (12b)

Yield: 61%; m.p. 110-112°C. IR (KBr, cm^{-1}): 1698, 1700 (2C=O), 1702, 1677 (2C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.71 (s, 6H, $2CH_3$), 2.06-2.43 (m, 14H, pentanone-H), 3.20-3.23 (m, 2H, propyl-H), 3.30 (dd, *J* = 11.88, 3.00 Hz, 2H, propyl-H), 3.45 (dd, *J* = 12.94, 3.25 Hz, 2H, propyl-H), 4.43 (s, 1H, pyridine-H), 7.00-7.19 (m, 12H, Ar-H), 9.80 (s, 1H, NH, D_2O exchangeable). MS *m*/*z* (%): 717 (90). Analysis: calcd. for $C_{41}H_{40}Cl_3NO_4$ (717.11): C, 68.67, H, 5.6,2 N, 1.95%; found: C, 68.70, H, 5.60, N, 1.90%.

Antimicrobial activity

The antibacterial activity of the synthesized compounds was tested against *Bacillus subtilis* NRRL 543 , *Staphylococcus aureus* NRRL B-313 (Gram-positive bacteria), *Escherichia coli* NRRL B-210, *Pseudomonas aeruginosa* NRRL B-23 (Gram-negative bacteria) using nutrient agar medium. The

antifungal activity of the compounds was tested against *Candida albicans* NRRL Y-477 *and Aspergillus niger* NRRL 599 using Sabouraud dextrose agar medium.

Agar diffusion medium

All compounds were screened *in vitro* for their antimicrobial activity by agar diffusion method (42). A suspension of the organisms were added to sterile nutrient agar media at 45°C and the mixture was transferred to sterile Petri dishes and allowed to solidify. Holes of 10 mm in diameter were made using a cork borer. An amount of 0.1 mL of the synthesized compounds was poured inside the holes. A hole filled with DMSO was also used as control. The plates were left for 1 h at room temperature as a period of pre-incubation diffusion to minimize the effects to variation in time between the applications of the different solutions. The plates were then incubated at 37°C for 24 h and observed for antibacterial activity. The diameters of zone of inhibition were measured and compared with that of the standard; the values were tabulated. Ciprofloxacin (50 µg/mL) and fluconazole (50 µg/mL) were used as standard for antibacterial and antifungal activity, respectively. The observed zones of inhibition are presented in Table 1.

Minimal inhibitory concentration

Minimal inhibitory concentration (MIC) of the test compounds were determined by agar streak dilution method. Stock solutions of the synthesized compounds (100 mg/mL) were made using DMSO as the solvent. From this stock solution, a range of concentrations from 5 to 0.05 mg/mL of the tested compounds solutions was mixed with the known quantities of molten sterile agar media aseptically. About 20 mL of nutrient agar medium for bacteria and Sabouraud dextrose agar medium for fungi containing the tested compound under study was dispensed into each sterile Petri dish. Then, the media were allowed to get solidified. Microorganisms were then streaked one by one on the agar plates aseptically. After streaking, all the plates were incubated at 30°C for 24 h/48 h for bacteria and fungi, respectively. Then, the plates were observed for the growth of microorganisms. The lowest concentration of the synthesized compounds inhibiting the growth of the given bacteria/fungus was considered as minimal inhibitory concentration (MIC) of the test compounds against that bacteria or fungi on the plate. The MIC values of each compound against various bacteria and fungi were tabulated in Table 2.

RESULTS AND DISCUSSION

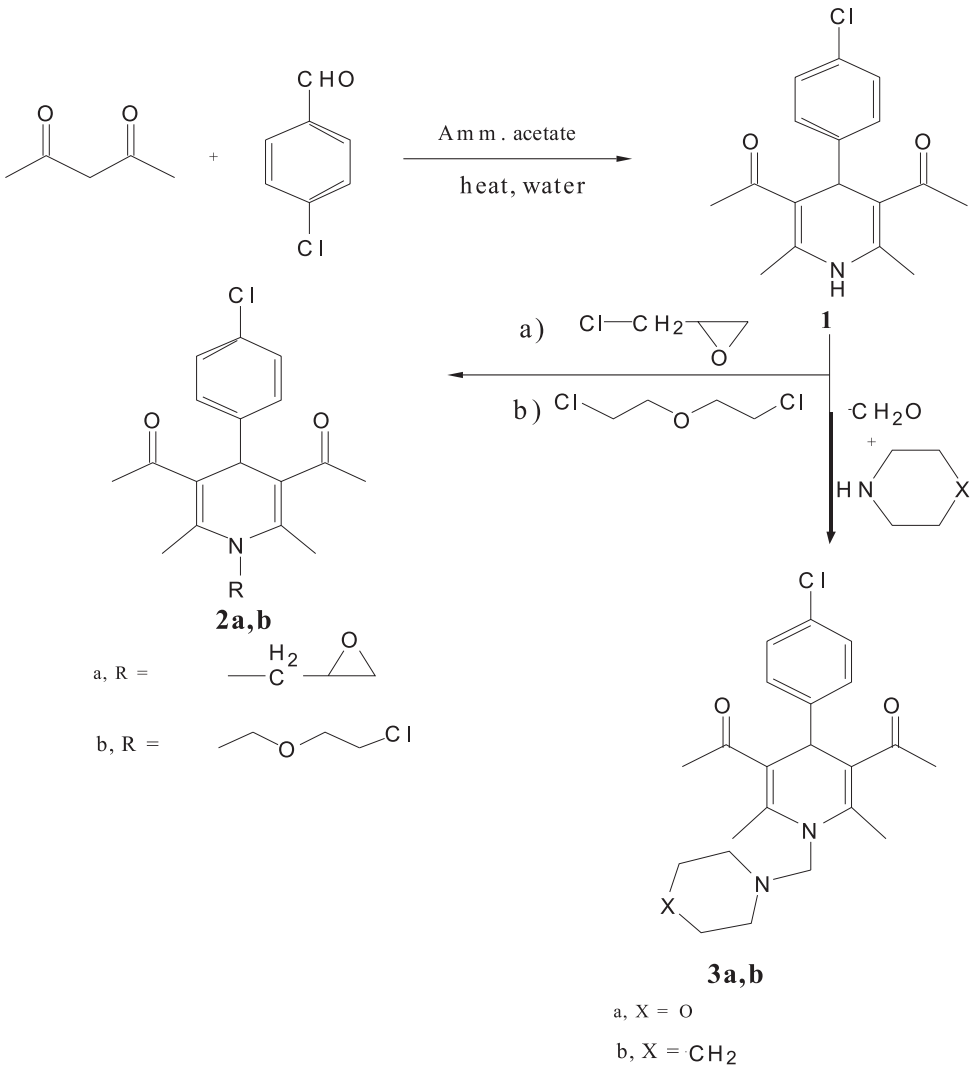
Chemistry

4-(4-Chlorophenyl)-2,6-dimethyl-3,5-bisdiacetyl-1,4-dihydropyridine (**1**) was prepared *via* condensation of 4-chlorobenzaldehyde and acetylacetone in the presence of ammonium acetate. The assignment of the structure was proved based on ele-

Table 1. Inhibition zone in mm as a criterion of antibacterial and antifungal activities of the newly synthesized compounds.

Compound	Microorganism inhibition zone diameter (mm)					
	Gram positive bacteria		Gram negative bacteria		Fungi	
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*
1	14	13	15	14	13	11
2a	13	12	14	13	14	12
3a	17	16	18	17	16	14
3b	23	21	24	22	22	19
8	19	18	20	18	19	15
9	17	17	18	18	18	16
10	17	16	17	17	15	13
12a	25	23	25	25	21	19
Ciprofloxacin	22	24	24	23	-	-
Fluconazole	-	-	-	-	22	24

Highly active = inhibition zone > 20 mm, moderately active = inhibition zone 15-20 mm, slightly active = inhibition zone 11-14 mm, inactive = inhibition zone < 11 mm.



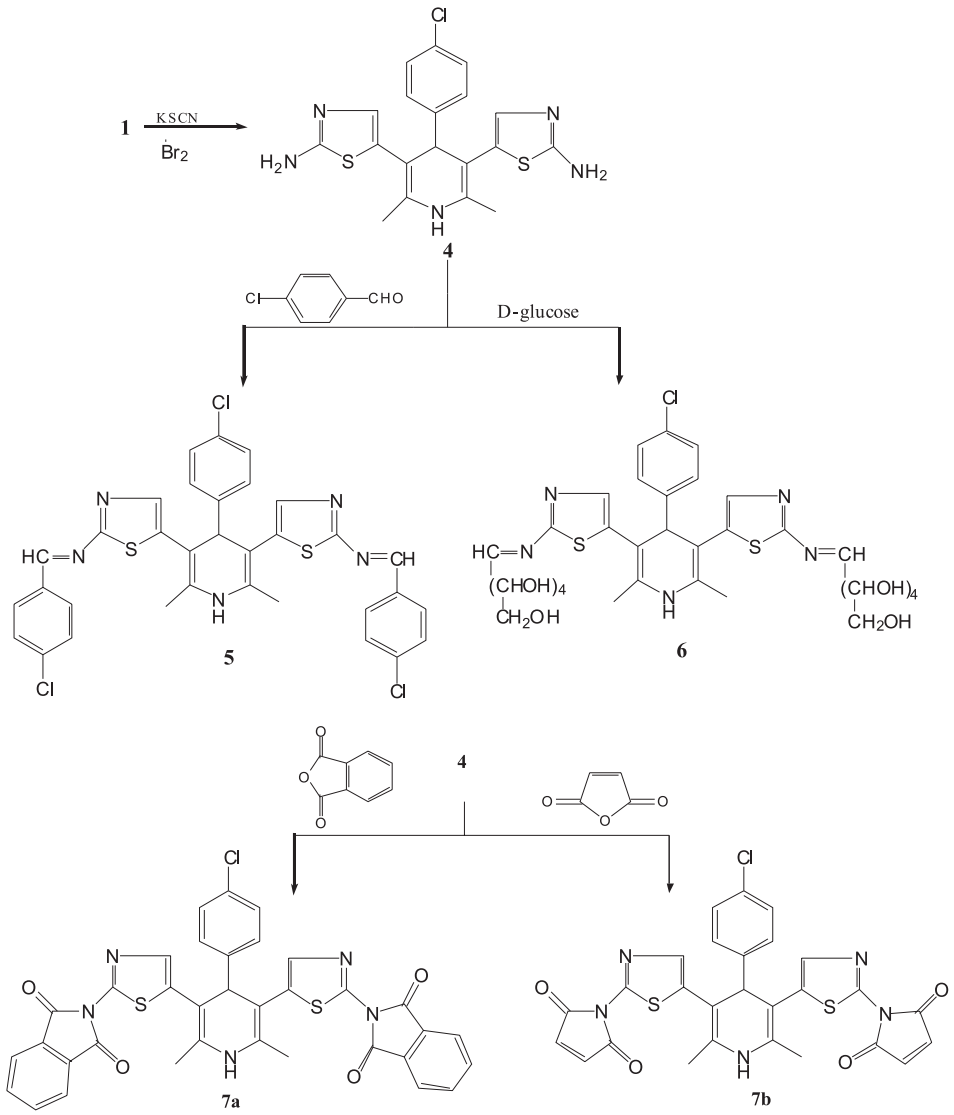
Scheme 1. Synthesis of compounds **1**-**3**

Table 2. MIC in µg/mL of the newly synthesized compounds against microorganisms.

Compound	Gram positive bacteria		Gram negative bacteria		Fungi	
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*
1	1.4	1.6	1.2	1.4	1.6	2
2a	1.8	1.8	1.4	1.6	1.4	1.8
3a	0.8	1	0.6	0.8	1	1.4
3b	0.14	0.18	0.12	0.16	0.16	0.4
8	0.4	0.6	0.2	0.6	0.4	1.2
9	0.8	0.8	0.6	0.6	0.6	1
10	0.8	1	0.8	0.8	1.2	1.6
12a	0.1	0.14	0.1	0.1	0.18	0.4

mental analysis and spectral data. The IR spectrum showed characteristic absorption bands at 1700, 1703 cm^{-1} (2C=O). The ^{1}H-NMR spectrum showed signals at 1.70 ($2CH_3$), 2.30 ($2CH_3$, acetyl), 4.43 (pyridine proton), 7.00-7.15 (Ar-H) and D_2O exchangeable signal at 9.70 ppm assigned for NH. The mass spectrum of **1** showed the molecular ion peak at m/z 303 [M^+, 100], also peak at m/z 305 [M^{2+}, 33] was observed. Compound **1** was transformed chemically *via* the reaction with acyclic alkyl halides yielding N-acyclic nucleoside of pyridine derivatives **2a**,**b**. Mannich adducts also were produced *via* the reaction of **1** with formaldehyde followed by the addition of different amines, namely: morpholine and piperidine affording **3a**,**b**, respectively (Scheme 1). The IR spectra showed no NH absorption for compounds **2** and **3**. The ^{1}H-NMR spectrum of **3b** as representative example showed signals at 1.17-1.49 (m, 6H, piperidine-H), 2.20-2.45 (m, 4H, piperidine-H) and 3.72 ppm (s, 2H, N-CH_2-N). The mass spectrum showed molecular ion peak at m/z 400 (80%).

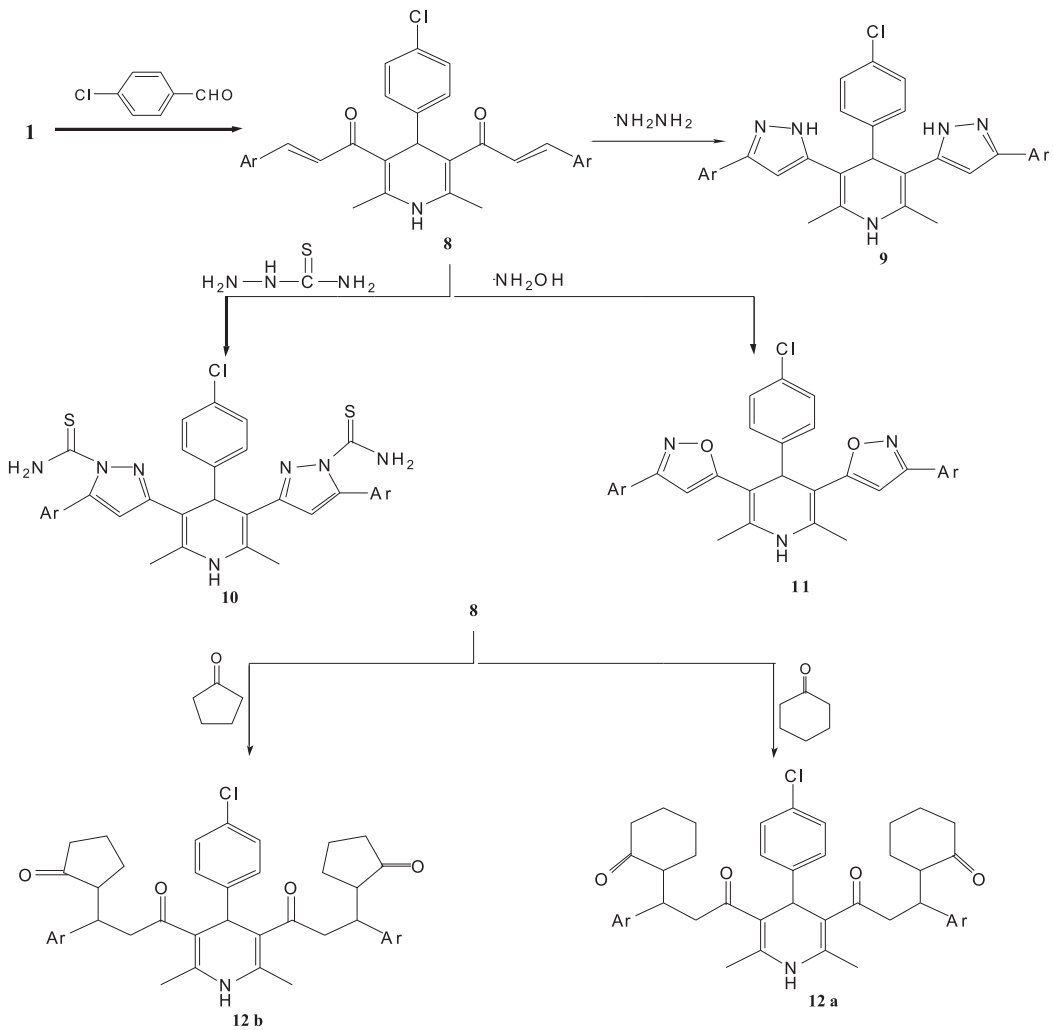
When compound **1** was reacted with potassium thiocyanate in the presence of bromine, 3-bisaminothiazole derivative **4** was produced. The IR spectrum of **4** showed absorption band at 3350 cm^{-1} (NH_2). The ^{1}H-NMR spectrum showed two characteristic signals at 4.00 (NH_2, D_2O exchangeable) and



Scheme 2. Synthesis of compounds **4**-**7**

at 7.50 ppm (thiazole protons). Compound **4** was transformed *via* condensation with 4-chlorobenzaldehyde in glacial acetic acid, glucose in ethanol containing drops of acetic acid and phthalic or maleic anhydrides in glacial acetic acid yielding compounds **5**, **6** and **7a**,**b**, respectively (Scheme 2). The structures of the aforementioned compounds were confirmed on the basis of microanalytical and spectral data. The ^{1}H-NMR spectrum of compound **5** showed a new singlet at 8.10 ppm due to –CH=N-. The mass spectrum of **5** showed a molecular ion peak at m/z 660 supporting its molecular formula. The IR spectrum of **6** was characterized by the appearance of a broad absorption bands of OH and NH groups at the range of 3451–3219 cm^{-1}, while the CH=N appeared at 1590 cm^{-1}. The ^{1}H-NMR spectrum of compound **6** showed the glucose protons as multiplet at the range 3.32–3.92 ppm and the OH groups at the range 4.19–5.00 ppm. The IR spectra of **7** showed bands at 1702–1698 cm^{-1} (C=O). The ^{1}H-NMR spectrum of compound **7b** revealed the presence of two doublets at 6.12 and 6.32 ppm assigned for vinylic protons.

On the other hand, condensation of compound **1** with 4-chlorobenzaldehyde gave bis arylmethylene derivative **8**. The ^{1}H-NMR spectrum of **8** showed absence of $2CH_3$ (acetyl) signals and presence of CH=CH (methylene) at 7.33 and 7.96 ppm. The mass spectrum showed molecular ion peak at m/z 548 (85%). Furthermore, condensation of **8** with different nucleophiles, namely: hydrazine hydrate, thiosemicarbazide and hydroxylamine *via* Micheal condensation reaction gave compounds **9–11,** respectively (Scheme 3).



Scheme 3. Synthesis of compounds **8**-**12**

The structures of compounds **9–11** were in agreement with their spectral and analytical data. The mass spectrum of compound **9** showed a molecular ion peak at m/z 572 (55%). Its ^{1}H-NMR spectrum showed singlet at 6.50 ppm characteristic for pyrazole ring protons. Compound **8**, when condensed with cyclohexanone and cyclopentanone, afforded compounds **12a**,**b**. The IR spectrum of **12a** showed absorption bands at1695, 1698, 1700–1705, 1710 cm^{-1} (C=O). The ^{1}H-NMR spectrum of **12a** showed multiplet at 1.80–2.59 for 18 protons of cyclohexanone, signals at 3.17–3.20 for 2CH-propyl protons and at 3.25–3.40 ppm for 2CH_2-propyl protons. The mass spectrum of **12a** showed molecular ion peak at m/z 745 (100%).

Antimicrobial activity

All the newly synthesized compounds were screened for their *in vitro* antibacterial activity against two strains of Gram positive bacteria *(Staphylococcus aureus, Bacillus subtilis*), and two strains of Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) using ciprofloxacin as a standard drug (100 μg/mL). They were also evaluated for their *in vitro* antifungal activity against the mycotic strains (*Candida albicans* and *Aspergillus niger*) using fluconazole as a standard antifungal drug (100 μg/mL). Agar-diffusion method was used in this investigation for determination of the preliminary antibacterial and antifungal activity and the results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the discs in mm (Table 1). The minimal inhibitory concentrations (MIC) were determined for compounds showing promising growth inhibition, using the twofold serial dilution method (43). The MIC (μg/mL) values against the tested bacterial and fungal isolates are presented in Table 2.

According to Tables 1 and 2, it is clear that compounds **1**, **2a** and **3a** showed low activities toward all types of microorganisms. Compounds **9** and **10** showed moderate antibacterial and antifungal activities. Bis-arylmethylene derivative **8** was found to be highly active against *Escherichia coli*, but showed moderate activity towards Gram positive bacteria, Gram negative bacteria and fungi. Derivatives **3b**, 3,5-bis-pyridin-1H-morpholine and **12a**, dihydropyridine cyclohexanone, showed high activity toward all microorganisms.

CONCLUSION

In the present study, 2,6-dimethyl-3,5-bis-acetyl-1,4-dihydropyridine (**1**) was used to synthesize novel derivatives of N-acyclic nucleosides (**2a**,**b**), Mannich products (**3a**,**b**), 3,5-bis-aminothiazole (**4**), heterocyclic derivatives (**5**-**7**), 3,5-bisarylmethylene (**8**) and (**9**-**12**). The antimicrobial activity of some compounds was reported. Compounds **3b** and **12a** showed high activity against Gram positive bacteria, Gram negative bacteria and fungi.

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REFERENCES

1. Triggle D.J.: Biochem. Pharmacol. 74, 1 (2007).
2. Love B., Goodman M., Snader K., Tedeschi R., Macko E.: J. Med. Chem. 17, 956 (1974).
3. Bossert F., Meyer H., Wehinger E.: Angew. Chem. Int. Ed. Engl. 20, 762 (1981).
4. Breitenbucher, J.G., Figliozz G.: Tetrahedron Lett. 41, 4311 (2000).
5. Boer R., Gekeler V.: Drugs Fut. 20, 499 (1995).
6. Briukhanov V.M., Zverev Iaf, Elkin V.I.: Eksp. Klin. Farmakol. 57, 47 (1994).
7. Bahekar S., Shinde D.:, Acta Pharm. (Zagreb) A. 52, 281 (2002).
8. Wachter G.A., Davis M.C.: J. Med. Chem. 41, 2436 (1998).
9. Gullapalli S., Ramarao P.: Neuropharmacology 42, 467 (2002).
10. Sunkel C.E., De Casa Juana M.F., Santos L.: J. Med. Chem. 33, 3205 (1990).
11. Ono Handkinura M.: Arzneimittelforschung 3, 1131 (1981).
12. Tusell J.M., Barron S., Seratosa J.: J. Brain Res. 622, 99 (1993).
13. Tarasenko L.M., Neporada K.S., Klusha V.: Bull. Exp. Biol. Med. 133, 426 (2002).
14. Budriesi R., Ioan P., Locatelli A., Cosconati S., Leoni A., Maria P. Ugenti M.P., Andreani A. et al.: J. Med. Chem. 51, 1592 (2008).
15. Kagabu S., Azuma A., Nishimura K.: J. Pest. Sci. 27, 267 (2002).
16. Nishiwaki H., Nakagawa Y., Ueno T., Kagabu S., Nishimura K.: Science 57, 810 (2001).
17. Raj M.P.P., Rao J.T.: Asian J. Chem. 25, 492 (2003).
18. Reddy D.B., Seshamma T., Seenaiah B.: Indian J. Chem. 30B, 46 (1991).
19. Kennedy A.D., Summers A.L.: J. Heterocycl. Chem. 18, 409 (1981).
20. Attia A., Abo-Ghalia M.H., Abd El-Salam O.I.: Pharmazie 50, 455 (1995).

21. De Filippis P., Bovina E., Da Ros L., Fiori J., Cavrini V.: J. Pharm. Biomed. Anal. 27, 803 (2002).
22. Testa R., Dolfini E., Reschiotto C., Secchi C., Biondi P.A.: Farmaco Ed. Prat. 34, 463 (1979).
23. Jakobsen P., Lederballe Pedersen O., Mikkelsen E.J.: J. Chromatogr. 162, 81 (1979).
24. Pietta P., Rava A., Biondi P.: J. Chromatogr. 210, 516 (1981).
25. Majeed I.A., Murray W.J., Newton D.W., Othman S., Al-Turk W.A.: J. Pharm. Pharmacol. 39, 1044 (1987).
26. Krivopalov V.P., Sedova V.F., Shkurko O.P.: Russ. Chem. Bull. Int. Ed. 52, 2440 (2003).
27. Ragno G., Garofalo A., Vetuschi C.: J. Pharm. Biomed. Anal. 27, 19 (2002).
28. Marinkovic V., Agbaba D., Karljikovic-Rajic K., Comor J., Zivanov-Stakic D.: Farmaco 55, 128 (2000).
29. Mielcarek J., Stobiecki M., Franski R.: J. Pharm. Biomed. Anal. 24, 71 (2000).
30. Zanocco A., Diaz L., López M., Nuñez-Vergara L.J., Squella J.A.: J. Pharm. Sci. 81, 920 (1992).
31. Afrasiabi Z., Sinn E., Padhye S., Dutta S., Newton C., Anson C.E., Powell A.K.: J. Inorg. Biochem. 95, 306 (2003).
32. Rakhi C., Shelly C.: Res. J. Chem. Sci. 1, 1 (2011).
33. Berhardt P.V., Caldwell L.M., Lovejoy D.B., Richardson D.R.: Acta Cryst. 59 C, 629 (2003).
34. Afrasiabi Z., Sinn E., Chen J.: Inorg. Chim. Acta 357, 271 (2004).
35. Lakobidou Z., Mioglou E., Mourelatos D., Kotsis A., Demertzis M.A., Papagoergiou A., Miller J.M., Demertzi D.K.: Anticancer Drugs, 12, 65 (2001).
36. Singh S., Bharti N., Naqvi F., Azam A.: Eur. J. Med. Chem. 39, 459 (2004).
37. Sharma S., Athar F., Maurya M.R., Naqvi F., Azam A.: Eur. J. Med. Chem., 40, 557 (2005).
38. Quenelle D.C., Keith K.A., Kern E.R.: Antivir. Res. 71, 24 (2006).
39. Bharti N., Athar F., Maurya M.R., Azam A.: Bioorg. Med. Chem. 12, 4679 (2004).
40. Jeragh B.J.A., El-Dissouky A.: J. Coord. Chem. 58, 1029 (2005).
41. Labisbal E., Haslow K.D., A Sousa-Pedrares., Valdés-Martínez J., Hernández-Ortega S., West D.X.: Polyhedron 22, 2831 (2003).
42. Gross D.C., De Vay S.E.: Physiol. Plant Pathol. 11, 13 (1977).
43. Bauer A.W., Kirby W.W.M., Sherris J.C., Turck M.: Am. J. Clin. Pathol. 45, 493 (1966).

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