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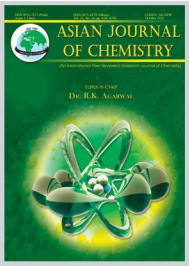

Synthesis and Antibacterial Activity of Novel Hydrazones Derived from 4,5-Diazafluoren-9-hydrazone

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An environmentally benign series of novel hydrazones were synthesized by condensation of 4,5-diazafluoren-9-hydrazone with various aldehydes and ketones by using acetic acid as a catalyst under solvent free condition. This protocol gives excellent yield of the products. All synthesized products were characterized by IR, NMR, mass and also tested for antibacterial (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella pneumonae*) activities by disc diffusion method.

Key Words: 4,5-Diazafluoren-9-hydrazone, Grinding, Acetic acid, Hydrazones, Antibacterial.

INTRODUCTION

Hydrazone is an important class in organic chemistry. These compounds have interesting biological activities such as antimycobacterial[1], antimicrobial[2-4], antituberculosis[5], anti-tumorals[6], antiinflammatory[7,8], antimalarial[9], anticonvulasnt[10] and anticancer-anti HIV[11].

At present, a broad range of methods for synthesis of hydrazones were reported such as microwave irradiation [polystyrene sulfonic acid][12], ultrasound irradiation[13], Ball-mill process[14], grinding method [PTSA][15] and also by refluxing method[16-18]. But these known methods of hydrazones synthesis suffers from one or other limitations such as harsh reaction conditions, expensive reagents, low yields and relatively long reaction time.

Because of that the researcher still continuous to synthesize novel hydrazones with better methodology in terms of simplicity, eco-friendly and economic viability which is achieved by using acetic acid. Thus, in this article, we report the synthesis of novel hydrazones derived from 4,5-diazafluorene-9-hydrazone in acetic acid as a catalyst by grinding method. Synthesis of organic compounds by grinding method has the advantages of shorter time, higher yield, mild reaction condition as well as being environmentally friendly[19]. Thus grinding method comes under the title of green chemistry. This synthesized hydrazones are also assayed for antibacterial activities.

EXPERIMENTAL

Melting points of the synthesized compounds were determined in open-glass capillaries on a stuart-SMP10 melting point apparatus and are uncorrected. IR absorption spectra were recorded on a Perkin Elmer 1650 FTIR using KBr pellets in the range of 4000-450 cm^{-1}. ^1H NMR was recorded on a Bruker spectrometer operating at 300 MHz. The ^1H NMR chemical shifts are reported as parts per million (ppm) downfield from TMS (Me_4Si) used as an internal standard. Mass spectra were recorded on LCQ ion trap mass spectrometer. Purity of the compounds were checked by thin layer chromatography (TLC) on Merck silica gel 60 F_{254} pre-coated sheets in benzene/methanol mixture and spots were developed using iodine vapour as visualizing agents.

General procedure

Synthesis of 4,5- diazafluoren-9-one (2): The 4,5-diazafluoren-9-one was synthesized using method reported in the literature[20].

Synthesis of 4,5-diazafluoren-9-hydrazone (3): A mixture of 4,5-diazafluoren-9-one (1 mmol) and hydrazine hydrate (1.1 mmol) was crushed in mortar with a pestle at room temperature and acetic acid (20 mol %) was added and crushed then the progress of reaction was monitored by TLC. After completion of reaction (3 min) the crude product was washed with water, dried and purified by column chromatography.

Synthesis of hydrazones on grinding (4a-o and 5a-e): A mixture of 4,5-diazafluoren-9-hydrazone (1 mmol) and aldehyde/ketone (1 mmol) was crushed in mortar with a pestle at room temperature and acetic acid (20 mol %) was added and crushed then the progress of reaction was monitored by TLC. After completion of reaction (3 min) the crude product

was washed with water, dried and purified by column chromatography using 20 % methanol in benzene as eluent. Synthetic pathway for preparation of title compounds is shown in **Scheme-I**.

5-[(2E)-(4-Bromobenzylidene)hydrazinylidene]-5*H*-pyrido[3',2':4,5]cyclopenta[1,2-b]pyridine (4a): IR (KBr, ν_{max}, cm^{-1}): 3044 (Ar-H), 1640, 1612 (C=N/C=C), 1595 (CH=N), 1509 (C=N-N), 1464 (C=N/C=C), 557 (C-Br); ^{1}H NMR: ($CDCl_3$, 300 MHz), δ: 8.74 (2H, m), 8.60 (1H, s, CH=N), 8.57 (1H, dd, *J* = 7.8 Hz, 1.5 Hz), 8.22 (1H, dd, *J* = 7.8 Hz, 1.5 Hz), 7.90 (2H, d, *J* = 8.1 Hz, bromobenzylidene), 7.78 (2H, d, *J* = 8.1 Hz, bromobenzylidene), 7.34 (2H, m); MS (ES) found[calcd. (%)]: m/z 363.21 [363.17] (MH^+).

4-[(E)-(5*H*-Pyrido[3',2':4,5]cyclopenta[1,2-b]pyridin-5-ylidenehydrazinylidene)methyl]phenol (4b): IR (KBr, ν_{max}, cm^{-1}): 3439 (O-H), 3046 (Ar-H), 1640, 1607 (C=N/C=C), 1561 (CH=N), 1555 (C=N-N), 1458 (C=N/C=C); ^{1}H NMR: (DMSO-d_6, 300 MHz) δ: 8.85 (1H, d, *J* = 7.5 Hz), 8.60 (2H, m), 8.35 (1H, s, CH=N), 8.22 (1H, d, *J* = 7.5 Hz), 7.70 (2H, d, *J* = 7.7 Hz, phenol), 7.32 (2H m), 7.10 (1H, s, -OH) 6.82 (2H, d, *J* = 7.7 Hz, phenol); MS (ES) found [calcd. (%)]: m/z 300.12 [300.31] (MH^+).

5-[(2E)-(2,4-Dichlorobenzylidene)hydrazinylidene]-5*H*-pyrido[3',2':4,5]cyclopenta[1,2-b]pyridine (4c): IR (KBr, ν_{max}, cm^{-1}): 3046 (Ar-H), 1640, 1610 (C=N/C=C), 1597 (CH=N), 1507 (C=N-N), 1466 (C=N/C=C), 787, 754.49 (C-Cl); ^{1}H NMR: ($CDCl_3$, 300 MHz), δ: 9.01 (1H, s, dichlorobenzylidene), 9.00 (1H, s, CH=N), 8.79 (2H, m), 8.66 (1H, dd, *J* = 7.8 Hz, 1.2 Hz), 8.29 (1H, dd, *J*= 7.8 Hz, 1.2 Hz), 8.23 (1H, d, *J* = 8.6 Hz, dichlorobenzylidene), 8.15 (1H, d, *J* = 8.6 Hz, dichlorobenzylidene), 7.4 (2H, m); MS (ES) found [calcd. (%)]: m/z 353.17 [353.20] (MH^+), 355 (MH^{2+})

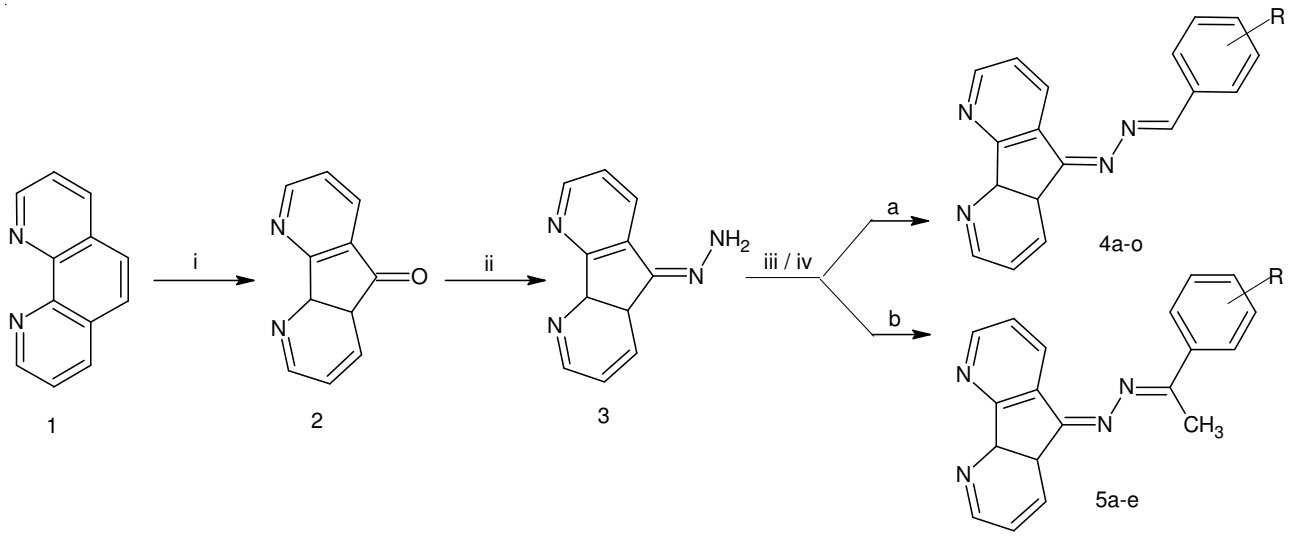
5-[(2E)-(4-Chlorobenzylidene)hydrazinylidene]-5*H*-pyrido[3',2':4,5]cyclopenta[1,2-b]pyridine (4d): IR (KBr, ν_{max}, cm^{-1}): 3038 (Ar-H), 1647, 1620 (C=N/C=C), 1598 (CH=N), 1511 (C=N-N), 1469 (C=N/C=C), 790, 757 (C-Cl); ^{1}H NMR: ($CDCl_3$, 300 MHz), δ: 8.71 (2H, m), 8.61 (1H, s, CH=N), 8.58 (1H, dd, *J* = 7.8 Hz, 1.5 Hz), 8.20 (1H, dd, *J* = 7.8 Hz, 1.5 Hz), 7.85 (2H, d, *J* = 8.0 Hz, chlorobenzylidene), 7.61 (2H, d, *J* = 8.0 Hz, chlorobenzylidene), 7.31 (2H, m); MS (ES) found [calcd. (%)]: m/z 318.75 [318.71] (MH^+), 319.70 (MH^{1+}).

5-[(2E)-(4-Nitrobenzylidene)hydrazinylidene]-5*H*-pyrido[3',2':4,5]cyclopenta[1,2-b]pyridine (4e): IR (KBr, ν_{max}, cm^{-1}): 3149 (Ar-H), 1632, 1607 (C=N/C=C), 1560 (CH=N), 1520 (C=N-N), 1483 (C=N/C=C), 1378 (NO_2); ^{1}H NMR: (DMSO-d_6, 300 MHz), δ: 8.79 (2H, m), 8.61 (1H, s, CH=N), 8.59 (1H, dd, *J* = 7.7 Hz, 1.5 Hz), 8.25 (1H, dd, *J* = 7.7 Hz, 1.5 Hz), 8.01 (2H, d, *J* = 8.3 Hz, nitrobenzylidene), 7.85 (2H, d, *J* = 8.3 Hz, nitrobenzylidene), 7.36 (2H, m); MS (ES) found [calcd. (%)]: m/z 329.19 [329.31] (MH^+).

N,N-Dimethyl-4-[(E)-(5*H*-pyrido[3',2':4,5]cyclopenta[1,2-b]pyridin-5-ylidenehydrazinylidene)methyl]aniline (4f): IR (KBr, ν_{max}, cm^{-1}): 3085 (Ar-H), 1638, 1603 (C=N/C=C), 1573 (CH=N), 1560 (C=N-N), 1499 (C=N/C=C), 1180 (C-N); ^{1}H NMR: ($CDCl_3$, 300 MHz), δ: 9.02 (1H, d, *J* = 7.8 Hz), 8.80 (2H, m), 8.65 (1H, s, CH=N), 8.28 (1H, d, *J* = 7.8 Hz), 7.85 (2H, d, *J* = 7.7 Hz, aniline), 7.38 (2H, m), 6.72 (2H, d, *J* = 7.7 Hz, aniline), 3.11 (6H, s, 2 × CH_3); MS (ES) found [calcd. (%)]: m/z 327.28 [327.38] (MH^+), 328.1 (MH^{1+}).

5-[(2E)-(3-Nitrobenzylidene)hydrazinylidene]-5*H*-pyrido[3',2':4,5]cyclopenta[1,2-b]pyridine (4g): IR (KBr, ν_{max}, cm^{-1}): 3148 (Ar-H), 1633, 1607 (C=N/C=C), 1564 (CH=N), 1521 (C=N-N), 1482 (C=N/C=C), 1377 (NO_2); ^{1}H NMR: ($CDCl_3$, 300 MHz), δ: 8.81 (2H, m), 8.74 (1H, s, nitrobenzylidene), 8.68 (1H, s, CH=N), 8.62 (1H, dd, *J* = 7.8 Hz, 1.5 Hz), 8.38 (1H, dd, *J* = 7.8 Hz, 1.0 Hz, nitrobenzylidene), 8.30 (1H, dd, *J* = 7.8 Hz, 1.5 Hz), 8.12 (1H, dd, *J* = 7.8 Hz, 1.0 Hz, nitrobenzylidene), 7.82 (1H, ddd, *J* = 8.0 Hz, 7.5 Hz, 1.0 Hz, nitrobenzylidene), 7.30 (2H, m); MS (ES) found [calcd. (%)]: m/z 329.31 [329.35] (MH^+).

2-Methoxy-4-[(E)-(5*H*-pyrido[3',2':4,5]cyclopenta[1,2-b]pyridin-5-ylidenehydrazinylidene)methyl]phenol (4h): IR (KBr, ν_{max}, cm^{-1}): 3436 (O-H), 3044 (Ar-H), 1642, 1609 (C=N/C=C), 1560 (CH=N), 1557 (C=N-N), 1456 (C=N/C=C); ^{1}H NMR: ($CDCl_3$, 300 MHz), δ: 8.78 (2H, m), 8.64 (1H, d, *J* =



i: $KMnO_4$, water, reflux ii: NH_2-NH_2·H_2O, HOAc, grinding iii: HOAc, grinding, r.t. iv: HOAc, ethanol, reflux
a: substituted aromatic aldehydes b: substituted aromatic ketones

Scheme-I: Synthesis of novel hydrazones derived from 4,5-diazafluoren-9-hydrazone

7.8 Hz,), 8.35 (1H, s, CH=N), 8.20 (1H, d, *J* = 7.8 Hz), 7.30 (2H, m), 7.21 (1H, dd, *J* = 7.4 Hz, 1.2 Hz, phenol), 7.11 (1H, s. -OH), 6.98 (1H, s, phenol), 6.67 (1H, dd, *J* = 7.4 Hz, 1.2 Hz, phenol), 4.12 (3H, s, CH_3); MS (ES) found [calcd. (%)]: m/z 330.34 [330.36] (MH^+).

5-[(2E)-Benzylidenehydrazinylidene]-5*H*-pyrido-[3',2':4,5]cyclopenta[1,2-b]pyridine (4i): IR (KBr, ν_{max}, cm^{-1}): 3138 (Ar-H), 1649, 1609 (C=N/C=C), 1535 (CH=N), 1504 (C=N-N), 1489 (C=N/C=C), 1202 (N-N); ^{1}H NMR: ($CDCl_3$, 300 MHz), δ: 8.81- 8.74 (3H, m), 8.67 (1H, s, CH=N), 8.25 (1H, dd, *J*= 8.7 Hz, 1.2 Hz), 7.94 (2H, m, benzylidene), 7.55 (3H, m, benzylidene), 7.37 (2H, m); MS (ES) found [calcd. (%)]: m/z 284.29 [284.31] (MH^+).

4-[(E)-(5*H*-Pyrido[3',2':4,5]cyclopenta[1,2-b]pyridin-5-ylidenehydrazinylidene)methyl]benzonitrile (4j): IR (KBr, ν_{max}, cm^{-1}): 3152 (Ar-H), 2226 (CN), 1636, 1611 (C=N/C=C), 1562 (CH=N), 1520 (C=N-N), 1483 (C=N/C=C), 1105 (C-N); ^{1}H NMR: ($CDCl_3$, 300 MHz), δ: 8.77 (2H, m), 8.65 (1H, s, CH=N), 8.62 (1H, dd, *J* = 7.8 Hz, 1.5 Hz), 8.24 (1H, dd, *J* = 7.8 Hz, 1.5 Hz), 8.04 (2H, d, *J* = 8.1 Hz, benzonitrile), 7.83 (2H, d, *J* = 8.1 Hz, benzonitrile), 7.37 (2H, m); MS (ES) found [calcd. (%)]: m/z 309.25 [309.32] (MH^+).

5-[(2E)-(Thiophen-2-ylmethylidene)hydrazinylidene]-5*H*-pyrido[3',2':4,5]cyclopenta[1,2-b]pyridine (4k): IR (KBr, ν_{max}, cm^{-1}): 3130 (Ar-H), 1642, 1609 (C=N/C=C), 1578 (CH=N), 1532 (C=N-N), 1050 (C-S); ^{1}H NMR: ($CDCl_3$, 300 MHz) δ: 8.89 (1H, dd, *J* = 7.8 Hz, 1.5 Hz), 8.85 (1H, s, CH=N), 8.75 (2H, m), 8.23 (1H, dd, *J* = 7.8 Hz, 1.5 Hz), 7.62 (1H, d, *J*= 5.1 Hz, thiophen), 7.57 (1H, d, *J* = 3.9 Hz, thiophen), 7.37 (2H, m), 7.20 (1H, m, thiophen); MS (ES) found [calcd. (%)]: m/z 290.17 [290.34] (MH^+), 291.27 (MH^{1+}).

5-[(2E)-(2-Nitrobenzylidene)hydrazinylidene]-5*H*-pyrido[3',2':4,5]cyclopenta[1,2-b]pyridine (4l): IR (KBr, ν_{max}, cm^{-1}): 3114 (Ar-H), 1628, 1603 (C=N/C=C), 1578 (CH=N), 1507 (C=N-N), 1471 (C=N/C=C), 1340 (NO_2); ^{1}H NMR: ($CDCl_3$, 300 MHz) δ: 9.12 (1H, s, CH=N), 8.77 (2H, m), 8.60 (1H, dd, *J* = 7.8 Hz, 1.0 Hz), 8.32 (1H, dd, *J*= 7.5 Hz, 1.0 Hz, nitrobenzylidene), 8.27 (1H, dd, *J* = 7.8 Hz, 1.0 Hz), 8.14 (1H, dd, *J*= 8.1 Hz, 1.0 Hz, nitrobenzylidene), 7.83 (1H, ddd, *J* = 7.5 Hz, 7.5 Hz, 1.0 Hz, nitrobenzylidene), 7.71 (1H, ddd, *J* = 8.1 Hz, 7.5 Hz, 1.0 Hz, nitrobenzylidene), 7.40 (1H, m), 7.33 (1H, m); MS (ES) found [calcd. (%)]: m/z 329.24 [329.31] (MH^+).

5-[(2E)-(2-Chlorobenzylidene)hydrazinylidene]-5*H*-pyrido[3',2':4,5]cyclopenta[1,2-b]pyridine (4m): IR (KBr, ν_{max}, cm^{-1}): 3047 (Ar-H), 1642, 1611 (C=N/C=C), 1595 (CH=N), 1507 (C=N-N), 1467 (C=N/C=C), 752 (C-Cl); ^{1}H NMR: ($CDCl_3$, 300 MHz) δ: 8.90 (1H, s, CH=N), 8.70 (2H, m), 8.54 (1H, dd, *J* = 7.8 Hz, 1.2 Hz), 8.16 (1H, dd, *J*= 7.8 Hz, 1.2 Hz), 7.92 (1H, dd, *J*= 7.4 Hz, 1.0 Hz, chlorobenzylidene), 7.68 (1H, dd, *J*= 8.0 Hz, 1.0 Hz, chlorobenzylidene), 7.48 (1H, ddd, *J* = 7.5 Hz, 7.5 Hz, 1.2 Hz, chlorobenzylidene), 7.38 (2H, m), 7.30 (1H, ddd, *J* = 8.0 Hz, 7.4 Hz, 1.0 Hz, chlorobenzylidene); MS (ES) found [calcd. (%)]: m/z 318.75 [318.78] (MH^+).

5-[(2E)-(Pyridin-2-ylmethylidene)hydrazinylidene]-5*H*-pyrido[3',2':4,5]cyclopenta[1,2-b]pyridine (4n): IR (KBr, ν_{max}, cm^{-1}): 3124 (Ar-H), 1647, 1607 (C=N/C=C), 1572 (CH=N), 1561 (C=N-N), 1445 (C=N/C=C) 1223, 1130 (C-N); ^{1}H NMR: ($CDCl_3$, 300 MHz) δ: 8.72-8.78 (3H, m), 8.66 (1H, s, CH=N), 8.61 (1H, dd, *J* = 7.8 Hz, 1.5 Hz), 8.22-8.27 (2H, m, pyridin), 7.91 (1H, ddd, *J* = 7.8 Hz, 7.8 Hz, 1.5 Hz, pyridin), 7.45 (1H, m, pyridin), 7.35 (2H, m); MS (ES) found [calcd. (%)]: m/z 285.19 [285.30] (MH^+).

5-[(2E)-(4-Methoxybenzylidene)hydrazinylidene]-5*H*-pyrido[3',2':4,5]cyclopenta[1,2-b]pyridine (4o): IR (KBr, ν_{max}, cm^{-1}): 3134 (Ar-H), 1648, 1607 (C=N/C=C), 1537 (CH=N), 1502 (C=N-N), 1488 (C=N/C=C), 1072 (C-O); ^{1}H NMR: (DMSO-d_6, 300 MHz) δ: 8.80 (1H, d, *J* = 7.8 Hz), 8.64 (2H, m), 8.37 (1H, s, CH=N), 8.26 (1H, d, *J* = 7.8 Hz), 7.75 (2H, d, *J* = 7.7 Hz, methoxybenzylidene), 7.30 (2H m), 6.80 (2H, d, *J* = 7.7 Hz, methoxybenzylidene), 3.92 (3H, s, -CH_3); MS (ES) found [calcd. (%)]: m/z 314.34 [314.30] (MH^+).

5-{(2E)-[1-(Pyridin-2-yl)ethylidene]hydrazinylidene}-5H-pyrido[3',2':4,5]cyclopenta[1,2-b]pyridine (5a): IR (KBr, ν_{max}, cm^{-1}): 3153 (Ar-H), 1659, 1616 (C=N/C=C), 1578 (CH=N), 1561 (C=N-N), 1438 (C=N/C=C), 1133 (C-N); ^{1}H NMR: ($CDCl_3$, 300 MHz) δ: 8.78 (1H, dd, *J* = 7.8 Hz, 1.2 Hz), 8.71-8.74 (2H, m), 8.22-8.34 (2H, m, pyridin and 1H, pyrido-pyridine), 7.89 (1H, ddd, *J* = 7.5 Hz, 7.5 Hz, 1.5 Hz, pyridin), 7.36-7.45 (2H, m), 7.24 (1H, m, pyridine), 2.56 (3H, s, -CH_3); MS (ES) found [calcd. (%)]: m/z 299.27 [299.32] (MH^+).

5-[(2E)-(1-Phenylethylidene)hydrazinylidene]-5*H*-pyrido[3',2':4,5]cyclopenta[1,2-b]pyridine (5b): IR (KBr, ν_{max}, cm^{-1}): 3134 (Ar-H), 1655, 1611 (C=N/C=C), 1541 (CH=N), 1516 (C=N-N), 1467 (C=N/C=C); ^{1}H NMR: ($CDCl_3$, 300 MHz) δ: 8.74 (2H, m), 8.37 (1H, dd, *J* = 8.7 Hz, 1 Hz), 8.28 (1H, dd, *J* = 8.7 Hz, 1 Hz), 8.02 (2H, m, phenyl), 7.52 (3H, m, phenyl), 7.38 (1H, m), 7.25 (1H, m), 2.49 (3H, s, -CH_3); MS (ES) found [calcd. (%)]: m/z 298.26 [298.34] (MH^+).

5,5'-Hydrazine-1,2-diylidenebis(5*H*-pyrido[3',2':4,5]cyclopenta[1,2-b]pyridine) (5c): IR (KBr, ν_{max}, cm^{-1}): 3062 (Ar-H), 1630, 1608 (C=N/C=C), 1585 (CH=N), 1560 (C=N-N), 1410 (C=N/C=C) 734; ^{1}H NMR: ($CDCl_3$, 300 MHz), δ: 8.80 (2H, d), 8.78 (2H, d), 8.54 (2H, d), 8.36 (2H, d), 7.48 (2H, dd, *J* = 7.8 Hz, 1.0 Hz), 7.30 (2H, dd, *J* = 7.8 Hz, 1.0 Hz); MS (ES) found [calcd. (%)]: m/z 360.37 [360.40] (MH^+).

4-Methyl-2-[(1E)-1-(5*H*-pyrido[3',2':4,5]cyclopenta[1,2-b]pyridin-5-ylidenehydrazinylidene)ethyl]phenol (5d): IR (KBr, ν_{max}, cm^{-1}): 3394 (O-H), 3092 (Ar-H), 1647, 1609 (C=N/C=C), 1575 (CH=N), 1535 (C=N-N), 1457 (C=N/C=C), 1080 (C-O); ^{1}H NMR: ($CDCl_3$, 300 MHz) δ: 8.81 (2H, m), 8.60 (1H, d, *J* = 7.8 Hz), 8.24 (1H, d, *J* = 7.8 Hz), 7.38 (1H, s, phenol), 7.31 (2H, m), 7.10 (1H, s, -OH), 6.98 (1H, dd, *J* = 7.8 Hz, 1 Hz, phenol), 6.64 (1H, dd, *J* = 7.8 Hz, 1 Hz, phenol), 2.48 (3H, s, CH_3), 2.21 (3H, s, CH_3); MS (ES) found [calcd. (%)]: m/z 328.36 [328.38] (MH^+).

2-[(1E)-1-(5*H*-Pyrido[3',2':4,5]cyclopenta[1,2-b]pyridin-5-ylidenehydrazinylidene)ethyl]phenol (5e): IR (KBr, ν_{max}, cm^{-1}): 3393 (O-H), 3090 (Ar-H), 1640, 1607 (C=N/C=C), 1578 (CH=N), 1532 (C=N-N), 1458 (C=N/C=C), 1082 (C-O); ^{1}H NMR: ($CDCl_3$, 300 MHz) δ: 8.75 (2H, m), 8.43 (1H, dd, *J* = 9 Hz, 1.2 Hz), 8.26 (1H, dd, *J* = 9 Hz, 1.2 Hz), 7.65 (1H, dd, *J* = 9 Hz, 1.2 Hz, phenol), 7.45 (2H, m), 7.29 (1H, m, phenol), 7.13 (1H, s, OH) 6.96-7.09 (2H, m, phenol), 2.61 (3H, s, CH_3); MS (ES) found [calcd. (%)]: m/z 314. 30 [314.34] (MH^+).

Synthesis of hydrazones in ethanol reflux (4a-o and 5a-e): A mixture of 4,5-diazafluoren-9-hydrazone (1 mmol), aldehyde/ketone (1 mmol) and acetic acid (20 mol %) was refluxed in ethanol and progress of reaction was monitored by TLC. Having completed the reaction, the resulting reaction mixture was cooled at -20 ºC then filtered and washed with cold ethanol three times. It was finally dried under vacuum at 40 ºC to give desire product.

Antimicrobial activity assay procedure

Disc diffusion method: The antimicrobial activity of newly synthesized compounds was evaluated using the disc diffusion method as per the guidelines of the National Committee for Clinical Laboratory Standards [NCCLS, 1997][21]. Briefly, a 24/48 h old culture of selected bacteria was mixed with sterile physiological saline (0.85 %) and the turbidity was adjusted to the standard inoculum of McFarland scale 0.5 [*ca.* 106 colony forming units (CFU) per mL]. Petri plates containing 20 mL of Nutrient Agar (NA, Hi- Media) were used for all the bacteria tested. The inoculums was spread on the surface of the solidified media and Whatman No. 1 filter paper discs (6 mm in diameter) were impregnated with the test compound (20 µL/disc) and placed on the plates. Cefotaxime (10 µg/disc, Hi-Media) and Tetracycline (30 µg/disc, Hi-Media) were used as positive controls for bacteria. A paper disc impregnated with dimethylsulfoxide (DMSO) was used as negative control. Plates inoculated with the bacteria were incubated for 24 h at 37 ºC. The inhibition zone diameters were measured in millimeters. All the tests were performed in triplicate and the average was considered for final reading.

RESULTS AND DISCUSSION

The study was started by evaluating the efficiency of grinding in the reaction between 4,5-diazafluoren-9-hydrazone and 4-bromobenzaldehyde in solid state to obtain the hydrazone **4a** under the reaction conditions as described in the Table-1. Initially, the mixture was grounded in mortar with a pestle at room temperature under neat conditions. However, the results demonstrated the need of a catalyst, since the starting material was recovered (Table-1, entry 1). Thus, we chose four catalysts (PTSA, HOAc, $ZnCl_2$, $SnCl_2$) to be analyzed in this condensation reaction. The starting materials were mostly recovered in presence of PTSA (Table-1, entry 2, 3, 4), but at high mole % of PTSA, better yield of the product was obtained (Table-1, entry 5, 6).

The reaction failed in the presence of $ZnCl_2$ and starting materials were recovered (Table-1, entry 12, 13, 14, 15, 16). Thus, we found acetic acid, an inexpensive and common organic chemical which was used an efficient catalyst for the reaction. Acetic acid was used in 20-40 mol % which hastened yield improvement and completion of the reaction in 3 min (Table-1, entry 7, 8). For evaluating the amount of catalyst, HOAc was employed in 60, 80 and 100 mol % for 3 min, however there was no large effect on yield of the product (Table-1. entry 9, 10, 11). This reaction also failed when we used $SnCl_2$ as a catalyst at 60, 80 and 100 mol % (Table-1, entry 19, 20, 21), but it gave moderate yield of the product at 20 and 40 mol % (Table-1, entry 17, 18).

TABLE-1
OPTIMIZATION FOR SYNTHESIS OF **4a**

Entry	Catalyst	Catalyst amount (mol %)	Time (min)	Yield[a] (%)
1	Neat	–	6	–[b]
2	PTSA	20	3	45[c]
3	PTSA	40	3	45[c]
4	PTSA	60	3	60[c]
5	PTSA	80	3	78
6	PTSA	100	3	88
7	HOAc	20	3	92
8	HOAc	40	3	92
9	HOAc	60	3	91
10	HOAc	80	3	90
11	HOAc	100	3	90
12	$ZnCl_2$	20	6	–[b]
13	$ZnCl_2$	40	6	–[b]
14	$ZnCl_2$	60	6	–[b]
15	$ZnCl_2$	80	6	–[b]
16	$ZnCl_2$	100	6	–[b]
17	$SnCl_2$	20	3	75
18	$SnCl_2$	40	3	50
19	$SnCl_2$	60	3	–[b]
20	$SnCl_2$	80	3	–[b]
21	$SnCl_2$	100	3	–[b]

[a]Yield of isolated product, [b]The starting material was recovered, [c]The starting material were mostly recovered.

The generality of the reaction was authenticated by the use of aromatic aldehydes containing electron-donating groups or electron-withdrawing groups and also variety of ketones. As shown in Table-2, aldehydes containing electron-donating or electron withdrawing groups reacted well to give corresponding hydrazones (**4a-o**) in high yield. This finding demonstrated no influence of the electronic nature of the substituent on the reaction time or yield. In addition, we observed that the used varieties of ketones gave low yield as compared to aldehydes (Table-2).

In order to evaluate the scope and limitations of this solvent free reaction, we compared this grinding process with conventional thermal heating, with ethanol reflux and an acetic acid as a catalyst. From Table-2, it is possible to affirm that the grinding allowed the reaction to proceed in a shorter reaction time, furnishing better yields as compared to conventional thermal heating. A possible explanation for the shorter reaction time and better yield under solvent free condition is that the formation of liquid phase prior to the reaction. *i.e.*, formation of a eutectic mixture with uniform distribution of the reactants brings the reacting species into proximity than does a solvent[22].

Antibacterial activity: The antibacterial activities of the newly synthesized compounds **4a-o** and **5a-e** were evaluated against various pathogenic (gram-negative and gram-positive) bacterial strains *viz*., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella pneumonae*. The antibacterial activities were evaluated by the disc diffusion method. The solvent used for the preparation of compound solution (DMSO) did not show inhibition against the tested organisms (negative control).

The results of antibacterial screening of all newly synthesized compounds are presented in Table-3. Most of these tested compounds did not show any activity against *E. coli*,

TABLE-2
SYNTHESIS OF HYDRAZONES

Entry	a/b	Products	Grinding			Ethanol reflux			R_f^*
			Time (min)	Yield (%)	m.p. (ºC)	Time (min)	Yield (%)	m.p. (ºC)	
1	4-Bromobenzaldehyde	**4a**	3	92	238	14	78	238	0.64
2	4-Hydroxybenzaldehyde	**4b**	3	90	> 300	15	76	> 300	0.62
3	2,4-Dichlorobenzaldehyde	**4c**	3	94	210	12	78	210	0.62
4	4-Chlorobenzaldehyde	**4d**	3	92	242	13	75	240	0.60
5	4-Nitrobenzaldehyde	**4e**	3	94	> 300	12	78	> 300	0.56
6	4-N,N-Dimethylbenzaldehyde	**4f**	3	92	188	16	72	189	0.54
7	3-Nitrobenzaldehyde	**4g**	3	88	232	15	70	232	0.58
8	4-Hydroxy,3-Methoxybenzaldehyde	**4h**	3	89	212	15	72	212	0.50
9	Benzaldehyde	**4i**	3	90	182	13	74	182	0.48
10	4-Cyanobenzaldehyde	**4j**	3	93	268	12	80	266	0.58
11	2-Thiophene carboxaldehyde	**4k**	3	90	125	14	73	125	0.64
12	2-Nitrobenzaldehyde	**4l**	3	88	220	15	78	222	0.58
13	2-Chlorobenzaldehyde	**4m**	3	90	125	13	80	125	0.54
14	2-Pyridine carboxaldehyde	**4n**	3	89	190	12	72	190	0.50
15	4-Methoxybenzaldehyde	**4o**	3	95	145	14	81	145	0.67
16	2-Acetylpyridine	**5a**	3	85	160	15	71	162	0.56
17	Acetophenone	**5b**	3	82	192	15	65	192	0.58
18	4,5-Diazafluoren-9-hydrazone	**5c**	3	86	> 300	17	75	> 300	0.65
19	2-Hydroxy, 5-methylacetophenone	**5d**	3	84	115	16	68	116	0.56
20	2-Hydroxyacetophenone	**5e**	3	88	226	17	74	226	0.73

[a]Yield of isolated product, *Benzene: methanol (4:1).

TABLE-3
ANTIBACTERIAL ACTIVITIES OF THE COMPOUNDS **4a-o** AND **5a-e**

Comp.	*E. coli* (mm)	*S. aureus* (mm)	*B. subtilis* (mm)	*K. pneumonia* (mm)
4a	–	–	10	–
4b	8	9	8	11
4c	8	–	–	12
4d	–	8	–	11
4e	–	–	10	–
4f	–	9	8	–
4g	–	–	–	–
4h	9	–	–	–
4i	10	–	–	–
4j	9	–	–	–
4k	12	–	–	–
4l	12	–	–	–
4m	8	–	9	8
4n	–	9	–	–
4o	9	–	–	–
5a	–	–	–	9
5b	10	–	–	–
5c	–	9	8	–
5d	9	–	9	10
5e	–	–	–	10
S1	14	12	13	14
S2	12	15	14	14

–: Indicates bacteria are resistant to the compounds. S1: Cephotoxime used as standard drug at concentration of 10 µg/mL. S1: Tetracycline used as standard drug at concentration of 30 µg/mL. All compounds (**4a-o** and **5a-e**) tested at concentration of 250 µg/mL.

S. aureus, *B. subtilis* and *K. pneumonae* with concentration of 250 µg/mL in DMSO. But, thiophene and 2-nitro derivative of hydrazones (**4k** and **4l**) showed moderate activity (zone of inhibition up to 12 mm at concentration of 250 µg/mL) against *Escherichia coli*. Compounds **4b**, **4c** and **4d** (which bear 4-OH, 2,4-di-chloro, 4-chloro) showed moderate activity against *Klebsiella pneumonae* (zone of inhibition up to 11-12 mm at concentration of 250 µg/mL).

Conclusion

A number of novel hydrazones were synthesized under solvent free condition. This protocol furnishes the products very quickly, simplifies the work-up and does not harm the environment. In this protocol, there is no influence of the electronic nature of the substituent on the reaction time or yield but ketones gives low yield as compared to aldehydes. The antibacterial screening results reveal that the compounds that bear 4-OH, 2,4-di-chloro, 4-chloro, thiophene and 2-nitro substituents showed moderate antibacterial activities *i.e.*, **4b**, **4c**, **4d**, **4k** and **4l**.

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