CSA-Catalyzed Three-component Synthesis of Fused Polycyclic Pyrazolo[4,3-e]pyridines Under Ultrasonic Irradiation and Their Antioxidant Activity

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Abstract: New fused pyrazolo[4,3-e]pyridines were obtained by three-component reaction of 1,3-dimethyl-1*H*-pyrazol-5-amine or 3-phenyl-1*H*-pyrazol-5-amine, aromatic aldehydes and indan-1,3-dione in the presence of camphor-10-sulfonic acid (CSA) as an effective catalyst under ultrasound promoted conditions. The antioxidant activity of the pyrazolopyridine compounds **4b**, **5c**, **5e**, **7a**, **7b** and **7c** were determined.

Keywords: Pyrazolopyridines, three-component synthesis, ultrasonic irradiation, camphor-10-sulfonic acid (CSA), antioxidant activity.

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INTRODUCTION

In the past few decades, multi-component reactions have increasing attention in synthetic organic chemistry due to the building of several new bonds can easily be achieved in a single step (1, 2). These synthetic methodologies have great utility, especially for the construction of heterocyclic compounds which exhibit biological activity (3). Multi-component reactions act in accordance with green chemistry principles in terms of a high degree of atomic economy, easier progress of reactions, decreased reaction times, lack of waste products, and low power consumption (4, 5).

Pyrazolopyridine compounds have shown many biological properties such as anti-viral agent (6, 7), (CDK1) inhibitor (8), HIV inhibitors (9), CCR1 antagonists (10), protein kinase inhibitors (11), and they also exhibit parasiticide properties and antimalarial activities (12-14). Many of them show fluorescence in the blue-green region and have been used in organic light emitting experimental diodes (15). Therefore many synthetic methods for the synthesis of pyrazolopyridines have been reported (16-19).

Along with other reaction parameters, the nature of the catalyst plays an important role in reaction's yield, selectivity, and general applicability. Thus, development of an inexpensive, reusable, and non-toxic catalyst for multi-component reactions continues to be a subject of interest. CSA has been demonstrated to be an efficient, non-toxic, and reusable organocatalyst for several reactions such as synthesis of β-amino carbonyl compounds (20), Friedel-Crafts reactions (21, 22), synthesis of spirocyclic compounds (23), rearrangement of 1,2-dialkynylallyl alcohols (24), and it is widely used in the optical resolution of amines (25).

Ultrasonic irradiation is widely used in synthetic organic chemistry as it is comply with the principles of green chemistry (26-28). Ultrasound irradiation is able to activate many organic reactions due to cavitational collapse (29, 30). Compared with traditional methods, many organic synthesis can be efficiently carry out in higher yields, higher selectivity, shorter reaction times, and milder reaction conditions under ultrasonic irradiation (31, 32).

This work aims to the preparation of pyrazolopyridines under ultrasonic irradiation in the presence of CSA as a catalyst. There is also a rising significance in antioxidants due to prevention of harmful effects of free radicals in human body (33, 34). Therefore in an attempt to extend biological interest to this new group of compounds most of the pyrazolopyridine compounds were tested for their free radical scavenging activity (determined for DPPH), reducing activity (reduction of the Fe^{3+}/ferricyanide complex to its

ferrous form), metal-chelating (chelating activity capacity of ferrous ions), superoxide scavenging activity, and total antioxidant activity.

MATERIALS AND METHODS

NMR spectra were determined on a Bruker Avance III-500 MHz NMR. Chemical shifts are given in ppm downfield from Me_4Si in DMSO-d_6 or $CDCl_3$ solution. Coupling constants are given in Hz. The FTIR spectra were recorded on a Perkin-Elmer FT-IR spectrometer (ATR) and absorption frequencies are reported in cm^{-1}. MS spectra were recorded on a Thermo Elemental X Series ICP-MS or AB Sciex 3200 QTRAP LC-MS/MS. Elemental analyses were measured with Flash EA 1112 Series or CHNS-932 LECO apparatus and were in good agreement (± 0.2 %) with the calculated values. Ultrasonication was performed in a Alex Ultrasonic Bath with a frequency of 32 kHz. The internal dimensions of the ultrasonic cleaner tank were 240x140x100 mm with liquid holding capacity of 3L. The reactor was a 100 mL pyrex round-bottom flask. The reaction flasks were suspended in the center of the bath, and the addition or removal of water controlled the temperature of the water bath. Melting points were measured on a Gallenkamp melting-point apparatus. TLC was conducted on standard conversion aluminum sheets pre-coated with a 0.2-mm layer of silica gel. All reagents were commercially available.

General Procedure for the Synthesis of Pyrazolopyridine Compounds (4a-b, 5c-e, 7a-d) Under Ultrasonic Irradiation

A mixture of CSA (7.3 mg, 0.03 mmol), 1,3-dimethyl-1*H*-pyrazol-5-amine or 3-phenyl-1*H*-pyrazol-5-amine, (1.00 mmol), indan-1,3-dione (1.00 mmol), and aromatic aldehyde (1.00 mmol) in 5 mL of EtOH was irradiated with ultrasound of low power (with a frequency of 32 kHz) at 40 °C for the period of time indicated in Table 3. The reaction flask was located at the maximum energy area in the cleaner and the surface of the reactants was placed slightly lower than the level of the water. The addition or removal of water controlled the temperature of the water bath. After completion of the reaction, as indicated by TLC monitoring, the resultant solid was washed with water and crystallized from ethanol to give products **4a**-**b**, **5c**-**e** and **7a**-**d**.

Antioxidant Activity

α,α-Diphenyl-β-picryl-hydrazyl (DPPH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), trolox, resorcinol, gallic acid, linoleic acid, ethylenediaminetetraacetic acid (EDTA), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonicacid)-1,2,4-triazine (Ferrozine), polyoxyethylenesorbitan monolaurate (Tween-20) and trichloroacetic acid (TCA), Ferrozine, Folin Ciocalteu solution, nicotinamide adenine

dinucleotide (NADH), phenazine methosulphate (PMS), nitroblue tetrazolium (NBT) were obtained from Sigma-Aldrich. Ammonium thiocyanate was purchased from Merck. All other chemicals used were in analytical grade and obtained from either Sigma–Aldrich or Merck.

Free Radical Scavenging Activity

The free radical scavenging activity was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH•). The activity was measured by following the methodology described by Brand-Williams et al (35). Briefly, 20 mg/L DPPH• in methanol was prepared and 1.5 mL of this solution was added to 0.75 mL of pyrazolopyrine compounds solution in water at different concentrations (25-400 µg/mL). After 30 minutes, the absorbance was measured at 517 nm. Water (0.75 mL) in place of the sample was used as control. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percent inhibition activity was calculated using the following equation:

$$\text{Free radical scavenging effect \%} = [(A_0 - A_1)/A_0].100$$

(A_0= the control absorbance and A_1= the sample solution absorbance).

Reducing Power Assay

The reducing power of pyrazolopyridine compounds was determined by the method of Oyaizu (36). Different concentrations of pyrazolopyridine compounds (25-400 µg/mL) in 1 mL of water were mixed with phosphate buffer (2.5 mL, 0.2 M pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%, w/v). The mixture was incubated at 50°C for 30 min. A portion of trichloroacetic acid (2.5 mL, 10%, w/v) was added to the mixture which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of upper-layer solution was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%, w/v), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power.

Metal Chelating Activity

The ferrous ions (Fe^{2+}) chelating activities of pyrazolopyridine compounds were measured according to the method of Decker and Welch (37). 1 mL of different concentrations of pyrazolopyrine compounds (25–400 µg/mL) were mixed with 3.7 mL of deionized water. The mixture was incubated with $FeCl_2$ (2 mM, 0.1 mL) for 30 min. After incubation, the reaction was initiated by addition of ferrozine (5 mM and 0.2 mL), and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured at 562 nm. A lower absorbance indicates a higher chelating

power. The Fe^{2+} chelating activity of the compounds was compared with EDTA at the same concentrations.

$$\text{Metal chelating activity (\%)} = [(A_0\text{-}A_1)/A_0].100$$

Superoxide Scavenging Activity

Measurement of superoxide anion scavenging activity of pyrazolopyridine compounds was done based on the method described by Liu (38). Superoxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). In this experiments, the superoxide radicals were generated in 3 mL of *Tris*-HCl buffer (16 mM, pH 8.0) containing 1 mL of NBT (50 µM) solution, 1 mL of NADH (78 µM) solution and sample solution of pyrazolopyridine compounds (from 25-400 µg/mL) in water. The reaction started by adding 1 mL of phenazine methosulphate (PMS) solution (10 µM) to the mixture. The reaction mixture was incubated at 25 ºC for 5 min and the absorbance at 560 nm was measured. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\%\text{Inhibition} = [(A_0\text{-}A_1)/A_0].100$$

Total Antioxidant Activity Assay

The total antioxidant activity of pyrazolopyridine compounds was measured according to the thiocyanate method described by Mitsuda et al (39). The solution of pyrazolopyridine compounds (150 µg/mL) in 2.5 mL of potassium phosphate buffer (0.04 M, pH 7.4) was added to 2.5 mL of linoleic acid emulsion in potassium phosphate buffer (0.04 M, pH 7.4). The mixed solution (5 mL) was incubated at 37 ºC. During incubation at regular interval moments, a 0.1 mL of the mixture was diluted with 3.7 mL of methanol, followed by the addition of 0.1mL of 30 % ammonium thiocyanate and 0.1 mL of 20 mM ferrous chloride in 3.5 % hydrochloric acid. The peroxide level was determined by measurement of the absorbance at 500 nm in. This step was repeated every 10 h until the control reached its maximum absorbance value. High absorbance indicates high linoleic acid oxidation. The per cent inhibition of lipid peroxidation in linoleic acid emulsion was calculated by the following equation:

$$\text{Inhibition of lipid peroxidation (\%)} = [(A_0\text{-}A_1)/A_0].100$$

1,3-Dimethyl-4-phenylindeno[1,2-b]pyrazolo[4,3-e]pyridin-5(1H)-one (4a)

Red powder; m.p. 246-248 °C; FTIR (ATR, cm^{-1}): 3056, 3028, 2925, 2850, 1709, 1606, 1562, 1499, 1435, 1326, 1245, 1132, 1009, 776. ^{1}H NMR (500 MHz, $CDCl_3$, delta, ppm): 7.85 (d, *J*= 6.50 Hz, 1H, Ar-*H*), 7.53-7.43 (m, 4H, Ar-*H*), 7.36-7.18 (m, 4H, Ar-*H*), 4.02 (s, 3H, N-C*H*$_3$), 1.90 (s, 3H, C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.05 (*C*=O), 164.77 (Ar-*C*), 153.28 (Ar-*C*), 145.92 (Ar-*C*), 144.36 (Ar-*C*), 142.38 (Ar-*C*), 137.54 (Ar-*C*), 134.57 (Ar-*C*H), 132.99 (Ar-*C*), 131,34 (Ar-*C*H), 129.08 (Ar-*C*H), 128.66 (Ar-*C*H), 127.91 (Ar-*C*H), 123.38 (Ar-*C*H), 121.13 (Ar-*C*H), 119.15 (Ar-*C*), 113.72 (Ar-*C*), 33.81 (N-*C*H$_3$), 14.62 (*C*H$_3$). MS: m/z (ESI) 326.2 $[M+H]^+$, 310.0, 297.0, 282.0, 252.8, 230.2, Anal. Calc. for $C_{21}H_{15}N_3O$: C, 77.52; H, 4.65; N, 12.91. Found: C, 77.38; H, 4.68; N, 12.86.

4-(1,3-dimetyl-5-oxo-1,4,5,10-tetrahydroindeno[1,2-b]pyrazolo[4,3-e]pyridin-4-yl)benzonitrile (4b)

Red powder; m.p. 273-275 °C; FTIR (ATR, cm^{-1}): 3396, 3086, 3053, 2927, 2227, 1706, 1604, 1567, 1507, 1490, 1324, 1238, 1182, 1025, 857, 771. ^{1}H NMR (500 MHz, DMSO-*d*$_6$, delta, ppm): 8.03 (d, *J*= 8.20 Hz, 2H, Ar-*H*), 7.99 (d, *J*= 7.50 Hz, 1H, Ar-*H*), 7.78-7.73 (m, 3H, Ar-*H*), 7.63-7.57 (m, 2H, Ar-*H*), 4.07 (s, 3H, N-C*H*$_3$), 1.88 (s, 3H, C*H*$_3$). ^{13}C NMR (125 MHz, DMSO-*d*$_6$): δ 189.04 (*C*=O), 162.47 (Ar-*C*), 157.00 (Ar-*C*), 151.10 (Ar-*C*), 143.02 (Ar-*C*), 142.20 (Ar-*C*), 137.75 (Ar-*C*), 135.77 (Ar-*C*), 133.00 (Ar-*CH*), 131.74 (Ar-*CH*), 129.96 (Ar-*C*H), 129.04 (Ar-*C*H), 127.03 (Ar-*C*H), 124.72 (Ar-*C*H), 123.32 (Ar-*C*H), 121.13 (Ar-*C*H), 118.55 (*C*≡N), 111.74 (Ar-*C*), 106.65 (Ar-*C*), 33.82 (N-*C*H$_3$), 14.06 (*C*H$_3$). MS: m/z (ESI) 351.2 $[M^+]$, 248.3, 233.0, 205.5, 102.1, Anal. Calc. for $C_{22}H_{14}N_4O$: C, 75.42; H, 4.03; N, 15.99. Found: C, 75.38; H, 4.15; N, 16.11.

4-(2,4-Difluorophenyl)1,3-dimetyl-4,10-dihydroindeno[1,2-b]pyrazolo[4,3-e]pyridin-5(1H)-one (5c)

Red powder; m.p. 274-276 °C; FTIR (ATR, cm^{-1}): 3630, 3138, 3070, 3028, 2937, 1689, 1655, 1600, 1542, 1497, 1450, 1350, 1287, 1197, 1134, 1088, 963, 866, 772. ^{1}H NMR (500 MHz, DMSO-*d*$_6$, delta, ppm): 10.76 (s, 1H, N*H*), 7.70 (d, *J* = 7.20 Hz, 1H, Ar-*H*), 7.46 (t, *J* = 7.40 Hz, 1H, Ar-*H*), 7.34 (t, *J* = 7.40 Hz, 1H, Ar-*H*), 7.27-7.17 (m, 2H, Ar-*H*), 7.15-7.07 (m, 1H, Ar-*H*), 6.95 (t, *J* = 8.40 Hz, 1H, Ar-*H*), 5.13 (s, 1H, C-*H*), 3.78 (s, 3H, N-C*H*$_3$), 1.73 (s, 3H, C*H*$_3$). ^{13}C NMR (125 MHz, DMSO-*d*$_6$): δ 189.85 (*C*=O), 161.56 (Ar-*C*), 160.27 (Ar-*C*), 159.62 (Ar-*C*), 158.30 (Ar-*C*), 155.90 (Ar-*C*), 143.81 (Ar-*C*), 137.75 (Ar-*C*), 136.40 (Ar-*C*), 134.10 (Ar-*C*), 131.35 (Ar-*C*H), 130.06 (Ar-*C*H), 119.98 (Ar-*C*H), 119.35 (Ar-*C*H), 111.40 (Ar-*C*H), 106.09 (Ar-*C*), 103.16 (Ar-*C*H), 102.18 (Ar-*C*), 35.16 (N-*C*H$_3$), 27.39 (*C*-H), 11.53 (*C*H$_3$). MS: m/z (ESI) 364.0 $[M+H]^+$, 344.2, 302.5, 268.2, 250.2, 236.0, 126.9, Anal. Calc. for $C_{21}H_{15}F_2N_3O$: C, 69.41; H, 4.16; N, 11.56. Found: C, 69.23; H, 4.14; N, 11.53.

1,3-Dimethyl-4-p-tolyl-4,10-dihydroindeno[1,2-b]pyrazolo[4,3-e]pyridin-5(1H)-one **(5d)**

Red powder; m.p. 251-253 °C; FTIR (ATR, cm^{-1}): 3242, 3173, 3144, 3043, 2921, 2849, 1739, 1653, 1599, 1536, 1499, 1349, 1196, 1108, 865, 761; ^{1}H NMR (500 MHz, $CDCl_3$, delta, ppm): 10.90 (s, 1H, N*H*), 8.17-7.74 (m, 2H, Ar-*H*), 7.65-7.28 (m, 3H, Ar-*H*), 6.88 (d, *J*= 6.50 Hz, 1H, Ar-*H*), 6.80 (d, *J*= 6.50 Hz, 1H, Ar-*H*), 4.80 (s, 1H, C-H), 4.14 (s, 3H, N-CH_3), 2.49 (s, 3H, CH_3), 2.06 (s, 3H, Ph-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 189.90 (*C*=O), 155.73 (Ar-*C*), 152.95 (Ar-*C*), 143.24 (Ar-*C*), 139.27 (Ar-*C*), 136.55, (Ar-*C*), 135.82 (Ar-*C*), 135.10 (Ar-*C*), 133.74 (Ar-*C*H), 129.09 (Ar-*C*H), 128.60 (Ar-*C*H), 126.38 (Ar-CH), 125.45 (Ar-*C*H), 123.18 (Ar-*C*H), 104.86 (Ar-*C*), 103.50 (Ar-*C*), 35.67 (N-*C*H_3), 33.30 (*C*-H), 21.16 (Ph-*C*H_3), 11.58 (*C*H_3). MS: m/z (ESI) 342.2 $[M+H]^+$, 298.0, 285.3, 250.1, 236.3, 180.1, Anal. Calc. for $C_{22}H_{19}N_3O$: C, 77.40; H, 5.61; N, 12.31. Found: C, 77.61; H, 5.49; N, 12.35.

1,3-Dimetyl-4-(5-nitrofuran-2-yl)-4,10-dihydroindeno[1,2-b]pyrazolo[4,3-e]pyridin-5(1H)-one (5e)

Red powder; m.p. 300-302 °C; FTIR (ATR, cm^{-1}): 3371, 3156, 3117, 3068, 2975, 2936, 1702, 1683, 1602, 1590, 1497, 1479, 1353, 1232, 1188, 1091, 966, 813, 757. ^{1}H NMR (500 MHz, DMSO-d_6, delta, ppm): 10.97 (s, 1H, N*H*), 7.79 – 7.30 (m, 5H, Ar-*H*), 6.66 (br s, 1H, Ar-*H*), 5.25 (s, 1H, C-*H*), 3.79 (s, 3H, N-CH_3), 1.96 (s, 3H, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6): δ 189.54 (*C*=O), 156.65 (Ar-*C*), 152.02 (Ar-*C*), 149.47 (Ar-*C*), 144.39 (Ar-*C*), 137.79 (Ar-*C*), 136.12 (Ar-C), 131.69 (Ar-*C*), 130.51 (Ar-*C*), 123.79 (Ar-*C*H), 120.41 (Ar-*C*H), 119.81 (Ar-*C*H), 114.50 (Ar-*C*H), 110.31 (Ar-*C*H), 108.15 (Ar-*C*H), 103.76 (Ar-*C*), 35.33 (N-*C*H_3), 29.00 (*C*-H), 11.83 (*C*H_3). MS: m/z (ESI) 363.3 $[M+H]^+$, 316.2, 272.0, 250.2, 192.0, Anal. Calc. for $C_{19}H_{14}N_4O_4$: C, 62.98; H, 3.89; N, 15.46. Found: C, 63.14; H, 4.08; N, 15.60.

3,4-Diphenyl-4,10-dihydroindeno[1,2-b]pyrazolo[4,3-e]pyridin-5(1H)-one (7a)

Red powder; m.p. 287-289 °C; FTIR (ATR, cm^{-1}): 3418, 3175, 3058, 3025, 2966, 2888, 1658, 1595, 1566, 1494, 1429, 1347, 1192, 1128, 1074, 970, 919, 760. ^{1}H NMR (500 MHz, DMSO-d_6, delta, ppm): 12.90 (s, 1H, N*H*), 11.41 (s, 1H, N*H*), 7.68-7.30 (m, 7H, Ar-*H*), 7.28-7.09 (m, 6H, Ar-*H*), 6.99 (d, *J*= 6.50 Hz, 1H, Ar-*H*), 5.34 (s, 1H, C-*H*). ^{13}C NMR (125 MHz, DMSO-d_6): δ 189.31 (*C*=O), 156.05 (Ar-*C*), 148.58 (Ar-*C*), 145.87 (Ar-*C*), 138.78 (Ar-*C*), 136.32 (Ar-*C*), 134.78 (Ar-*C*), 131.08 (Ar-*C*H), 130.07 (Ar-*C*H), 129.02 (Ar-*C*), 128.58 (Ar-*C*H), 128.06 (Ar-*C*H), 127.84 (Ar-*C*H), 127.57 (Ar-*C*H), 126.31 (Ar-*C*H), 125.78 (Ar-*C*H), 119.64 (Ar-*C*H), 119.01 (Ar-*C*H), 105.86 (Ar-*C*), 103.52 (Ar-*C*),

34.88 (*C*-H). MS: m/z (ESI) 376.3 $[M+H]^+$, 298.2, 268.8, 242.3, 227.2, 215.3, 180.1, Anal. Calc. for $C_{25}H_{17}N_3O$: C, 79.98; H, 4.56; N, 11.19. Found: C, 80.05; H, 4.52; N, 11.26.

4-(2,4-difluorophenyl)-3-phenyl-4,10-dihydroindeno[1,2-b]pyrazolo[4,3-e]pyridine-5(1H)-one (7b)

Red powder; m.p. 293-295 °C; FTIR (ATR, cm^{-1}): 3406, 3378, 3197, 3059, 3027, 2971, 2888, 1657, 1614, 1595, 1566, 1488, 1443, 1349, 1172, 1135, 1088, 972, 848, 770. ^1H NMR (500 MHz, DMSO-d_6, delta, ppm): 12.85 (s, 1H, N*H*), 11.44 (s, 1H, N*H*), 7.69 (d, *J*= 7.10 Hz, 1H, Ar-*H*), 7.47 (d, *J*= 7.50 Hz, 2H, Ar-*H*), 7.42 (t, *J*= 7.40 Hz, 1H, Ar-*H*), 7.36-7.29 (m, 4H, Ar-*H*), 7.24-7.11 (m, 2H, Ar-*H*), 6.92 (t, *J*= 9.10 Hz, 1H, Ar-*H*), 6.78 (t, *J*= 8.00 Hz, 1H, Ar-*H*), 5.56 (s, 1H, C-*H*). ^{13}C NMR (125 MHz, DMSO-d_6): δ 189.06 (*C*=O), 161.38 (Ar-*C*), 160.25 (Ar-*C*), 159.34 (Ar-*C*), 158.38 (Ar-*C*), 156.46 (Ar-*C*), 148.33 (Ar-*C*), 139.06 (Ar-*C*), 136.28 (Ar-*C*), 134.74 (Ar-*C*), 131.48 (Ar-*C*H), 131.08 (Ar-*C*H), 130.16 (Ar-*C*H), 128.84 (Ar-C*H*), 128.45 (Ar-C*H*), 128.17 (Ar-C*H*), 126.38 (Ar-*C*H), 123.26 (Ar-*C*H), 119.63 (Ar-*C*H), 119.14 (Ar-*C*H), 111.08 (Ar-*C*H), 104.34 (Ar-*C*), 102.95 (Ar-*C*H), 102.77 (Ar-*C*), 28.17 (C-*H*). MS: m/z (ESI) 412.1 $[M+H]^+$, 298.2, 269.2, 241.1, 180.0, 168.0. Anal. Calc. for $C_{25}H_{15}F_2N_3O$: C, 72.99; H, 3.68; N, 10.21. Found: C, 73.06; H, 3.57; N, 10.32.

3-phenyl-4-(thiophen-2-yl)-4,10-dihydroindeno[1,2-b]pyrazolo[4,3-e]pyridin-5(1H)-one (7c)

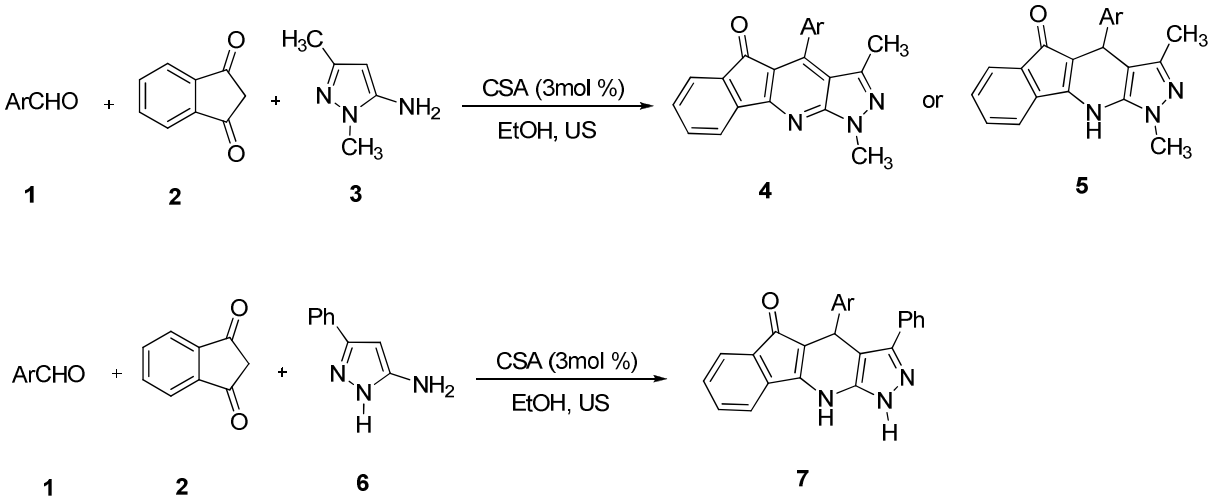
Red powder; m.p. 290-292 °C; FTIR (ATR, cm^{-1}): 3378, 3152, 3091, 3024, 2967, 2885, 1657, 1595, 1564, 1486, 1428, 1345, 1189, 1123, 1099, 1075, 1036, 966, 911, 756. ^1H NMR δ_H (500 MHz, DMSO-d_6, delta, ppm): 12.95 (s, 1H, N*H*), 11.45 (s, 1H, N*H*), 7.96-7.90 (m, 1H, Ar-*H*), 7.68 (d, *J*= 7.10 Hz, 1H, Ar-*H*), 7.61 (d, *J*= 7.50 Hz, 2H, Ar-*H*), 7.44-7.27 (m, 5H, Ar-*H*), 7.10 (d, *J*= 5.10 Hz, 1H, Ar-*H*), 6.79 (d, *J*= 3.10 Hz, 1H, Ar-*H*), 6.74 (dd, J_1= 4.90 Hz, J_2= 3.60 Hz, 1H, Ar-*H*), 5.70 (s, 1H, C-*H*). ^{13}C NMR (125 MHz, DMSO-d_6): δ 189.25 (*C*=O), 156.08 (Ar-*C*), 150.58 (Ar-*C*), 147.98 (Ar-*C*), 143.42 (Ar-*C*H), 141.38 (Ar-*C*), 139.20 (Ar-*C*), 136.16 (Ar-*C*), 135.64 (Ar-*C*H), 134.77 (Ar-*C*), 131.16 (Ar-*C*H), 130.20 (Ar-*C*H), 128.70 (Ar-*C*H), 128.23 (Ar-*C*H), 126.43 (Ar-*C*H), 123.74 (Ar-*C*H), 123.51 (Ar-*C*H), 122.80 (Ar-*C*H), 119.84 (Ar-*C*H), 119.19 (Ar-*C*H), 106.15 (Ar-*C*), 103.37 (Ar-*C*), 29.69 (*C*-H). MS: m/z (ESI) 382.3 $[M+H]^+$, 297.8. Anal. Calc. for $C_{23}H_{15}N_3OS$: C, 72.42; H, 3.96; N, 11.02. Found: C, 72.61; H, 3.90; N, 10.98.

4-(Furan-2-yl)-3-phenyl-4,10-dihydroindeno[1,2-b]pyrazolo[4,3-e]pyridin-5(1H)-one (7d)

Red powder; m.p. 294-296 °C; FTIR (ATR, cm^{-1}): 3376, 3188, 3139, 3096, 3062, 2945, 1727, 1691, 1606, 1589, 1467, 1351, 1220, 1161, 1017, 884, 774, 737. ^{1}H NMR δ_H (500 MHz, DMSO-d_6, delta, ppm): 12.89 (s, 1H, N*H*), 11.42 (s, 1H, N*H*), 7.81-7.77 (m, 1H, Ar-*H*), 7.64-7.54 (m, 3H, Ar-*H*), 7.40-7.20 (m, 5H, Ar-*H*), 7.08 (d, J= 5.30 Hz, 1H, Ar-*H*), 6.75 (d, *J*= 7.50 Hz, 1H, Ar-*H*), 6.60 (d, *J*= 7.40 Hz, 1H, Ar-*H*), 5.60 (s, 1H, C-*H*). ^{13}C NMR (125 MHz, DMSO-d_6): δ 189.43 (*C*=O), 156.25 (Ar-*C*), 150.20 (Ar-*C*), 148.10 (Ar-*C*), 143.36 (Ar-*C*), 142.18 (Ar-*C*H), 136.50 (Ar-*C*), 135.76 (Ar-*C*), 134.60 (Ar-*C*), 133.12 (Ar-*C*H), 130.05 (Ar-*C*H), 128.60 (Ar-*C*H), 127.51 (Ar-*C*H), 127.02 (Ar-*C*H), 126.30 (Ar-*C*H), 123.45 (Ar-*C*H), 122.90 (Ar-*C*H), 119.10 (Ar-*C*H), 118.85 (Ar-*C*H), 107.70 (Ar-*C*H), 104.83 (Ar-*C*), 102.40 (Ar-*C*), 29.60 (*C*-H). MS: m/z (ESI) 366.2 $[M+H]^+$, 298.9. Anal. Calc. for $C_{23}H_{15}N_3O_2$: C, 75.60; H, 4.14; N, 11.50. Found: C, 75.54; H, 4.20; N, 11.55.

RESULTS AND DISCUSSION

Initially, the condensation reaction of benzaldehyde (1.00 mmol), indan-1,3-dione (1.00 mmol), and 1,3-dimethyl-1*H*-pyrazol-5-amine (1.00 mmol) was examined under ultrasonic irradiation without a catalyst at 60 °C in 5 mL of EtOH. The reaction was completed in 15 minutes with a yield of 80%. In order to observe the effect of CSA as a catalyst, the same reaction was examined under ultrasound irradiation (Table 1) in the presence of 3 mol % CSA at 40 °C. The reaction was completed in 7 minutes with a yield of 88%. Higher amount of catalyst or higher temperature did not lead to significant change in the reaction yields.



Scheme 1: Multi-component synthesis of pyrazolopyridine derivatives.

Table 1: Effect of amount of CSA on the synthesis of **4a.**

CSA (mol %)	Temperature (°C)	Time (min)	Yield (%)[a]
-	60	15	80
3	40	7	88
3	60	7	88
5	40	7	88
10	40	7	89

[a]Isolated yield.

With the best optimized condition in hand, several pyrazolopyridines were synthesized at 40 °C using CSA catalyst in EtOH under ultrasonic irradiation (Scheme 1). The results are summarized in Table 2.

It is important to point out the fact that when 1,3-dimethyl-1*H*-pyrazol-5-amine, indan-1,3-dione and benzaldehyde or electron withdrawing 4-cyanobenzaldehyde were sonicated for required reaction time, the reaction leads to the formation of the aromatized pyrazolopyridine derivatives **4**, which were isolated and characterized, but in the case of using aromatic aldehydes containing electron releasing substituents, dihydropyrazolopyridine derivatives **5** were observed.

Then the same reaction was performed with 3-phenyl-1*H*-pyrazol-5-amine, indan-1,3-dione and aromatic aldehydes under the same conditions, this time only dihydropyrazolopyridine derivatives **7** were observed (Scheme 1, Table 2) as products.

Table 2: Synthesis of pyrazolopyridine using catalytic amount of CSA.

Product	Ar	Time (min)	Yield %)[a]
4a	Phenyl	7	88
4b	4-Cyanophenyl	7	94
5c	2,4-Difluorophenyl	7	92
5d	4-Methylphenyl	7	90
5e	5-Nitrofuran-2-yl	7	96
7a	Phenyl	7	92
7b	2,4-Difluorophenyl	7	96
7c	Thiophen-2-yl	7	97
7d	Furan-2-yl	7	95

[a]Isolated yield

The structure of the newly generated compounds have been confirmed by Fourier transform-infrared (FTIR), mass and NMR techniques. In the ^{1}H NMR spectra of dihydropyrazolopyridines **5c-e** and **7a-d**, benzylic C-H proton resonated at near *δ* 4.80-

5.70 and in their ^{13}C NMR spectra, the benzylic C—H carbon resonated at near δ 27-35. The mass spectra of all new compounds showed the expected molecular ion peak.

Mainly effective HAT agents are compounds with high hydrogen atom donating ability, which is compounds with low dissociation energies of heteroatom-H bond and/or compounds from which abstraction of hydrogen leads to C-centered radicals stabilized by resonance or compounds from which hydrogen abstraction leads to sterically hindered radicals (40). In this study, the antioxidant activity of compounds **4b**, **5c**, **5e**, **7a**, **7b** and **7c** were examined for free radical scavenging activity (determined for DPPH), reducing activity (reduction of the Fe^{3+}/ferricyanide complex to its ferrous form), metal chelating (chelating activity capacity of ferrous ions) capacity, superoxide scavenging activity, and total antioxidant activity.

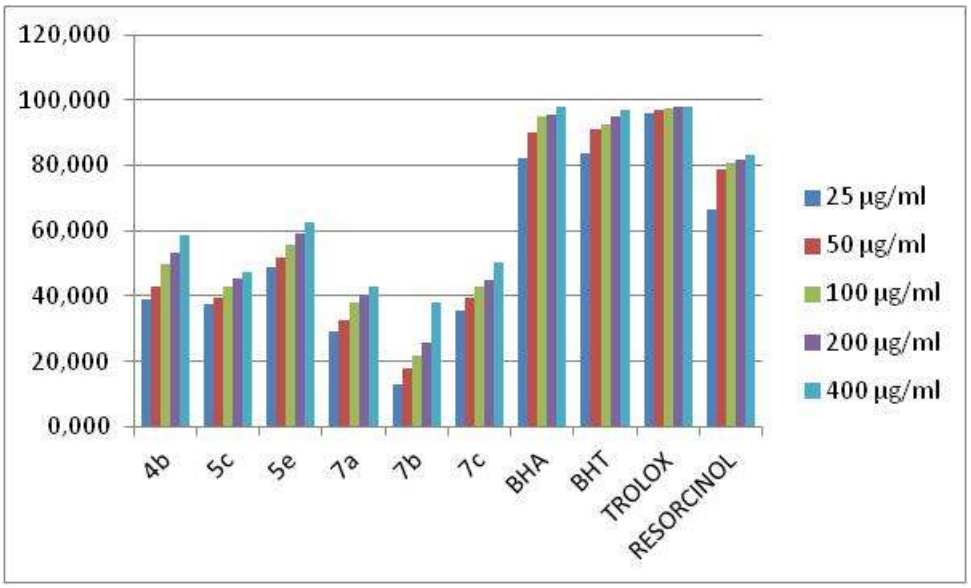


Figure 1: DPPH radical scavenging activity of pyrazolopyridine compounds. BHA, BHT, Trolox and Resorcinol were used as reference antioxidants.

DPPH• (1,1-diphenyl-2-picryl-hydrazyl) is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The scavenging effect of pyrazolopyridine compounds (**4b**, **5c**, **5e**, **7a**, **7b** and **7c**) and standards (BHA, BHT, trolox, and resorcinol) on the DPPH• radical decreased in order of: BHA > trolox > BHT > resorcinol > **5e** > **4b** > **5c** > **7c** > **7a** > **7b** (Figure 1). These results indicate that pyrazolopyridine compounds had a moderate effect on scavenging free radical, and free radical scavenging activity was increased with increasing concentration. A higher DPPH radical scavenging activity is associated with a lower EC_{50} value.

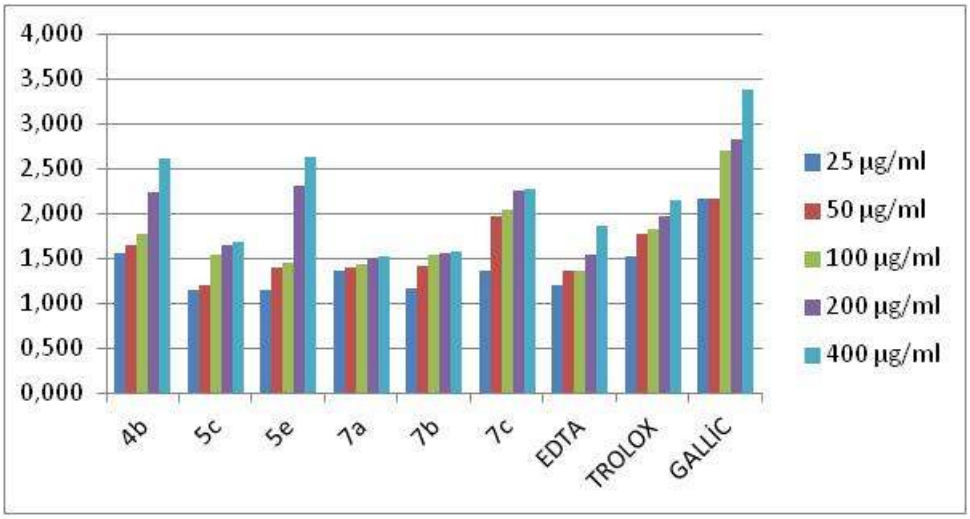


Figure 2: Reducing power of pyrazolopyridine compounds. EDTA, Trolox and Gallic acid were used as reference antioxidants.

In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of ferric iron to ferrous iron by electron donation. Pyrazolopyridine compounds **4b**, **5e**, and **7c** showed higher reducing power than the other pyrazolopyridine compounds (Fig. 2), and reducing power was increased with increasing concentration.

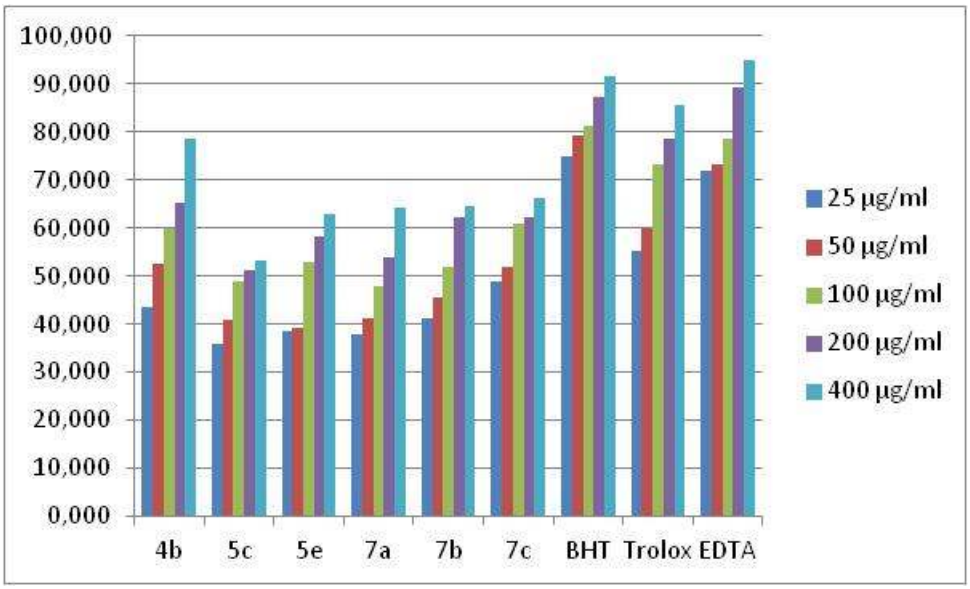


Figure 3: Metal chelating activity of pyrazolopyridine compounds. BHT, Trolox and EDTA were used as reference antioxidants.

The metal chelating activity for ferrous ion of the pyrazolopyridine compounds (**4b**, **5c**, **5e**, **7a**, **7b** and **7c**) was assayed by the inhibition of red-colored ferrozine/$FeCl_2$ complex. EDTA, Trolox and BHT were used as standard compounds. The pyrazolopyridine compounds showed moderate metal chelating activity at 25, 50,100, 200 and 400 µg/mL (Fig. 3).

Nevertheless, compound **4b** exhibited the highest chelating activity among the tested pyrazolopyridine compounds at 400 µg/mL (78.58 %).

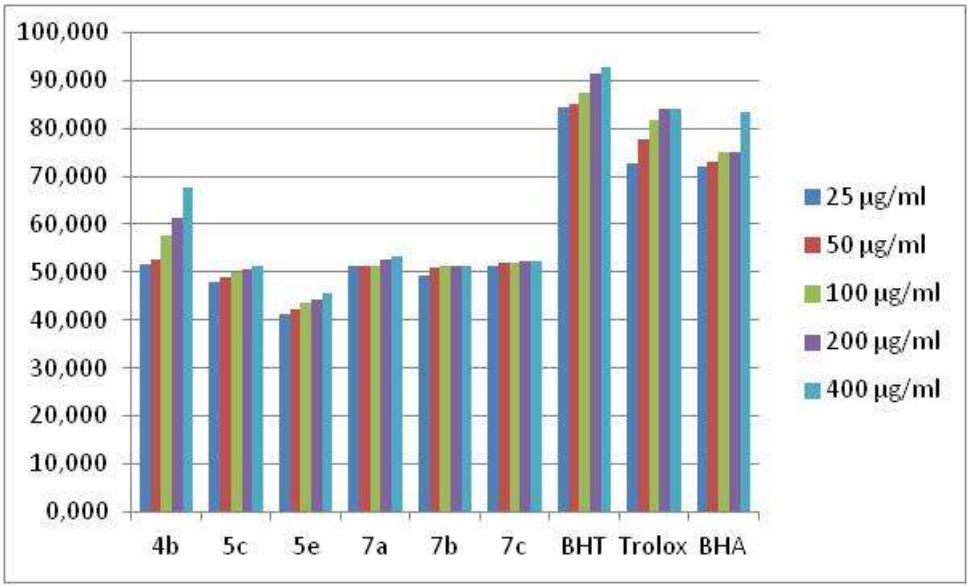


Figure 4: Superoxide scavenging activity of pyrazolopyridine compounds. BHT, Trolox and BHA were used as reference antioxidants.

Fig. 4 shows the % inhibition of superoxide radical generation by 25, 50, 100, 200 and 400 µg/mL of pyrazolopyridine compounds (**4b**, **5c**, **5e**, **7a**, **7b** and **7c**) and comparison with same concentrations of BHT, trolox and BHA. None of the compounds showed greater superoxide scavenging activity than the standards. The best inhibition (67.61%) was measured for the compound **4b** at 400 µg/mL.

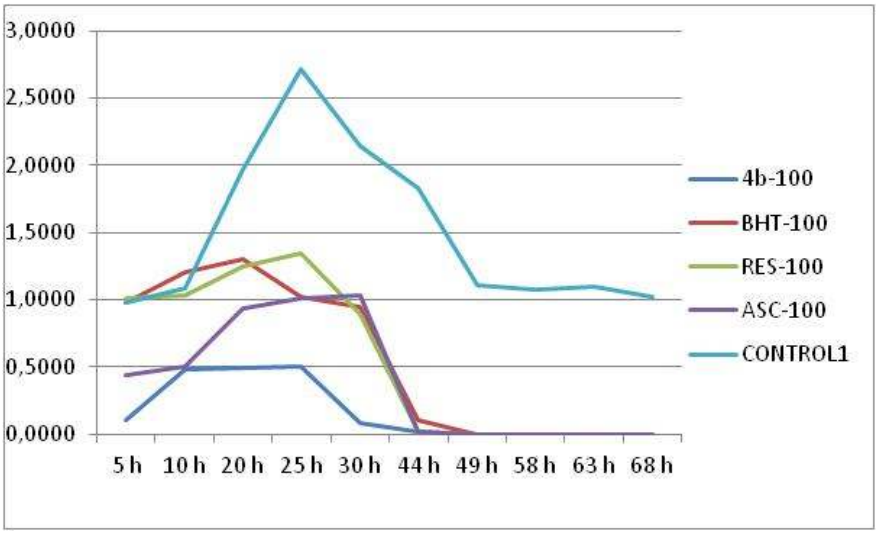


Figure 5: Total antioxidant activities of pyrazolopyridine compounds. BHT, Resorcinol and Ascorbic acid were used as reference antioxidants.

Total antioxidant activity of pyrazolopyridine compounds was determined by the thiocyanate method. Compound **4b** exhibited effective antioxidant activity. The effect of 100 µg/mL concentration of compound **4b** on lipid peroxidation of linoleic acid emulsion are shown in Figure 5. The results clearly showed that compound **4b** had stronger total antioxidant activity than BHT, resorcinol and ascorbic acid at the same concentration (100 µg/mL). The effects on lipid peroxidation of linoleic acid pyrazolopyridine compound 4b and standards decreased in that order: compound **4b** > ascorbic acid > BHT > resorcinol.

In conclusion, this one-pot three component protocol using CSA as an organocatalyst in ethanol provides a practical method for the preparation of fused pyrazolo pyridines from 1,3-dimethyl-1*H*-pyrazol-5-amine or 3-phenyl-1*H*-pyrazol-5-amine, indan-1,3-dione and various aromatic aldehydes in short reaction times and excellent yields. The simplicity, high atom economy, easy execution, and work up are the notable features of this procedure. The antioxidant activity of compounds **4b**, **5c**, **5e**, **7a**, **7b** and **7c** were determinate. Most potent was found to be compound **4b** followed by **5e** and **7c**.

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