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Synthesis and Antiproliferative Activity of Some Dihydro-1*H*-furo[2,3-*c*]pyrazole-Flavone Hybrids

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A new series of dihydro-1*H*-furo[2,3-*c*]pyrazole-flavone hybrids were synthesized from one-pot four-component reaction of β-keto ester (**1**), hydrazine (**2**),7-hydroxy 8-formyl flavones (**3**), pyridiniumylide (**4**) in presence of NEt_3 as catalyst under ethanol reflux conditions and their antiproliferative properties were evaluated against human cancer cell lines, namely, laryngeal carcinoma (Hep2), lung adenocarcinoma (A549) and cervical cancer (HeLa). The best among them, furo[2,3-*c*]pyrazole-flavone with C4'-methoxy substitution was selected for further structure activity relationship (SAR) studies. Among the derivatives, (4*S*,5*S*)-ethyl 4-(7-hydroxy-5-methoxy-4-oxo-2-(2,4,6-trimethoxyphenyl)-4*H*-chromen-8-yl)-3-methyl-4,5-dihydro-1*H*-furo[2,3-*c*]pyrazole-5-carboxylate (**8r**) showed most potent cytotoxic activity against all three cancer cell lines. Toxicity studies revealed that the dihydro-1*H*-furo[2,3-*c*]pyrazole-flavones are specifically target the cancer cell lines.

Keywords: Furo[2,3-*c*]pyrazole-flavone hybrids, Pyridiniumylide, 7-Hydroxy 8-formyl flavone, Antiproliferative.

INTRODUCTION

Flavones are widely distributed heterocyclic natural products with varied biological activities [1]. In technological and medicinal fields, flavones, find extensive uses [2]. Flavones, particularly those with several phenolic hydroxyl groups are used as antioxidants. Flavopyridol, demifin, flavone-*c*-glycoside, cannflavin, mitoflaxone, flavoxate are some of the commercially available flavone drugs [3-10]. Several reports are available for antiprolifirative activity of natural and synthetic flavones [11-26]. The ability to manipulate flavone activity through structural variation motivates research on the synthesis of flavanone derivatives and evaluation of their bioactivity. Beutler *et al.* [27] reported results of a comparative cytotoxicity screening and subsequent tubulin polymerization studies carried out with a series of 79 natural and synthetic flavones. Maximum potencies for cytotoxicity and tubulin interaction were found only with compounds bearing an OMe group at C-5 on the A-ring, 3'-hydroxy-4'-methoxy groups on the B-ring and an OCH_3 at C-3 on the C-ring [28]. In the continuous efforts towards the synthesis of different biologically active heterocyclic molecules [29-32] in our laboratory recently we have developed a new methodology for synthesis of biologically active dihydro-1*H*-furo[2,3-*c*]-pyrazole flavones from four-component reaction of β-keto ester, hydrazine, aromatic aldehydes, pyridinium ylide utilizing microwave irradiation under solvent-free conditions. Schlager *et al.* [34] reported that spiro cyclicfuropyrazoles acts as σ-receptor ligands. Chavan *et al.* [35] reported anti-inflammatory activity of pyrazole amalgamated flavones. Now we are contemplated to synthesize furopyrazole-flavone hybrids by in incorporating furopyrazole on 7-hydroxy flavone by employing our newly developed methodology and in order to study their biological activity. Such hybrid molecules represent both 3-furopyrazoles and flavones characteristics. Moreover, furopyrazole-flavone hybrids, target molecules of present study are not known.

EXPERIMENTAL

The progression of all the reactions were monitored by TLC using hexanes (60-80 °C boiling mixture)/ethyl acetate mixture as eluent. Column chromatography was carried on silica gel (100-200 mesh, SRL chemicals) using increasing percentage of ethyl acetate in hexanes. ^{1}H NMR spectra (400 MHz) and ^{13}C NMR (100 MHz) and DEPT-135 spectra were recorded for $CDCl_3$ solutions on a Bruker-400 spectrometer with tetramethylsilane (TMS) as internal standard; *J*-values are in Hz. Number of hydrogens attached to each carbon was determined from DEPT spectra and are given next to the corresponding ^{13}C NMR spectral data. IR spectra were recorded as KBr pellets on a Nicolet-6700 spectrometer. Melting points

were recorded using open-ended capillary tubes on VEEGO VMP-DS instrument. High resolution mass spectra were recorded on a Waters Micromass Q-TOF micro mass spectrometer using electron spray ionization mode. Organic solvents were distilled and dried before use.

General procedure for synthesis of dihydro-1*H*-furo[2,3-*c*]pyrazole-flavone hybrids (8a-r)

(4R,5R)-Ethyl 4-(7-hydroxy-4-oxo-2-phenyl-4*H*-chromen-8-yl)-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8a): Mixture of ethyl acetoacetate (**1**, 211 mg, 0.153 mmol), hydrazine (**2**, 81 mg, 0.153), 7-hydroxy-4-oxo-2-phenyl-4*H*-chromene-8-carbaldehyde (**4a**, 406 mg, 0.153 mmol), 1-(2-ethoxy-2-oxoethyl)pyridinium ylide (**4**, 272 mg, 0.153 mmol), 0.1 equivalents of trimethylamine (16 mg, 0.015 mmol) in15 mL EtOH were refluxed in a pre-heated oil bath (80 °C) under the blanket of nitrogen for 30 min till the completion of reaction (TLC, 20 % dicholromethane in hexanes; R_f = 0.3). The reaction mixture was diluted with dichloromethane (10 mL) and the organic solution was washed with water (20 mL) and brine (20 mL) and dried over anhydrous Na_2SO_4. Column chromatographic purification on silica gel with increasing amount of dichlormethane in hexanes provided **8a** as a free flowing solid in about 88 % yield. Analytical samples were obtained through from the recrystallization in EtOH. Light yellow colour solid. Yield 581 mg (88 %); m.p.: 136.5 °C, IR (KBr, ν_{max}, cm^{-1}): 3310, 3206, 3035, 2998, 2680, 2622, 1746, 1670, 1642, 1490, 1421, 1374, 1343, 1213, 1182, 1114, 1076, 1022, 982, 880, 822, 775; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.26 (s, 1H), 8.25 (d, *J* = 8.8 Hz, 1H), 7.79 (d, *J* = 8.7 Hz, 2H), 7.44-7.42 (m, 3H), 7.14 (d, *J* = 7.9 Hz, 1H), 6.80 (s, 1H), 6.09 (br s, 1H), 5.29 (d, *J* = 4.6 Hz, 1H), 5.16 (d, *J* = 4.6 Hz, 1H), 4.16 (q, *J* = 6.6 Hz, 2H), 1.91 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ191.7, 176.8, 165.6, 163.3, 161.5, 157.8, 139.6, 132.1, 129.9, 126.7, 124.9, 122.3, 121.7, 118.6, 117.3, 112.6, 105.2, 88.0, 62.7, 26.7, 14.2, 12.3 ppm; HRMS (ESI, *m/z*) 455.1214 calcd. for $C_{24}H_{20}N_2O_6$ (M+Na) found 455.1212; Anal. calcd. for $C_{24}H_{20}N_2O_6$; C, 66.66; H, 4.66; N, 6.48; Found; C, 66.65; H, 4.65; N, 6.46.

(4R,5R)-Ethyl 4-[2-(4-chlorophenyl)-7-hydroxy-4-oxo-4*H*-chromen-8-yl]-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8b): Light yellow colour solid, m.p.: 138.1 °C, IR (KBr, ν_{max}, cm^{-1}): 3312, 3212, 3075, 2987, 2686, 2630, 1743, 1672, 1644, 1496, 1406, 1377, 1330, 1215, 1179, 1115, 1081, 1023, 995, 963, 881, 848, 769; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.28 (s, 1H), 8.25 (d, *J* = 8.8 Hz, 1H), 7.79 (d, *J* = 7.1 Hz, 2H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.05 (d, *J* = 7.9 Hz, 2H), 6.80 (s, 1H), 6.06 (br s, 1H), 5.29 (d, *J* = 4.6 Hz, 1H), 5.16 (d, *J* = 4.6 Hz, 1H), 4.16 (q, *J* = 6.6 Hz, 2H), 1.91 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ191.3, 176.6, 166.9, 163.3, 161.3, 157.6, 139.2, 133.3, 129.4, 126.7, 124.6,123.3, 121.7, 118.6, 117.3, 112.3, 105.1, 88.0, 62.7, 26.7, 14.2, 12.3 ppm; HRMS (ESI, *m/z*) 489.0824 calcd. for $C_{24}H_{19}N_2O_6Cl$ (M+Na) found 489.0821; Anal. calcd. for $C_{24}H_{19}N_2O_6Cl$; C, 61.74; H, 4.10; N, 6.00; Found; C, 61.72; H, 4.10; N, 5.98.

(4R,5R)-Ethyl 4-[2-(4-bromophenyl)-7-hydroxy-4-oxo-4*H*-chromen-8-yl]-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8c): Light yellow colour solid, m.p.: 141.4 °C, IR (KBr, ν_{max}, cm^{-1}): 3315, 3205, 3075, 2987, 1746, 1681, 1649, 1490, 1422, 1350, 1335, 1203, 1123, 1102, 1089, 1043, 996, 885, 842, 765; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.16 (s, 1H), 8.27 (d, *J* = 8.4 Hz, 1H), 7.61 (d, *J* = 7.8 Hz, 2H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.05 (d, *J* = 7.8 Hz, 2H), 6.79 (s, 1H), 6.09 (br s, 1H), 5.29 (d, *J* = 4.6 Hz, 1H), 5.16 (d, *J* = 4.6 Hz, 1H), 4.16 (q, *J* = 6.6 Hz, 2H), 1.91 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ191.0, 175.3, 166.8, 163.3, 161.4, 157.6, 139.2, 133.7, 129.4, 126.7, 124.6,123.3, 121.7, 118.6, 117.3, 112.3, 105.1, 88.0, 62.7, 26.7, 14.2, 12.3 ppm; HRMS (ESI, *m/z*) 533.0319 calcd. for $C_{24}H_{19}N_2O_6Br$ (M+Na) found 533.0317; Anal. calcd. for $C_{24}H_{19}N_2O_6Br$; C, 56.37; H, 3.75; Br, 15.63; N, 5.48; Found; C, 56.36; H, 3.74; N, 5.46.

(4R,5R)-Ethyl 4-(7-hydroxy-4-oxo-2-*p*-tolyl-4*H*-chromen-8-yl)-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8d): Light yellow colour solid, m.p.: 130.7 °C, IR (KBr, ν_{max}, cm^{-1}): 3313, 3202, 3032, 2987, 1746, 1672, 1644, 1483, 1411, 1365, 1338, 1210, 1184, 1123, 1075, 1034, 987, 893; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.04 (s, 1H), 8.25 (d, *J* = 8.4 Hz, 1H), 7.79 (d, *J* = 7.8 Hz, 2H), 7.43 (d, *J* = 8.8 Hz, 1H), 7.05 (d, *J* = 7.7 Hz, 2H), 6.81 (s, 1H), 6.11 (br s, 1H), 5.29 (d, *J* = 4.6 Hz, 1H), 5.16 (d, *J* = 4.6 Hz, 1H), 4.16 (q, *J* = 6.6 Hz, 2H), 1.91 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ191.1, 176.7, 166.3, 163.3, 161.3, 157.4, 141.7, 139.2, 129.4, 126.3, 124.6, 124.6, 123.3, 121.7, 118.6, 117.7, 112.3, 105.1, 88.0, 62.8, 28.6, 21.7, 14.1, 12.1 ppm; HRMS (ESI, *m/z*) 469.1370 calcd. for $C_{25}H_{22}N_2O_6$ (M+Na) found 469.1367; Anal. calcd. for $C_{25}H_{22}N_2O_6$; C, C, 67.26; H, 4.97; N, 6.27; Found; C 67.25; H, 4.96; N, 6.25.

(4R,5R)-Ethyl 4-(7-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H*-chromen-8-yl)-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8e): Light yellow colour solid, m.p.: 138.2 °C, IR (KBr, ν_{max}, cm^{-1}): 3341, 3231, 3023, 2987, 1746, 1672, 1644, 1496, 1398, 1330, 1218, 1198, 1145, 1081, 1023, 995, 878; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.29 (s, 1H), 9.09 (s, 1H), 8.30 (d, *J* = 8.8 Hz, 1H), 7.63 (d, *J* = 7.1 Hz, 2H), 7.34 (d, *J* = 8.8 Hz, 1H), 7.15 (d, *J* = 7.9 Hz, 2H), 6.79 (s, 1H), 6.13 (br s, 1H), 5.28 (d, *J* = 4.6 Hz, 1H), 5.16 (d, *J* = 4.6 Hz, 1H), 4.17 (q, *J* = 6.8 Hz, 2H), 1.92 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ191.2, 178.7, 168.3, 164.3, 161.0, 159.7, 158.5, 138.4, 129.6, 127.7, 125.6, 123.7, 121.7, 119.5, 118.3, 113.3, 105.7, 88.3, 62.6, 26.6, 14.4, 12.2 ppm; HRMS (ESI, *m/z*) 471.1163 calcd. for $C_{24}H_{20}N_2O_7$ (M+Na) found 471.1161; Anal. calcd. for $C_{24}H_{20}N_2O_7$; C, 64.28; H, 4.50; N, 6.25; Found; C, 64.27; H, 4.49; N, 6.23.

(4R,5R)-Ethyl 4-[7-hydroxy-2-(4-methoxyphenyl)-4-oxo-4*H*-chromen-8-yl]-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8f): Light yellow colour solid, m.p.: 135.7 °C, IR (KBr, ν_{max}, cm^{-1}): 3327, 3212, 3137, 3075, 2987, 1746, 1687, 1644, 1498, 1407, 1379, 1336, 1207, 1179, 1129, 1081, 1023, 992, 875, 778; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.26 (s, 1H), 8.30 (d, *J* = 8.2 Hz, 1H), 7.83 (d, *J* = 7.8 Hz, 2H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.05 (d, *J* = 7.8 Hz, 2H), 6.79 (s, 1H), 6.09 (br s, 1H), 5.29 (d, *J* = 4.6 Hz, 1H), 5.16 (d, *J* =

4.6 Hz, 1H), 4.16 (q, *J* = 6.6 Hz, 2H), 1.91 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ191.2, 177.7, 167.3, 163.3, 161.4, 159.4, 157.7, 139.4, 128.6, 126.7, 124.6, 123.7, 121.7, 118.5, 117.3, 112.3, 105.1, 88.3, 62.6, 59.4, 26.6, 14.1, 12.1 ppm; $C_{25}H_{22}N_2O_7$, C, 64.93; H, 4.80; N, 6.06; O, 24.22, (M + Na) 485.1319. HRMS (ESI, *m/z*) 485.1319 calcd. for $C_{25}H_{22}N_2O_7$ (M+Na) found 485.1316; Anal. calcd. for $C_{25}H_{22}N_2O_7$; C, 64.93; H, 4.80; N, 6.06; O, 24.22. Found; C, 64.91; H, 4.78; N, 6.05.

(4R,5R)-Ethyl 4-[2-{4-(dimethylamino)phenyl}-7-hydroxy-4-oxo-4*H*-chromen-8-yl]-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8g): Light yellow colour solid, m.p. 143.1 °C, IR (KBr, ν_{max}, cm^{-1}): 3304, 3212, 3075, 2987, 2686, 2630, 1746, 1672, 1644, 1496, 1406, 1377, 1330, 1215, 1179, 1115, 1081, 1023, 995, 963, 881, 848, 769; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.27 (s, 1H), 8.30 (d, *J* = 8.8 Hz, 1H), 7.53 (d, *J* = 7.1 Hz, 2H), 7.34 (d, *J* = 8.8 Hz, 1H), 7.05 (d, *J* = 7.9 Hz, 2H), 6.79 (s, 1H), 6.12 (br s, 1H), 5.28 (d, *J* = 4.6 Hz, 1H), 5.15 (d, *J* = 4.6 Hz, 1H), 4.16 (q, *J* = 6.6 Hz, 2H), 3.16 (s, 6H), 1.91 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.2, 177.4, 166.3, 163.7, 161.3, 156.5, 150.3, 140.4, 127.6, 126.5, 124.6, 123.7, 121.7, 118.5, 117.7, 112.3, 105.2, 88.3, 62.6, 41.1, 26.6, 14.1, 12.1 ppm; HRMS (ESI, *m/z*) 498.1636 calcd. for $C_{26}H_{25}N_3O_6$ (M+Na) found 498.1634; Anal. calcd. for $C_{26}H_{25}N_3O_6$; C, 65.67; H, 5.30; N, 8.84. Found; C, 65.66; H, 5.28; N, 8.82.

(4R,5R)-Ethyl 4-(7-hydroxy-2-(4-nitrophenyl)-4-oxo-4*H*-chromen-8-yl)-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8h): m.p. 139.3 °C, IR (KBr, ν_{max}, cm^{-1}): 3340, 3210, 3021, 2994, 1745, 1672, 1644, 1496, 1406, 1377, 1330, 1215, 1179, 1115, 1081, 1023, 995, 963, 881; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.26 (s, 1H), 8.20 (d, *J* = 8.8 Hz, 1H), 7.54 (d, *J* = 7.2 Hz, 1H), 7.36 (d, *J* = 8.4 Hz, 2H), 7.07 (d, *J* = 8.1 Hz, 2H), 6.86 (s, H), 6.11 (br s, 1H), 5.41 (d, *J* = 4.6 Hz, 1H), 5.16 (d, *J* = 4.6 Hz, 1H), 4.19 (q, *J* = 6.6 Hz, 2H),1.91 (s, 3H), 0.97 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ191.0, 175.3, 166.8, 163.3, 161.3, 157.7, 147.3, 139.2, 136.7, 126.7, 124.6, 123.3, 121.7, 118.6, 117.3, 112.3, 105.1, 88.0, 62.6, 26.7, 14.3, 12.3 ppm; HRMS (ESI, *m/z*) 500.1064 calcd. for $C_{24}H_{19}N_3O_8$ (M+Na) found 500.1064; Anal. calcd. for $C_{24}H_{19}N_3O_8$; C, 60.38; H, 4.01; N, 8.80; Found; C, 60.36; H, 4.00; N, 8.79.

(4R,5R)-Ethyl 4-(2-(2,4-dimethylphenyl)-7-hydroxy-4-oxo-4*H*-chromen-8-yl)-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8i): Light yellow colour solid, m.p.: 141.6 °C, IR (KBr, ν_{max}, cm^{-1}): 3337, 3212, 3075, 2987, 1746, 1672, 1644, 1487, 1404, 1383, 1330, 1210, 1183, 1126, 1085, 1018, 969, 881, 848, 769; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.14 (s, 1H), 8.27 (d, *J* = 8.8 Hz, 1H), 7.58 (s, 1H), 7.43 (d, *J* = 8.8 Hz, 2H), 7.07 (d, *J* = 8.2 Hz, 2H), 6.77 (s, H), 5.29 (d, *J* = 4.6 Hz, 1H), 6.14 (br s, 1H), 5.15 (d, *J* = 4.6 Hz, 1H), 4.16 (q, *J* = 6.6 Hz, 2H), 2.38 (s, 3H), 2.26 (s, 3H), 1.91 (s, 3H), 0.97 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.3, 175.3, 166.8, 163.3, 161.3, 157.7, 142.2, 139.7, 133.4, 129.8, 127.7, 125.6, 124.3, 123.7, 122.6, 118.7, 117.3, 112.3, 105.1, 88.1, 62.8, 26.7, 21.84, 21.82, 14.3, 12.3 ppm; HRMS (ESI, *m/z*) 483.1524 calcd. for $C_{26}H_{24}N_2O_6$ (M+Na) found 483.1527; Anal. calcd. for $C_{26}H_{24}N_2O_6$; C, 67.82; H, 5.25; N, 6.08; Found; C, 67.80; H, 5.24; N, 6.06.

(4R,5R)-Ethyl 4-(7-hydroxy-2-mesityl-4-oxo-4*H*-chromen-8-yl)-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]-pyrazole-5-carboxylate (8j): Light yellow colour solid, m.p. 144.2 °C, IR (KBr, ν_{max}, cm^{-1}): 3354, 3212, 3075, 2987, 1746, 1672, 1644, 1496, 1404, 1379, 1337, 1212, 1184, 1127, 1094, 1017, 989, 958, 887, 851, 769; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.19 (s, 1H), 8.27 (d, *J* = 8.8 Hz, 1H), 7.79 (s, 2H), 7.43 (d, *J* = 8.8 Hz, 1H), 6.79 (s, H), 6.16 (br s, 1H), 5.29 (d, *J* = 4.6 Hz, 1H), 5.15 (d, *J* = 4.6 Hz, 1H), 4.16 (q, *J* = 6.6 Hz, 2H), 2.29 (s, 3H), 2.22 (s, 6H), 1.91 (s, 3H), 0.97 (t, *J* = 7.4 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.0, 175.3, 166.8, 163.3, 161.3, 157.7, 139.3, 136.2, 133.7, 127.4, 125.8, 124.6, 121.7, 118.6, 117.3, 112.3, 105.1, 88.0, 62.6, 26.7, 21.7, 21.1, 14.3, 12.3 ppm; HRMS (ESI, *m/z*) 497.1683 calcd. for $C_{27}H_{26}N_2O_6$ (M+Na) found 497.1680; Anal. calcd. for $C_{27}H_{26}N_2O_6$; C, 68.34; H, 5.52; N, 5.90. Found; C, 68.32; H, 5.50; N, 5.89.

(4R,5R)-Ethyl 4-(2-(2,4-dichlorophenyl)-7-hydroxy-4-oxo-4*H*-chromen-8-yl)-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8k): Light yellow colour solid, m.p.: 135.4 °C, IR (KBr, ν_{max}, cm^{-1}): 3310, 3205, 3085, 2981, 2690, 2639, 1746, 1670, 1644, 1491, 1404, 1370, 1338, 1215, 1179, 1119, 1086, 1026, 998, 965, 883, 842, 769, 742, 692; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.14 (s, 1H), 8.27 (d, *J* = 8.8 Hz, 1H), 7.81 (d, *J* = 7.1 Hz, 1H), 7.59 (s, 1H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.05 (d, *J* = 7.9 Hz, 1H), 6.79 (s, 1H), 6.09 (br s, 1H), 5.29 (d, *J* = 4.6 Hz, 1H), 5.16 (d, *J* = 4.6 Hz, 1H), 4.16 (q, *J* = 6.6 Hz, 2H), 1.91 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.0, 175.3, 166.8, 163.3, 161.3, 157.7, 143.3, 139.2, 133.7, 129.4, 127.8, 125.7, 124.6, 123.3, 121.7, 118.6, 117.3, 112.3, 105.1, 88.0, 69.6, 26.7, 14.3, 12.3 ppm; HRMS (ESI, *m/z*) 523.0434 calcd. for $C_{24}H_{18}N_2O_6Cl_2$ (M+Na) found 523.0431. Analysis calcd. for $C_{24}H_{18}N_2O_6Cl_2$: C, 57.50; H, 3.62; N, 5.59; Found C, 57.49; H, 3.60; N, 5.57.

(4S,5S)-Ethyl 4-[7-hydroxy-4-oxo-2-(2,4,6-trichlorophenyl)-4*H*-chromen-8-yl]-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8l): Light yellow colour solid, m.p.: 138.1 °C, IR (KBr, ν_{max}, cm^{-1}): 3320, 3221, 3072, 2970, 2692, 2641, 1746, 1672, 1643, 1490, 1408, 1375, 1333, 1212, 1177, 1119, 1085, 1027, 994, 964, 886, 842, 764, 744, 698; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.12 (s, 1H), 8.28 (d, *J* = 8.8 Hz, 1H), 7.79 (d, *J* = 7.1 Hz, 1H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.05 (d, *J* = 7.9 Hz, 1H), 6.79 (s, 1H), 6.09 (br s, 1H), 5.29 (d, *J* = 4.6 Hz, 1H), 5.16 (d, *J* = 4.6 Hz, 1H), 4.16 (q, *J* = 6.6 Hz, 2H), 1.92 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ191.3, 176.2, 167.2, 163.5, 161.3, 157.9, 139.1, 136.3, 133.2, 127.2, 125.8, 124.6, 121.7, 118.6, 117.3, 112.4, 105.2, 88.2, 62.7, 26.8, 14.3, 12.3 ppm; HRMS (ESI, *m/z*) 557.0044 calcd. for $C_{24}H_{17}N_2O_6Cl_3$ (M+Na) found 557.0042. Analysis calcd. for $C_{24}H_{17}N_2O_6Cl_3$: C, 53.80; H, 3.20; N, 5.23; Found C, 53.78; H, 3.19; N, 5.22.

(4R,5R)-Ethyl 4-[7-hydroxy-4-oxo-2-(pyridin-3-yl)-4*H*-chromen-8-yl]-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8m): Light yellow colour solid, m.p.: 140.5 °C, IR (KBr, ν_{max}, cm^{-1}): 3324, 3234, 3046, 2970, 1746, 1672, 1648, 1490, 1405, 1378, 1331, 1225, 1164, 1119, 1081, 1034, 994, 964, 886, 842, 764; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.28 (s, 1H), 8.42 (s, 1H), 8.25 (d, *J* = 8.8 Hz, 1H), 7.79 (d, *J* = 8.7 Hz, 1H), 7.45-7.42(m, 2H), 7.13 (d, *J* = 7.9

Hz, 1H), 6.80 (s, 1H), 6.11 (br s, 1H), 5.29 (d, *J* = 4.6 Hz, 1H), 5.16 (d, *J* = 4.6 Hz, 1H), 4.16 (q, *J* = 6.6 Hz, 2H), 1.92 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ191.0, 175.6, 168.8, 165.3, 164.3, 160.7, 157.4, 151.8, 139.3, 135.72, 135.71, 125.8, 124.3, 118.67, 118.64, 117.3, 112.3, 105.1, 88.0, 62.6, 26.7, 14.3, 12.3 ppm; HRMS (ESI, *m/z*) 456.1166 calcd. for $C_{23}H_{19}N_3O_6$ (M+Na) found 456.1162; Anal. calcd. for $C_{23}H_{19}N_3O_6$; C, 63.74; H, 4.42; N, 9.70; Found; C, 63.72; H, 4.41; N, 9.68.

(4R,5R)-Ethyl 4-(2-(furan-2-yl)-7-hydroxy-4-oxo-4*H*-chromen-8-yl)-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8n): Light yellow colour solid, m.p.: 138.9 °C, IR (KBr, ν_{max}, cm^{-1}): 3342, 3214, 3058, 2970, 1748, 1671, 1643, 1490, 1403, 1375, 1333, 1212, 1177, 1104, 1085, 1027, 994, 921, 854, 876, 744; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.26 (s, 1H), 8.25 (d, *J* = 8.8 Hz, 1H), 7.60 (d, *J* = 8.7 Hz, 1H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.16-7.12 (m,2H), 6.80 (s, 1H), 6.16 (br s, 1H), 5.29 (d, *J* = 4.6 Hz, 1H), 5.16 (d, *J* = 4.6 Hz, 1H), 4.16 (q, *J* = 6.6 Hz, 2H), 1.92 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ191.0, 175.6, 168.8, 164.3, 162.6, 160.7, 157.4, 147.1, 143.8, 139.3, 125.8, 118.67, 118.64, 117.3, 115.3, 110.6, 105.1, 88.0, 62.6, 26.7, 14.3, 12.3 ppm. HRMS (ESI, *m/z*) 445.1012 calcd. for $C_{22}H_{18}N_2O_7$ (M+Na) found 445.1010; Anal. calcd. for $C_{22}H_{18}N_2O_7$; C, 62.56; H, 4.30; N, 6.63; Found; C, 62.54; H, 4.28; N, 6.62.

(4R,5R)-Ethyl 4-(7-hydroxy-4-oxo-2-(thiophen-2-yl)-4*H*-chromen-8-yl)-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8o): Light yellow colour solid, m.p.: 140.8 °C, IR (KBr, ν_{max}, cm^{-1}): 3318, 3243, 3042, 2970, 1746, 1681, 1644, 1490, 1408, 1373, 1321, 1212, 1178, 1120, 1085, 1025, 994, 963, 887, 843, 764; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.20 (s, 1H), 8.26-8.20 (m, 2H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.16-7.12 (m, 2H), 6.80 (s, 1H), 6.12 (br s, 1H), 5.29 (d, *J* = 4.6 Hz, 1H), 5.16 (d, *J* = 4.6 Hz, 1H), 4.16 (q, *J* = 6.6 Hz, 2H), 1.92 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.2, 175.7, 168.6, 164.5, 162.6, 160.7, 157.1, 147.3, 143.8, 139.3, 125.6, 118.34, 118.32, 117.4, 115.3, 110.6, 105.1, 88.0, 62.6, 26.7, 14.3, 12.3 ppm. HRMS (ESI, *m/z*) 461.0778 calcd. for $C_{22}H_{18}N_2O_6S$ (M+Na) found 461.0773; Anal. calcd. for $C_{22}H_{18}N_2O_6S$; C, 60.27; H, 4.14; N, 6.39; S, 7.31; Found; C, 60.26; H, 4.14; N, 6.37; S, 7.30.

(4R,5R)-Ethyl 4-[2-(2,4-dimethoxyphenyl)-7-hydroxy-4-oxo-4*H*-chromen-8-yl]-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8p): Light yellow colour solid, m.p.: 137.2 °C, IR (KBr, ν_{max}, cm^{-1}): 3327, 3212, 3075, 2987, 1746, 1672, 1644, 1490, 1403, 1370, 1326, 1217, 1183, 1124, 1081, 1020, 995, 961, 881, 842, 769; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.19 (s, 1H), 8.27 (d, *J* = 8.8 Hz, 1H), 7.71 (d, *J* = 7.1 Hz, 1H), 7.61 (s, 1H), 7.43 (d, *J* = 6.9 Hz, 1H), 7.04 (d, *J* = 7.9 Hz, 1H), 6.81 (s, H), 6.18 (br s, 1H), 5.28 (d, *J* = 4.6 Hz, 1H), 5.17 (d, *J* = 4.6 Hz, 1H), 4.16 (q, *J* = 6.6 Hz, 2H), 3.79 (s, 3H), 3.78 (s, 3H), 1.91 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.3, 175.4, 168.3, 165.3, 164.3, 160.7, 160.0, 157.4, 139.0, 130.0, 125.8, 119.0, 118.1, 117.3, 112.3, 110.0, 105.1, 101.4, 88.0, 62.6, 61.84, 61.82, 26.7, 14.3, 12.3 ppm; $C_{26}H_{24}N_2O_8$, C, 63.41; H, 4.91; N, 5.69; O, 25.99, (M + Na) 515.1425.HRMS (ESI, *m/z*) 515.1425 calcd. for $C_{26}H_{24}N_2O_8$ (M+Na) found 515.1423; Anal. calcd. for $C_{26}H_{24}N_2O_8$; C, 63.41; H, 4.91; N, 5.69; Found; C, 63.40; H, 4.90; N, 5.67.

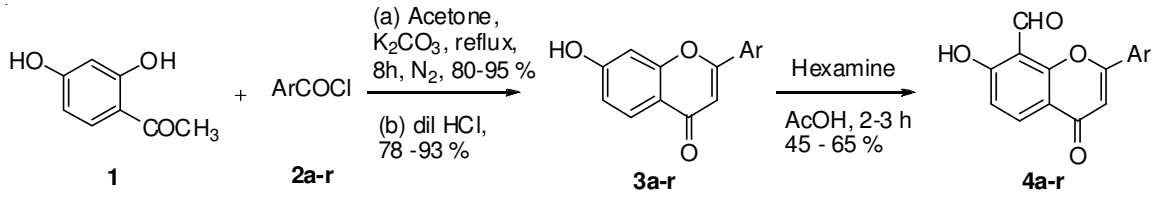
(4S, 5S)-Ethyl 4-(7-hydroxy-4-oxo-2-(2,4,6-trimethoxyphenyl)-4*H*-chromen-8-yl)-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8q): Light yellow colour solid, m.p.: 141.7 °C, IR (KBr, ν_{max}, cm^{-1}): 3210, 3072, 2989, 2676, 2621, 1746, 1672, 1644, 1491, 1402, 1379, 1333, 1217, 1177, 1115, 1086, 1023, 997, 963, 871, 845, 765; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.09 (s, 1H), 8.30 (d, *J* = 8.8 Hz, 1H), 7.72 (s, 2H), 7.42 (d, *J* = 8.8 Hz, 2H), 6.79 (s, 1H), 6.19 (br s, 1H), 5.32 (d, *J* = 4.7 Hz, 1H), 5.19 (d, *J* = 4.7 Hz, 1H), 4.19 (q, *J* = 6.6 Hz, 2H), 3.727 (s, 6H), 3.726 (s, 3H), 1.92 (s, 3H), 0.97 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.0, 175.6, 168.8, 165.3, 164.3, 160.7, 157.4, 155.8, 139.3, 125.8, 118.67, 118.64, 117.3, 112.3, 105.1, 88.0, 62.6, 61.89, 61.87, 26.74, 14.3 ppm; HRMS (ESI, *m/z*) 545.1531 calcd. for $C_{27}H_{26}N_2O_9$ (M+Na) found 545.1528; Anal. calcd. for $C_{27}H_{26}N_2O_9$; C, 62.06; H, 5.02; N, 5.36;. Found; C, 62.04; H, 5.01; N, 5.35.

(4S,5S)-Ethyl 4-(7-hydroxy-5-methoxy-4-oxo-2-(2,4,6-trimethoxyphenyl)-4*H*-chromen-8-yl)-3-methyl-4,5-dihydro-1*H*-furo[2,3-c]pyrazole-5-carboxylate (8r): Light yellow colour solid, m.p.: 148.5 °C, IR (KBr, ν_{max}, cm^{-1}): 3327, 3212, 3075, 2987, 1746, 1672, 1644, 1490, 1403, 1370, 1326, 1217, 1183, 1124, 1081, 1020, 995, 961, 881, 842, 769; ^{1}H NMR (400 MHz, $CDCl_3$) δ12.11 (br s, 1H), 7.04 (s, 1H), 6.79 (s, 1H), 6.02 (br s, 1H),5.32 (d, *J* = 4.7 Hz, 1H), 5.19 (d, *J* = 4.7 Hz, 1H), 4.19 (q, *J* = 6.6 Hz, 2H), 3.727 (s, 3H), 3.726 (s, 6H), 3.723 (S, 3H), 1.92 (s, 3H), 0.97 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ191.0, 175.6, 168.8, 165.3, 164.3, 160.7, 157.4, 155.8, 154.8, 139.3, 119.7, 118.67, 118.64, 117.3, 105.1, 97.1, 95.8, 88.0, 62.8, 61.89, 61.87, 60.7, 26.7, 14.3, 12.3 ppm; HRMS (ESI, *m/z*) 575.1636 calcd. for $C_{28}H_{28}N_2O_{10}$ (M+Na) found 575.1633; Anal. calcd. for $C_{28}H_{28}N_2O_{10}$; C, 60.87; H, 5.11; N, 5.07;. Found; C, 60.85; H, 5.10; N, 5.06.

RESULTS AND DISCUSSION

Synthesis commenced with reaction of resacetophenone (**1**) with benzoyl chloride in acetone, K_2CO_3 medium by modified Baker-Venkataraman reaction conditions provided acylated flavones. These products were treated with 5 % methanolic KOH followed by acidification with dil. HCl to give 7-hydroxy flavones (**3a-r**). 7-Hydroxy 8-formyl flavones (**4a-r**) was synthesized by the Duff reaction. A mixture of 7-hydroxy flavones (**3a-r**) and hexamethelenetetramine were refluxed in glacial acetic acid medium for 12 h. The resulting imminium complex was hydrolyzed by the addition of 0.1 N aq. HCl to furnish 8-formyl-7-hydroxy flavones (**4a-r**) (**Scheme-I**) [36].

In the next step of the reaction protocol, when equimolar amounts of 7-hydroxy 8-formylflavones (**4**), β-keto ester (**5**), hydrazine (**6**), ethyl ester pyridinium bromides (**7**) were reacted in the presence of 0.1 equivalents of Et_3N in a sealed vial under microwave-irradiation at 90 °C for 5 min afforded after work-up, dihydrofuro[1,2-*b*]pyrazole flavone derivative (**8a**) as a



Scheme-I: Formylation of 7-hydroxy, 8-formyl flavones

racemic *trans* stereo isomer in very good yield. A series of substituted 7-hydroxy 8-formyl flavone derivatives were reacted with ethylacetoacetate, hydrazine, pyridinium ylides to prepare a series of dihydro-1*H*-furo[2,3-*c*]pyrazole flavone hybrid (**8a-o**) with different substituents on their B-ring (**Scheme-II**).

The structure of the compounds was fully characterized by ^{1}H NMR and ^{13}C NMR, MS and IR spectra and elemental analysis. In the ^{1}H NMR spectra, two protons at 2,3-position of dihydrofuran ring display two doublets at 5.29 and 5.15 ppm with the vicinal coupling constant J = 4.6 Hz and 4.6 Hz, respectively. It has been documented that in *cis*-2,3-dihydrofuran the *vicinal* coupling constant of the two methine protons J = 7-10 Hz, while in *trans*-2,3-dihydrofuran vicinal coupling constant J = 4-7 Hz. Appearance of two singlet signals at about δ 8.2 and 6.8 ppm in the ^{1}H NMR spectra assignable to two olefinic CH groups confirmed formation of the product. As anticipated all the 22 signals are appeared in ^{13}C NMR. Moreover, the ^{13}C NMR spectra displayed ester carbonyl at about δ 191 ppm and α,β-unsaturated carbonyl carbon at 163 ppm. So we concluded that thermodynamically stable *trans* isomer of 2,3-dihydrofuropyrazole unit incorporated on flavone moity. Further, it was confirmed from the analysis of the NOESY spectrum of the compound. The mass spectrum shows, a sharp distinguishable peak of compound **8a** at 455.1212 $[M+Na]^{+}$.

Similarly, all the synthesized compounds **8b-r** was characterized. Newly prepared dihydro-1*H*-furo[2,3-*c*]pyrazole flavone derivatives (**8a-r**) was subjected to *in vitro* screening against three cancer cell lines namely Hep2 (human laryngeal carcinoma), A549 (human lung adenocarcinoma) and HeLa (human cervical cancer). Out of which, the compound **8f** emerged as the most promising lead compound open for further structure activity relationship (SAR) studies (Table-1). Two domains in compound **8f** namely, the aromatic ring (ring A & B, Fig. 1) of flavone were agreeable for alteration with different substituents while keeping rest of the molecule intact. Then, the substrate scope of the reaction was explored by using various 7-hydroxy, 8-formyl flavones in the model reaction. In all cases the substitution reaction provided the product dihydro-1*H*-furo[2,3-*c*]pyrazole flavone hybrid (**8a-r**) without any difficulty (**Scheme-III**). Based on the biological activity of compound **8f** and substitution pattern of natural flavonoids [37], we changed different substituents on ring A (Fig. 2) to alter steric and electronic effects. Electronic effects were also observed in the reaction process. The electron-donating group (EDG) at 2,5 positions of *ortho*-hydroxy benzaldehyde required less reaction time to give comparatively high yields of the product while stronger EWG-substituted ones gave evidently poor yields. The electronic properties of the substi-

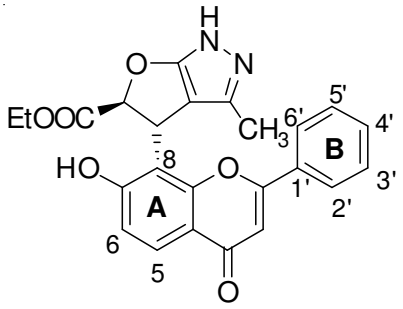
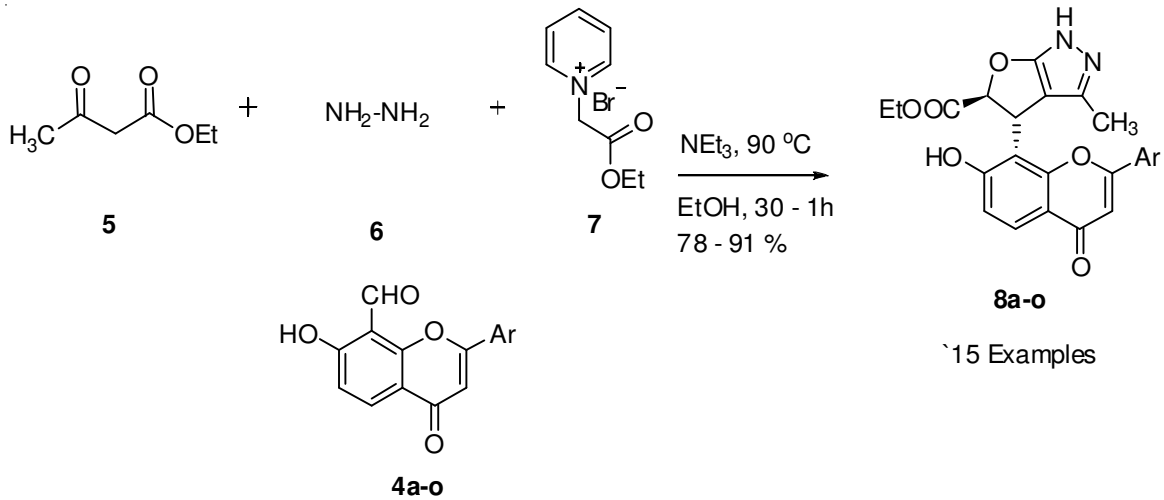


Fig. 1. Structure of 1*H*-furo[2,3-*c*]pyrazole flavone



Scheme-II: Synthesis of dihydro-1*H*-furo[2,3-*c*]pyrazole flavones

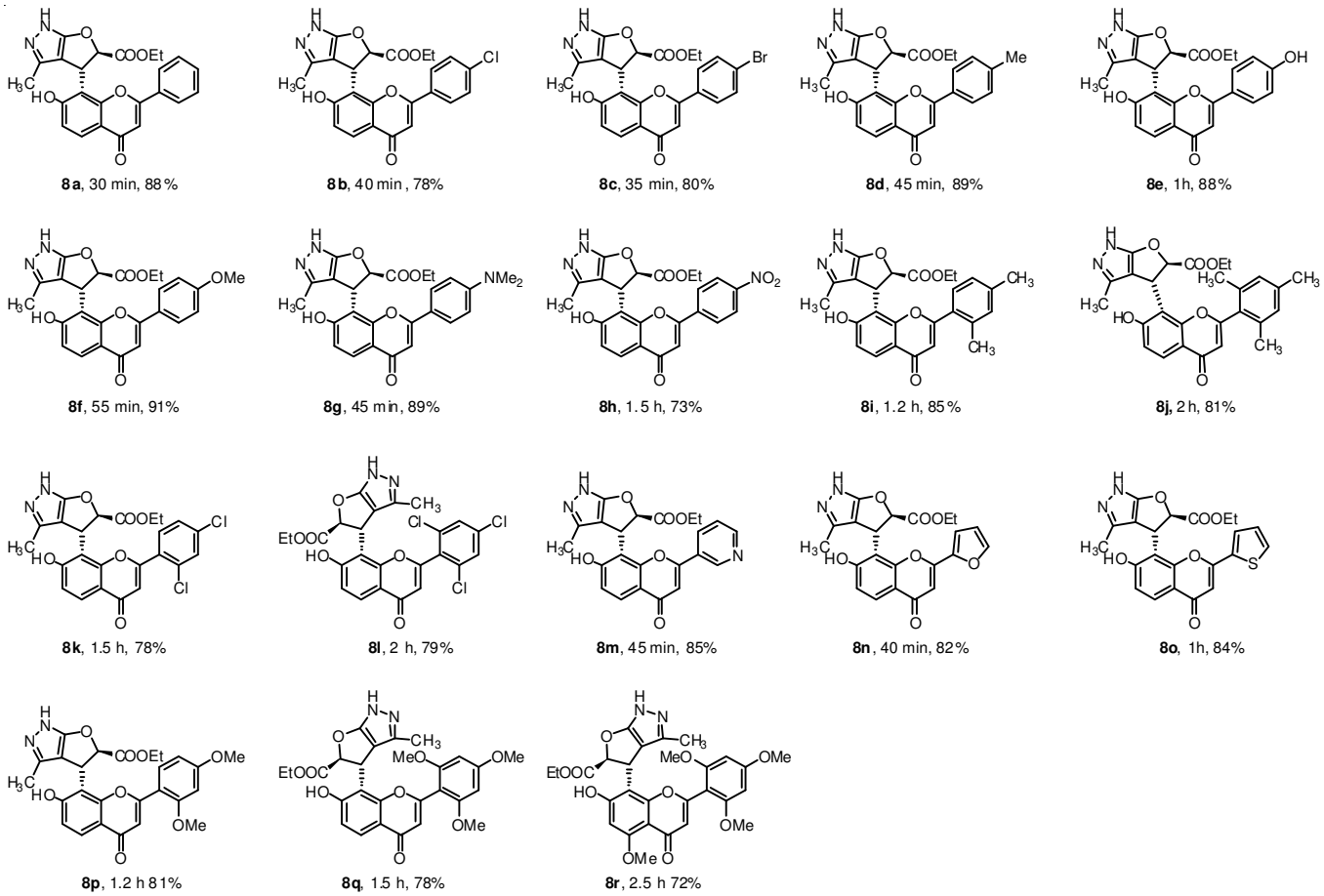


Fig. 2. Synthesis of SAR directed a combinatorial library of dihydro-1*H*-furo[2,3-*c*]pyrazole-flavones (**8a-r**)

TABLE-1
in vitro ANTIPROLIFERATIVE ACTIVITY OF DIHYDRO-1*H*-FURO[2,3-*c*]PYRAZOLE-FLAVONE DERIVATIVES (**8a-r**) AGAINST Hep2, A549, HeLa HUMAN CANCER CELLS BY MMT ASSAY EXPRESSED IN IC_{50} (μM)[a]

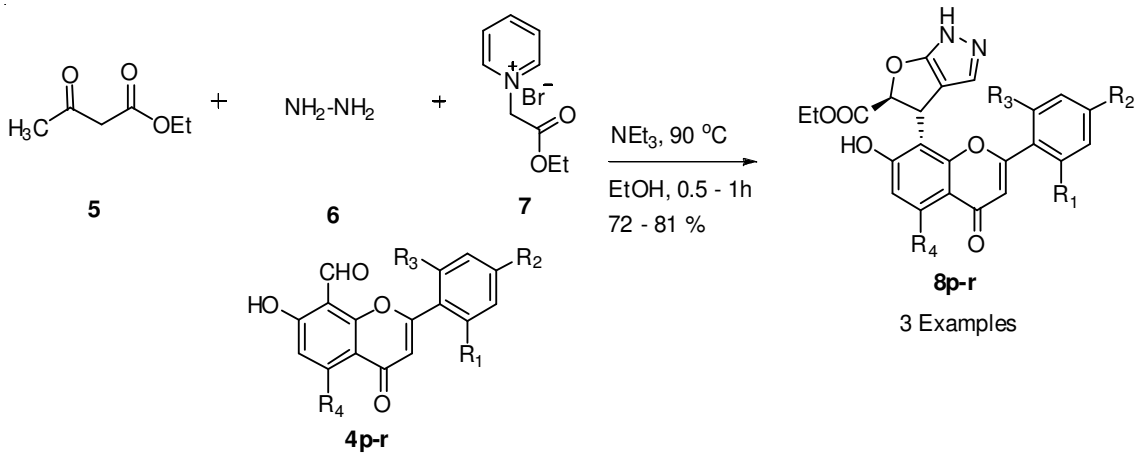
Entry	Compounds	Hep2[b]	A549[c]	HeLa[d]
1	**8a**	50 ± 0.91	>100	>100
2	**8b**	50 ± 1.85	>100	>100
3	**8c**	45 ± 1.32	30 ± 1.78	50 ± 1.12
4	**8d**	25 ± 2.5	20 ± 1.72	35 ± 4.78
5	**8e**	40 ± 0.80	>100	>100
6	**8f**	2.5 ± 0.20	24 ± 1.85	18 ± 1.31
7	**8g**	50 ± 3.63	24 ± 1.85	35 ± 2.8
8	**8h**	50 ± 4.78	10 ± 0.95	>100
9	**8i**	24 ± 1.65	35 ± 2.5	15 ± 0.96
10	**8j**	30 ± 1.65	35 ± 2.5	35 ± 0.96
11	**8k**	100 ± 9.83	25 ± 1.37	>100
12	**8l**	35 ± 2.8	60 ± 5.7	12 ± 1.0
13	**8m**	30 ± 1.45	35 ± 1.25	35 ± 1.90
14	**8n**	20 ± 0.52	20 ± 0.84	35 ± 0.96
15	**8o**	21 ± 1.45	23 ± 1.25	11 ± 1.90
16	**8p**	6 ± 0.24	8 ± 0.52	14 ± 0.82
17	**8q**	4 ± 0.85	7 ± 0.28	12 ± 0.78
18	**8r**	2.5 ± 0.20	6 ± 0.85	10 ± 1.31
19	Doxorubicin	10 ± 0.8	0.65 ± 0.04	1.54 ± 0.08
20	Paclitaxel	1.8 ± 0.12	0.175 ± 0.01	0.26 ± 0.01

[a]Results are the average of three independent experiments; [b]Hep2-Human laryngeal carcinoma; [c]A549-Human lung adenocarcinoma; [d]HeLa-Human cervical cancer.

tuents of aromatic aldehydes significantly affect the reactivity. The electron-donating group (NMe_2) at the *para* position of the aldehyde to give comparatively high yield of the product while stronger EWG-substituted one (NO_2) gave evidently poor yield. Spectral (IR, ^{1}H NMR, ^{13}C NMR and DEPT) and analytical (ESI-MS HRMS) data of the all the derivatives **8b-r** agreed well with the assigned structures. We gathered structures of all dihydro-1*H*-furo[2,3-*c*]pyrazole flavone hybrids (**8a-r**) along with the time taken for the substitution reaction and yield of the product in Fig. 2 to provide overall picture of the substitution pattern and to discern SAR results. List of synthetic compounds used for *in vitro* studies were given in Fig. 2.

Antiproliferative activity: In the first phase, antiproliferative activity of eighteen furo[2,3-*c*]pyrazole-flavone (**8a-r**, Fig. 2) was evaluated *in vitro* against a panel of three human cancer cell lines, namely human laryngeal carcinoma (Hep2), human lung adenocarcinoma (A549) and human cervical cancer (HeLa) cells and the results for inhibitory concentration (IC_{50}) values are gathered in Table-1. The studies reveal that methoxy group substituted derivative **8f** showed significant cytotoxic activity in comparison with other derivatives in all the three cancer cell lines (entry 5, Table-1).

For the next batch of three furo[2,3-*c*]pyrazole-flavones (**8p-r**) methoxy group substitutions were made in ring A & B. Antiproliferative evaluation of **8p-r** revealed that furo[2,3-*c*]pyrazole-flavone derivative **6r** (entry 18, Table-1) which has total four methoxy groups at aromatic ring A and B displays better activity compared to others. In summary *in vitro*



8p = R_1 = OMe; R_2 = OMe; R_3 = H, R_4 = H; **8q** = R_1 = OMe; R_2 = OMe; R_3 = OMe, R_4 = H;
8r = R_1 = OMe; R_2 = OMe; R_3 = OMe, R_4 = OMe;

Scheme-III: SAR oriented synthesis of furo[2,3-*c*]pyrazole-flavone hybrids (**8p-r**)

evaluation revealed that furo[2,3-*c*]pyrazole-flavone (**8r**) is the most potent molecule within the batch of **8a-r**. In order to determine the cytotoxic effects, all the eighteen compounds were subjected to *in vitro* cytotoxicity assay using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction test with the panel of three cancer cell lines for 48 h. All the compounds exhibited minimal cytotoxicity on 'human peripheral blood mononuclear cell (hPBMC), which indicates that furo[2,3-*c*]pyrazole-flavone (**8a-r**) are selectively toxic towards cancer cell lines.

MTT assay: Cell growth assays were carried out with the help of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) reduction test. The MTT colorimetric assay was performed as described previously [38]. Briefly, Hep2, A549 and HeLa cells (5×10^3/well) were plated in 0.2 mL (DMEM with 10 % FBS) in 96-well plates in the presence of indicated concentration of the compounds in three independent experiments. MTT was dissolved in phosphate buffered saline (PBS) at 5 mg/mL. After 48 h of incubation of Hep2, A549 and HeLa cells, MTT solution was added and the plate was incubated for 3 h and the cells were dissolved in 100 µL of DMSO. The conversion of MTT to formazan by metabolically viable cells was measured by the absorbance at 570 nm. The cell viability was expressed with the concentration that inhibits 50 % of growth (IC_{50}).

Conclusion

In summary, we have synthesized a combinatorial library of dihydro-1*H*-furo[2,3-*c*]pyrazole-flavone hybrids (**8a-r)** from one-pot four-component reaction of β-keto ester, hydrazine, 7-hydroxy 8-formyl flavones, pyridinium ylide in presence of NEt_3 as catalyst under ethanol reflux conditions. Evaluated their antiproliferative activity against human laryngeal carcinoma (Hep2), lung adenocarcinoma (A549) and cervical cancer (HeLa). Among these, compounds dihydro-1*H*-furo-[2,3-*c*]pyrazole-flavone derivative (**8r**) having four methoxy groups displayed the most potent antiproliferative activity against the three-cell lines uniformly. Toxicity studies revealed that dihydro-1*H*-furo[2,3-*c*]pyrazole-flavone hybrids (**8a-r**) are specifically target the cancer cell lines. Thus, compound **8r** is found to be the most potential anticancer molecule.

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