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Synthesis and antiproliferative activity of some diaryldiazepines and diarylpyrimidines

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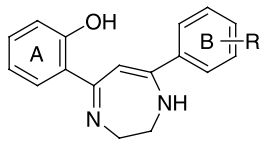
Abstract

Novel substituted 5,7-diaryl-2,3-dihydro-1,4-diazepines and 4,6-diaryl-2-aminopyrimidines were synthesized and tested for their antiproliferative activity. Title compounds were obtained by cyclocondensation of a substituted flavone with ethylenediamine and guanidine respectively. The cytotoxicity *in vitro* against various human leukemic cancer cell lines viz., Jurkat, HL60, MOLT3, NCEB-1, K562 was determined.

Keywords: *diaryldiazepines, diarylpyrimidines, antiproliferative activity*

Introduction

The present work is in conjunction with our ongoing programme [1–3] on the syntheses and biological studies of various diaryl substituted heterocyclic systems. There is an overwhelming evidence indicating that various heterocyclic cores attached to a diaryl system possess diverse pharmacological activities viz., COX II inhibition [4], allosteric modulation of $GABA_A$ receptor [5] and estrogen receptor [6], adenosine receptor antagonism [7], selective p38α inhibition [8], cannabinoid receptor antagonism [9], antimitotic activity [10] (combretastatin analogs), antiplatelet activity [11] and anti-HIV-1 activity [12] (TMC-125).



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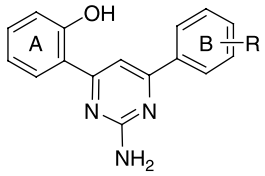

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Recent reports indicate the multifarious activities [13] of various benzodiazepines and diazepines condensed with different heterocyclic systems. In light of the reports available for diaryl heterocyclic systems, we envisaged the synthesis of diaryldiazepines and diaryl pyrimidines from a common intermediate. Here, we report some 5,7-diaryl-1,4-diazepine **(4)** and 4,6-diaryl-2-aminopyrimidine **(5)** compounds as antiproliferative agents.

Materials and methods

Chemistry

Melting points were determined on a Toshniwal melting point apparatus and are uncorrected. IR (cm^{-1}) spectra in KBr pellets were performed on a Shimadzu 8300 instrument and ^1H NMR spectra were recorded on a Brüker spectrometer (300 or 400 MHz) using d_6-DMSO or $CDCl_3$ as solvent and tetramethylsilane as an internal standard. Chemical shift data are reported in parts per million (δ in ppm)

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where s, bs, and m designate singlet, broad singlet, and multiplet respectively. Elemental analyses were recorded on a Perkin-Elmer PE 2400 CHNS analyzer. Mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer. Thin-layer chromatography (TLC) was performed on precoated Silica gel Merck plates. Compounds were visualized by illuminating with UV light (254 nm) or exposure to iodine vapors. Solvents were purified using standard methods.

Flavones **3** were prepared according to literature methods [14].

General method for the preparation of 5,7-diaryl-2,3-dihydro-1,4-diazepine derivatives. A mixture of the appropriate flavone **3a–k** in ethylenediamine (20 mL) was refluxed for 2 h on an oil bath. The cooled mixture was poured into ice-water and the precipitate was filtered. The crude product was recrystallized from methanol.

*5-(2-Hydroxyphenyl)-7-phenyl-2,3-dihydro-1*H*-1,4-diazepine (**4a**)*. Mp 208-210°C. IR (KBr), ν_{max} (cm^{-1}): 3231(N–H), 3000 (OH), 1605 (C=N). ^{1}H-NMR δ (400 MHz, DMSO-d_6): 3.65 and 3.95 (4H, bs, diazepine-C_2 & C_3-$(CH_2)_2$), 5.71(1H, s, diazepine-C_6-H), 6.45–7.75 (9H, m, ArH), 8.3 (1H, bs, NH). ES-MS (m/z): 264 (M^+, 100%). Anal. Calcd for $C_{17}H_{16}N_2O$ (264.32): C, 77.25; H, 6.10; N, 10.60. Found: C, 77.18; H, 6.24; N, 10.52%.

*5-(2-Hydroxyphenyl)-7-(4-methylphenyl)-2,3-dihydro-1*H*-1,4-diazepine (**4b**)*. Mp 235–237°C. IR (KBr), ν_{max}(cm^{-1}): 3176(N–H), 2916 (OH), 1595 (C=N). ^{1}H-NMR δ (400 MHz, DMSO-d_6): 2.39 (3H, s, CH_3), 3.72 and 3.88 (4H, bs, diazepine-C_2 & C_3-$(CH_2)_2$), 5.8 (1H, s, diazepine-C_6-H), 6.53–7.62 (8H, m, ArH), 8.25 (1H, bs, NH). ES-MS (m/z): 279 ($M^+ + 1$, 100%). Anal. Calcd for $C_{18}H_{18}N_2O$ (278.35): C, 77.67; H, 6.52; N, 10.06. Found: C, 77.74; H, 6.50; N, 9.88%.

*5-(2-Hydroxyphenyl)-7-(3-methylphenyl)-2,3-dihydro-1*H*-1,4-diazepine (**4c**)*. Mp 206–208°C. IR (KBr), ν_{max}(cm^{-1}): 3232 (N–H), 3000 (OH), 1608 (C=N). ^{1}H-NMR δ (400 MHz, DMSO-d_6): 2.45 (3H, s, CH_3), 3.65 and 3.9 (4H, bs, diazepine-C_2 & C_3-$(CH_2)_2$), 5.7 (1H, s, diazepine-C_6-H), 6.48–7.65 (8H, m, ArH), 8.27 (1H, bs, NH). ES-MS (m/z): 279 ($M^+ + 1$, 100%). Anal. Calcd for $C_{18}H_{18}N_2O$ (278.35): C, 77.67; H, 6.52; N, 10.06. Found: C, 77.70; H, 6.61; N, 9.99%.

*5-(2-Hydroxyphenyl)-7-(4-methoxyphenyl)-2,3-dihydro-1*H*-1,4-diazepine (**4d**)*. Mp 228–230°C. IR (KBr), ν_{max}(cm^{-1}): 3221(N–H), 2922 (OH), 1604 (C=N). ^{1}H-NMR δ (400 MHz, DMSO-d_6): 3.63 (2H, s, diazepine-C_3-H), 3.88 (5H, bs, OCH_3 & diazepine-C_2-H), 5.7 (1H, s, diazepine-C_6-H), 6.45–7.65 (8H, m, ArH), 8.25 (1H, bs, NH). ES-MS (m/z): 295 ($M^+ + 1$, 100%). Anal. Calcd for $C_{18}H_{18}N_2O_2$ (294.35): C, 73.45; H, 6.16; N, 9.52. Found: C, 73.49; H, 6.13; N, 9.64%.

*5-(2-Hydroxyphenyl)-7-(3-methoxyphenyl)-2,3-dihydro-1*H*-1,4-diazepine (**4e**)*. Mp 185–188°C. IR (KBr), ν_{max}(cm^{-1}): 3217 (N–H), 2985 (OH), 1600 (C=N) 1230 & 1048 (OCH_3). ^{1}H-NMR δ (400 MHz, DMSO-d_6): 3.63 (2H, s, diazepine-C_3-H), 3.9 (5H, bs, OCH_3 & diazepine-C_2-H), 5.7 (1H, s, diazepine-C_6-H), 6.45–7.66 (8H, m, ArH), 8.3 (1H, bs, NH). ES-MS (m/z): 295 ($M^+ + 1$, 100%). Anal. Calcd for $C_{18}H_{18}N_2O_2$ (294.35): C, 73.45; H, 6.16; N, 9.52. Found: C, 73.56; H, 6.19; N, 9.49%.

*5-(2-Hydroxyphenyl)-7-(4-chlorophenyl)-2,3-dihydro-1*H*-1,4-diazepine (**4f**)*. Mp 243–245°C. IR (KBr), ν_{max}(cm^{-1}): 3231(N–H), 3000 (OH), 1598 (C=N). ^{1}H-NMR δ (400 MHz, DMSO-d_6): 3.75 and 3.85 (4H, bs, diazepine-C_2 & C_3-$(CH_2)_2$), 5.74 (1H, s, diazepine-C_6-H), 6.61–7.74 (8H, m, ArH), 8.3 (1H, bs, NH). ES-MS (m/z): 298 (M^+, 100%). Anal. Calcd for $C_{17}H_{15}ClN_2O$ (298.77): C, 68.34; H, 5.06; N, 9.38. Found: C, 68.48; H, 5.04; N, 9.35%.

*5-(2-Hydroxyphenyl)-7-(3-chlorophenyl)-2,3-dihydro-1*H*-1,4-diazepine (**4g**)*. Mp 208–210°C. IR (KBr), ν_{max}(cm^{-1}): 3200(N–H), 2916 (OH), 1610 (C=N). ^{1}H-NMR δ (400 MHz, DMSO-d_6): 3.62 and 3.92 (4H, bs, diazepine-C_2 & C_3-$(CH_2)_2$), 5.70 (1H, s, diazepine-C_6-H), 6.5-7.7 (8H, m, ArH), 8.28 (1H, bs, NH). ES-MS (m/z): 298 (M^+, 100%). Anal. Calcd for $C_{17}H_{15}ClN_2O$ (298.77): C, 68.34; H, 5.06; N, 9.38. Found: C, 68.41; H, 4.97; N, 9.50%.

*5-(2-Hydroxyphenyl)-7-(2-chlorophenyl)-2,3-dihydro-1*H*-1,4-diazepine (**4h**)*. Mp 210–212°C. IR (KBr), ν_{max}(cm^{-1}): 3232(N–H), 2916 (OH), 1598 (C=N). ^{1}H-NMR δ (400 MHz, DMSO-d_6): 3.63 and 4.0 (4H, bs, diazepine-C_2 & C_3-$(CH_2)_2$), 5.37 (1H, s, diazepine-C_6-H), 6.49–7.64 (8H, m, ArH), 8.4 (1H, bs, NH). ES-MS (m/z): 298 (M^+, 100%). Anal. Calcd for $C_{17}H_{15}ClN_2O$ (298.77): C, 68.34; H, 5.06; N, 9.38. Found: C, 68.30; H, 5.08; N, 9.45%.

*5-(2-Hydroxyphenyl)-7-(4-fluorophenyl)-2,3-dihydro-1*H*-1,4-diazepine (**4i**)*. Mp 234–236°C. IR (KBr), ν_{max}(cm^{-1}): 3203(N–H), 2916 (OH), 1604 (C=N). ^{1}H-NMR δ (400 MHz, DMSO-d_6): 3.65 and 3.9 (4H, bs, diazepine-C_2 & C_3-$(CH_2)_2$), 5.68 (1H, s, diazepine-C_6-H), 6.5-7.8 (8H, m, ArH), 8.37 (1H, bs, NH). ES-MS (m/z): 282 (M^+, 100%). Anal. Calcd for $C_{17}H_{15}FN_2O$ (282.31): C, 72.32; H, 5.36; N, 9.92. Found: C, 72.31; H, 5.29; N, 10.01%.

*5-(2-Hydroxyphenyl)-7-(2-furyl)-2,3-dihydro-1*H*-1,4-diazepine (**4j**)*. Mp 168–170°C. IR (KBr), ν_{max} (cm^{-1}): 3206(N–H), 3000 (OH), 1608 (C=N). ^{1}H-NMR δ (400 MHz, DMSO-d_6): 3.65 and 3.9 (4H, bs, diazepine-C_2 & C_3-$(CH_2)_2$), 6.1 (1H, s, diazepine-C_6-H), 6.53–7.96 (7H, m, ArH), 8.28

(1H, bs, NH). ES-MS (m/z): 254 (M^+, 100%). Anal. Calcd for $C_{15}H_{14}N_2O_2$ (254.28): C, 70.85; H, 5.55; N, 11.02. Found: C, 70.96; H, 5.48; N, 10.98%.

*5-(2-Hydroxyphenyl)-7-(2-thienyl)-2,3-dihydro-1H-1,4-diazepine (**4k**)*. Mp 232–235°C. IR (KBr), ν_{max} (cm^{-1}): 3203(N–H), 2980 (OH), 1595 (C=N). ^{1}H-NMR δ (400 MHz, DMSO-d_6): 3.6 and 3.9 (4H, bs, diazepine-C_2 & C_3-$(CH_2)_2$), 5.92 (1H, s, diazepine-C_6-H), 6.58-7.8 (7H, m, ArH), 8.28 (1H, bs, NH). ES-MS (m/z): 270 (M^+, 100%). Anal. Calcd for $C_{15}H_{14}N_2OS$ (270.35): C, 66.64; H, 5.22; N, 10.36. Found: C, 66.71; H, 5.20; N, 10.47%.

General method for the preparation of 4,6-diaryl-2-amino pyrimidine derivatives. A mixture of the flavone **3a–k** (0.0019 mol), guanidine hydrochloride (0.01 mol) and potassium hydroxide (1.0 g) was refluxed in methanol (30 mL) for 4–6 h. After the completion of the reaction, the mixture was poured on to crushed ice containing acetic acid. The yellow solid obtained was filtered, washed with water and recrystallized from methanol to give **5a–k**.

*4-(2-Hydroxyphenyl)-6-phenyl-2-aminopyrimidine (**5a**)*. Mp 174–76°C. IR (KBr), ν_{max}(cm^{-1}): 3508, 3354 (NH_2), 3200 (OH), 1625 (C=N). ^{1}H-NMR δ (400 MHz, $CDCl_3$): 5.37 (2H, s, NH_2), 7.54 (1H, s, pyrimidine-C_5-H), 6.9–8.0 (9H, m, ArH), 14.1 (1H, br, OH). ES-MS (m/z): 264 ($M^+ + 1$, 100%). Anal. Calcd for $C_{16}H_{13}N_3O$ (263.29): C, 72.99; H, 4.98; N, 15.96. Found: C, 72.85; H, 5.02; N, 16.0%.

*4-(2-Hydroxyphenyl)-6-(4-methylphenyl)-2-amino-pyrimidine (**5b**)*. Mp 191–92°C. IR (KBr), ν_{max} (cm^{-1}): 3500, 3330 (NH_2), 3197 (OH), 1631(C=N). ^{1}H-NMR δ (400 MHz, $CDCl_3$): 2.43 (3H, s, CH_3), 5.7 (2H, s, NH_2), 7.54 (1H, s, pyrimidine-C_5-H), 6.93–8.0 (8H, m, ArH), 13.5 (1H, br, OH). ES-MS (m/z): 278 ($M^+ + 1$, 100%). Anal. Calcd for $C_{17}H_{15}N_3O$ (277.32): C, 73.63; H, 5.45; N, 15.45. Found: C, 73.46; H, 5.54; N, 15.52%.

*4-(2-Hydroxyphenyl)-6-(3-methylphenyl)-2-amino-pyrimidine (**5c**)*. Mp 124–26°C. IR (KBr), ν_{max} (cm^{-1}): 3490, 3394 (NH_2), 3200 (OH), 1639 (C=N). ^{1}H-NMR δ (400 MHz, $CDCl_3$): 2.46 (3H, s, CH_3), 5.35 (2H, s, NH_2), 7.54 (1H, s, pyrimidine-C_5-H), 6.92–7.88 (8H, m, ArH), 14.2 (1H, br, OH). ES-MS (m/z): 278 ($M^+ + 1$, 100%). Anal. Calcd for $C_{17}H_{15}N_3O$ (277.32): C, 73.63; H, 5.45; N, 15.45. Found: C, 73.52; H, 5.38; N, 15.40%.

*4-(2-Hydroxyphenyl)-6-(4??-methoxyphenyl)-2-aminopyrimidine (**5d**)*. Mp 161–63°C. IR (KBr), ν_{max} (cm^{-1}): 3492, 3327 (NH_2), 3201 (OH), 1639 (C=N), 1249, 1025 (OCH_3). ^{1}H-NMR δ (400 MHz, $CDCl_3$): 3.88 (3H, s, OCH_3), 5.4 (2H, s, NH_2), 7.50 (1H, s, pyrimidine-C_5-H), 6.7-8.2 (8H, m, ArH), 14.3 (1H, br, OH). ES-MS (m/z): 294 ($M^+ + 1$, 100%). Anal. Calcd for $C_{17}H_{15}N_3O_2$ (293.32): C, 69.61; H, 5.15; N, 14.33. Found: C, 69.74; H, 5.08; N, 14.38%.

*4-(2-Hydroxyphenyl)-6-(3-methoxyphenyl)-2-amino-pyrimidine (**5e**)*. Mp 203–06°C. IR (KBr), ν_{max} (cm^{-1}): 3400, 3313 (NH_2), 3176 (OH), 1647 (C=N), 1236, 1031 (OCH_3). ^{1}H-NMR δ (400 MHz, $CDCl_3$): 3.92 (3H, s, OCH_3), 6.0 (2H, s, NH_2), 7.52 (1H, s, pyrimidine-C_5-H), 6.9–7.8 (8H, m, ArH), 14.2 (1H, br, OH). ES-MS (m/z): 294 ($M^+ + 1$, 100%). Anal. Calcd for $C_{17}H_{15}N_3O_2$ (293.32): C, 69.61; H, 5.15; N, 14.33. Found: C, 69.68; H, 5.14; N, 14.30%.

*4-(2-Hydroxyphenyl)-6-(4-chlorophenyl)-2-amino-pyrimidine (**5f**)*. Mp 239–41°C. IR (KBr), ν_{max} (cm^{-1}): 3502, 3343 (NH_2), 3217 (OH), 1641 (C=N). ^{1}H-NMR δ (400 MHz, $CDCl_3$): 6.2 (2H, s, NH_2), 7.35 (1H, s, pyrimidine-C_5-H), 6.9–8.0 (8H, m, ArH), 13.5 (1H, br, OH). ES-MS (m/z): 298 ($M^+ + 1$, 100%). Anal. Calcd for $C_{16}H_{12}ClN_3O$ (297.74): C, 64.54; H, 4.06; N, 14.11. Found: C, 64.72; H, 4.17; N, 13.98%.

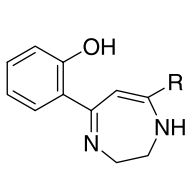
*4-(2-Hydroxyphenyl)-6-(3-chlorophenyl)-2-amino-pyrimidine (**5g**)*. Mp 183–85°C. IR (KBr), ν_{max} (cm^{-1}): 3502, 3346 (NH_2), 3203 (OH), 1616 (C=N). ^{1}H-NMR δ (400 MHz, $CDCl_3$): 5.24 (2H, s, NH_2), 7.52 (1H, s, pyrimidine-C_5-H), 6.9–8.0 (8H, m, ArH), 13.5 (1H, br, OH). ES-MS (m/z): 298 ($M^+ + 1$, 100%). Anal. Calcd for $C_{16}H_{12}ClN_3O$ (297.74): C, 64.54; H, 4.06; N, 14.11. Found: C, 64.65; H, 4.02; N, 14.14%.

*4-(2-Hydroxyphenyl)-6-(2-chlorophenyl)-2-amino-pyrimidine (**5h**)*. Mp 190–192°C. IR (KBr), ν_{max} (cm^{-1}): 3423, 3315 (NH_2), 3187 (OH), 1629 (C=N). ^{1}H-NMR δ (400 MHz, $CDCl_3$): 5.68 (2H, s, NH_2), 7.43 (1H, s, pyrimidine-C_5-H), 6.76–7.8 (8H, m, ArH), 14.3 (1H, br, OH). ES-MS (m/z): 298 ($M^+ + 1$, 100%). Anal. Calcd for $C_{16}H_{12}ClN_3O$ (297.74): C, 64.54; H, 4.06; N, 14.11. Found: C, 64.73; H, 3.86; N, 14.00%.

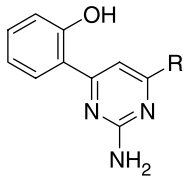
*4-(2-Hydroxyphenyl)-6-(4-fluorophenyl)-2-amino-pyrimidine (**5i**)*. Mp 221–23°C. IR (KBr), ν_{max} (cm^{-1}): 3490, 3321 (NH_2), 3201 (OH), 1647 (C=N). ^{1}H-NMR δ (400 MHz, $CDCl_3$): 5.5 (2H, s, NH_2), 7.51 (1H, s, pyrimidine-C_5-H), 6.9–8.1 (8H, m, ArH), 14.1 (1H, br, OH). ES-MS (m/z): 282 ($M^+ + 1$, 100%). Anal. Calcd for $C_{16}H_{12}FN_3O$ (281.28): C, 68.32; H, 4.30; N, 14.94. Found: C, 68.46; H, 4.52; N, 14.72%.

*4-(2-Hydroxyphenyl)-6-(2-furyl)-2-aminopyrimidine (**5j**)*. Mp 211–13°C. IR (KBr), ν_{max}(cm^{-1}): 3417, 3285 (NH_2), 3163 (OH), 1635 (C=N). ^{1}H-NMR δ (400 MHz, $CDCl_3$): 5.3 (2H, s, NH_2), 7.52 (1H, s, pyrimidine-C_5-H), 6.59–7.87 (7H, m, ArH), 14.3

Table I. Antiproliferative activity IC_{50} (μM) values of 5,7-diaryldiazepines **4a − k** and 4,6-diarylpyrimidines **5a − k**.



4a-k



5a-k

Compd	R	Jurkat	HL60	Molt3	NCEB1	K562
4a	C_6H_5	10.59	60	11.4	>100	9.12
4b	4-$CH_3C_6H_4$	50.79	>100	40.81	86	24.3
4c	3-$CH_3C_6H_4$	8.47	22	11.6	100	4.55
4d	4-$CH_3OC_6H_4$	>100	>100	61	>100	20.85
4e	3-$CH_3OC_6H_4$	5.39	23	14.33	26	8.28
4f	4-ClC_6H_4	81.58	>100	64	>100	65
4g	3-ClC_6H_4	>100	>100	>100	>100	>100
4h	2-ClC_6H_4	7.66	26	12.09	23	10.12
4i	4-FC_6H_4	22.75	80	38.48	70	29.46
4j	2-furyl	>100	>100	>100	>100	88.78
4k	2-thienyl	N.T	N.T	N.T	N.T	N.T
***5c**	3-$CH_3C_6H_4$	22.87	16	29.56	23	11.95

N.T-not tested.
*The remaining diarylpyrimidines did not show any inhibition at 10 μM level.

(1H, br, OH). ES-MS (m/z): 254 ($M^+ + 1$, 100%). Anal. Calcd for $C_{14}H_{11}N_3O_2$ (253.26): C, 66.40; H, 4.38; N, 16.59. Found: C, 66.24; H, 4.56; N, 16.66%.

*4-(2-Hydroxyphenyl)-6-(2-thienyl)-2-aminopyrimidine (**5k**)*. Mp 182–84°C. IR (KBr), $\nu_{max}(cm^{-1})$: 3502, 3438 (NH_2), 3342 (OH), 1635 (C=N). ^{1}H-NMR δ (400 MHz, $CDCl_3$): 5.27 (2H, s, NH_2), 7.44 (1H, s, pyrimidine-C_5-H), 6.9–7.8 (7H, m, ArH), 13.5 (1H, br, OH). ES-MS (m/z): 270 ($M^+ + 1$, 100%). Anal. Calcd for $C_{14}H_{11}N_3OS$ (269.32): C, 62.43; H, 4.12; N, 15.60. Found: C, 62.48; H, 4.01; N, 15.54%.

In vitro *cytotoxicity assay*

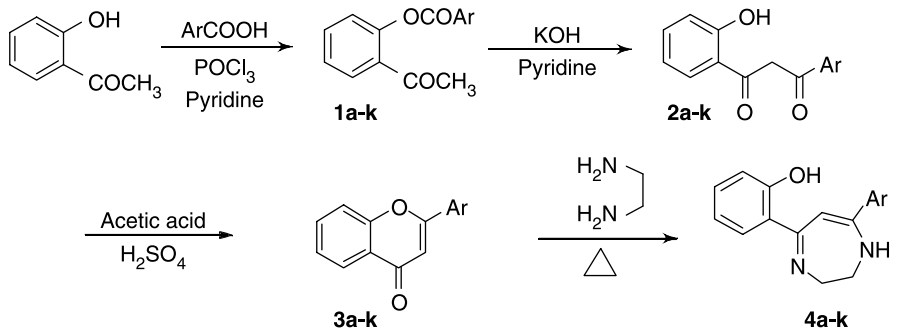
The results of the cytotoxic potencies of the synthesized compounds tested *in vitro* against five leukemic cell lines NCEB-1, HL60-DS, Jurkat E6-1, K562 and Molt-3 are summarized in Table I. The synthesized compounds were dissolved in DMSO (5 μL of each compound at a 10 μM final concentration) and dispensed into an assay plate using acustom built low volume 384-well head tool. The assay plates were then loaded with 45 μL of cells and allowed to incubate with the compounds for 48 h at 37°C. Then, 5 μL of alamar blue reagent was added to the assay plate and incubated for 24 h at 37°C. Alamar blue reduction [15] was measured on a CCD-based optical imaging reader.

For initial screening, 1% DMSO was utilized as the high control to represent maximum reduction of alamar blue from cellular metabolism. The cytotoxic agent staurosporine was utilized at 50 μM to represent minimal reduction of alamar blue as a result of total cellular killing. These controls are used to calculate Z′ as a test of the functionality of the assay and to determine its range, robustness and reliability. For these cell lines, the controls gave a Z′ factor of 0.598 or better and a good signal to noise ratio indicating a broad dynamic range making the study reliable in our initial screening.

Results and discussion

Chemistry

The diaryldiazepine heterocyclic derivatives presented in this paper were prepared according to the route described in Scheme 1. The 1,3-diketones required for the work were obtained from the esters, by the base-catalyzed Baker-Venkataraman transformation [16]. Condensation of 2-hydroxyacetophenones with various substituted benzoic acids in dry pyridine and $POCl_3$ furnished the esters (**1a–k**). In the IR spectra, the 1,3-diketones showed absorption bands for C=O in the range 1615–1625 cm^{-1}. Furthermore, characteristic absorption bands of C=O of ketone and ester in the region 1681 and 1740 cm^{-1} seen in (**1a–k**), are absent in the IR spectrum of the 1,3-diketones (**2a–k**). The traditional approach [17] to the synthesis of 2,3-dihydro-1,4-diazepines is based on the reaction of 1,3-diketones with ethylenediamine in acidic medium. However, a competitive reaction also occurs

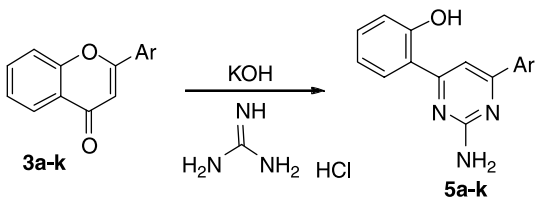


Scheme 1. Synthetic route to the diazepines (**4a–k**).

giving flavones as bi products. To circumvent this problem we transformed the 1,3-diketones to the flavones (**3a–k**) which were then reacted with ethylenediamine to afford the required diazepines. This approach is by analogy with the report [18] where substituted flavones undergo cyclocondensation reaction with amines in basic medium to form diazepines. The formation of the flavones [14] **3a–k** was confirmed by the appearance of C=O absorption bands at 1640–1660 cm^{-1}.

Substituted diazepines **4a–k** resulted when the corresponding flavones were condensed with ethylenediamine which gave intensely yellow or orange colored diaryldiazepines in fairly good yield. The structures of the 5,7-diaryldiazepines are consistent with the IR and NMR spectra. The ring expansion was confirmed by the appearance of C=N stretching at 1595–1610 cm^{-1} and NH stretching at 3200 cm^{-1} in the IR spectrum. The $-CH_2-CH_2-$ protons of the diazepine ring appeared as two broad singlets at δ 3.7 and 3.8, the N–H protons appeared as a broad singlet (δ 7.0–9.0) and the H-6 proton showed a sharp singlet at δ 5.8–6.0. The aromatic protons appeared as a multiplet. The hydroxyl proton could not be traced in the NMR spectra over the range 1–15 ppm. The mass spectra revealed the molecular ion as the base peak for most of the compounds.

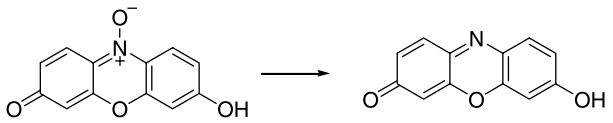
Treatment of the flavones **3a–k** with a slight excess of guanidine hydrochloride in alkaline medium afforded 4,6-diaryl-2-aminopyrimidines **5a–k** (Scheme 2). Compounds **5a–k** showed a characteristic peak at 3200 cm^{-1} for O–H str, asymmetric and symmetric stretching N–H bands at 3500 and 3350 cm^{-1} respectively in the IR spectrum. In the ^1H NMR spectra, hydroxyl protons were seen as a broad peak at δ 14–15, NH_2 protons δ 5.3–5.5 and the pyrimidinyl proton (C_5–H) appeared as a sharp singlet at δ 7.3–7.5. The aromatic protons appeared as a multiplet at δ 6.9–8.0.



Scheme 2. Synthetic route to the pyrimidines (**5a–k**).

Cytotoxicity

Initial evaluation of **4a–k** and **5a–k** were carried out at the Memorial Sloan Kettering Cancer Center (MSKCC), New York (USA). The cytotoxic effects of 5,7-diaryl-1,4-diazepine and 4,6-diarylpyrimidine derivatives were tested using Alamar blue assay. Alamar blue (Resazurin) is commonly employed as an indicator of cell number and viability, since it is reduced to a (Resorufin) pink fluorescent dye in the medium by cell activity (possibly by oxygen consumption through metabolism). Alamar Blue is nontoxic to cells and does not necessitate killing of cells to obtain measurements, as is the case with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT).



The various human leukemic cancer cell lines Jurkat, HL60, Molt3, NCEB-1 and K562 were incubated with various concentrations of the 5,7-diaryldiazepines and 4,6-diarylpyrimidines for 72 hours (48 h plus 24 h with dye). The resulting IC_{50} values for the compounds are summarized in Table I. Compound **4a** showed significant cytotoxic activity with IC_{50} values of 9.12 and 10.59 μM against K562 and Jurkat cell lines, respectively. Introduction of a methyl group at the meta position of the diaryldiazepine nucleus as in compound **4c,** demonstrated high cytotoxic activity against the K562 and Jurkat cell lines (70 & 77% cell death,

respectively, after 72 hours (48 h plus 24 h with dye) incubation time at 10 μM) with IC_{50} values of 4.55 and 8.47 μM respectively. Compound **4e,** in which the methyl group was replaced with a greater electron donating group, a methoxy group, was also found to be cytotoxic but significantly less so than compound **4c**. Results indicate that among the halo-substituted diaryldiazepines, compound **4 h**, bearing a chloro substituent at the ortho position showed significant activity with an IC_{50} value of 7.66 μM against Jurkat cell line.

The other diaryl series, compounds **5a–k,** in which the diazepine nucleus was replaced with a pyrimidine heterocyclic ring were all devoid of cell killing activity with the exception of compound **5c**. Compound **5c** showed considerable cytotoxic activity with an IC_{50} value of 11.95 μM against K562 cell line. The results indicate that some of the diazepines show moderate antiproliferative activity.

In conclusion, **4c** and **4e** are the most potent towards the K562 cell line, **4 h** is potent towards the Jurkat cell line and **5c** is quite good against all five types of cell lines.

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