EMSA assay for NF-κB-DNA binding: Human Jurkat leukemia T-cells (clone E6-1; Amer. Type Culture Collection, Rockville, MD) were grown in RPMI-1640 Media (Gibco-BRL, Rockville, MD) supplemented with 10% fetal bovine serum, penicillin (614 ng/mL), streptomycin (10 μg/mL) and HEPES buffer, pH 7.2 at 37°C, 5% CO_2. The Jurkat cells (1 X 10^6 cells/mL) were subsequently treated with various concentrations of the compounds for 30 min. at 37°C and 5% CO_2 followed by PMA (50 ng/mL) and PHA (1 μM/mL) stimulation for an additional 30 minutes. The cells were harvested by centrifugation, washed in ice cold PBS and the nuclear extracts were prepared as previously described. The protein concentration of the extracts was determined according to the Method of Bradford (1976) with BioRad reagents. Nuclear extracts are incubated for 20 minutes at room temperature with a double stranded Cy3 labeled NF-κB consensus oligonucleotide, 5'-AGTTGAGGGGACTTTC CCAGGC-3'. The binding mixture (25 μL) contained 10 mM HEPES-NaOH pH 7.9, 4 mM tris-HCl, pH 7.9, 6.0 mM KCl, 1 mM EDTA, 1 mM DTT, 10% glycerol, 0.3 mg/mL bovine serum albumin and 1 μg of poly (dI.dC). The binding mixtures (10 μg of nuclear extract protein) were incubated for 20 minutes at room temrperature with 0.16 pmol of Cy3 labeled oligonucleotide. The mixture was loaded on a 4% polyacrylamide gel prepared in 1X tris borate/EDTA buffer and was electrophoresed at 200 V for 20 minutes. After electrophoresis the gel was analyzed using a phosphorimager (Biorad FX plus) for detection of the NF-κB-DNA binding.

Competitive enzyme immuno assay (EIA) for IL-2 expression: Human Jurkat leukemia T-cells (clone E6-1; Amer. Type Culture Collection, Rockville, MD) were grown in RPMI-1640 Media (Gibco-BRL, Rockville, MD) supplemented with 10% fetal bovine serum, penicillin (614 ng/mL), streptomycin (10 μg/mL) and HEPES buffer, pH 7.2 at 37°C, 5% CO_2. To each well of a flat bottomed 96 well culture plate 0.2 mL $1X10^6$ Jurkat E6-1 cells/mL were added. Each sample was then treated in duplicate with the compounds at either 10 μM, 1 μM, 0.1 μM or 10 nM and allowed to incubate for thirty minutes at 37°C, 5% CO_2. Cell free supernatants were collected from stimulated cultures incubated for 24 hr at 37° C, 5% CO_2. Cultures were stimulated with phytohemagglutinin (PHA, Sigma-Aldrich, St. Louis, MO) at 1 μg/mL; and phorbol myristate acetate (PMA, Sigma-Aldrich, St. Louis, MO) at 50 ng/mL. The concentration of IL-2 in each sample was then measured using a competitive enzyme immunoassay (Neogen Corporation, Lansing, MI) according to the manufacturer's protocol. Known IL-2 concentrations were plotted and fit a 4 parameter logistic curve. Unknown concentrations were then extrapolated from the standard curve.

Competitive enzyme immuno assay (EIA) for TNF-α expression: THP-1 cells were grown in RPMI-1640 Media (Gibco-BRL, Rockville, MD) supplemented with 5% fetal bovine serum, penicillin (614 ng/mL), streptomycin (10 μg/mL) and HEPES buffer, pH 7.2 at 37°C, 5% CO_2. To each well of a flat bottomed 96 well culture plate 0.2 mL $1X10^6$ THP-1 cells/mL were added. Each sample was then treated in duplicate with the compounds at either 10 μM, 1 μM, 0.1 μM or 10 nM and allowed to incubate for thirty minutes at 37°C, 5% CO_2. Cell free supernatants were collected from stimulated cultures incubated for 3 hr at 37° C, 5% CO_2. Cultures were stimulated with LPS (Sigma-Aldrich, St. Louis, MO) at

1 μg/mL. The concentration of TNF-α in each sample was then measured using a competitive enzyme immunoassay (Neogen Corporation, Lansing, MI) according to the manufacturer's protocol. Known TNF-α concentrations were plotted and fit a 4 parameter logistic curve. Unknown concentrations were then extrapolated from the standard curve.

Inhibition of cell growth: CEM cells (CCRF-CEM; Amer. Type Culture Collection, Rockville, MD) were grown in RPMI-1640 Media (Gibco-BRL, Rockville, MD) supplemented with 10% Fetal Bovine Serum, penicillin (614 ng/mL), streptomycin (10 μg/mL) and HEPES buffer, pH 7.2 at 37°C, 5% CO_2. DMSO was used as the vector for all drugs and added in the control experiments. Cell cultures were then treated with 10 μM, 1 μM, 0.1 μM or 10 nM of the drug in duplicate and allowed to incubate at 37°C, 5% CO_2. Cells were stained with 4% trypan blue solution in PBS, and counted under the microscope (Thomas Scientific, Fisher Scientific) in duplicate. This was repeated for 0h, 2h, 6h, 12h, 24h and 48h. The data points obtained were then plotted and a point -to-point curve was drawn to determine the effect of the drug on the cells.

3-[(1H-Indole-2-carbonyl)-amino]-propionic acid ethyl ester, 4:

Mp 158-160°C. ^{1}H NMR: (500 MHz, $CDCl_3$ – 2 drops acetone) δ 1.17 (t, 3H, J = 7.5Hz), 2.58 (t, 2H, J = 6.5Hz), 3.67 (q, 2H, J = 6.0 Hz), 4.08 (q, 2H, J = 7 Hz), 6.81 (s, 1H), 7.01(t, 1H, J = 7.5 Hz), 7.09 (s, 1H), 7.14 (t, 1H, J = 7 Hz), 7.35 (d, 1H, J = 8.5 Hz), 7.52(d, 1H, J = 8.5 Hz), 9.86 (s, 1H). ^{13}C NMR: (74.47 MHz, $CDCl_3$) δ 13.9, 33.9, 35.0, 60.6, 102.3, 111.9, 120.3, 121.7, 124.2, 127.5, 130.6, 136.4, 161.7, 172.3. IR: (NaCl)

3377, 3352, 1716, 1624, 1552, 1325, 1207, 748 cm^{-1}. HRMS m/e ($C_{14}H_{16}N_2O_3$) (M) 260.1161, found 260.1159. Anal. ($C_{14}H_{16}N_2O_3$) calcd: C, 64.6; H, 6.20; N, 10.76; found: C, 64.22; H, 6.34; N, 10.67.

3-[(1-Methyl-1H-indole-2-carbonyl)-amino]-propionic acid ethyl ester, 5:

Mp 75-77°C. ^1H NMR: (300 MHz, $CDCl_3$) δ 1.28 (t, 3H, J = 7.2Hz), 2.67 (m, 2H), 3.72 (m, 2H), 4.07 (s, 3H), 4.22 (q, 2H, J = 6.9Hz), 6.86 (s, 1H), 7.05-7.65 (m, 4H). ^{13}C NMR: (74.47 MHz, $CDCl_3$) δ 14.1, 31.4, 33.9, 34.8, 60.7, 103.8, 110.0, 120.4, 121.7, 123.9, 125.9, 131.8, 138.9, 162.4, 172.7. IR (NaCl): 3265, 1730, 1630, 1554, 1460, 1383, 1319, 1184 cm^{-1}. HRMS m/e ($C_{15}H_{18}N_2O_3$)(M) 274.1317, found 274.1315. Anal. ($C_{15}H_{18}N_2O_3$) Calcd: C, 65.68; H, 6.61; N, 10.21; found: C, 64.55; H, 6.44; N, 10.10.

3-[(1-Methyl-1H-indole-2-carbonyl)-amino]-propionic acid, 7:

Mp 160-162°C. ^1H NMR: (300MHz, d^6-DMSO) δ 2.49 (t, 2H, J = 7.2 Hz), 3.46 (q, 2H, J = 6.6 Hz), 3.95 (s, 3H), 7.07-7.64 (m, 5H), 8.53 (t, 1H, J = 5.7 Hz). ^{13}C NMR: (74.47 MHz, d^6-DMSO) δ 31.1, 33.7, 35.0, 104.0, 110.2, 119.9, 121.3, 123.3, 125.5, 132.1, 138.2, 161.8, 172.6. IR (NaCl): 3377, 2922, 1714, 1601, 1550, 1464, 1423, 1280, 1226, 1190, 908 cm^{-1}. HRMS m/e ($C_{13}H_{14}N_2O_3$)(M) 246.1004, found 246.1016. Anal. ($C_{13}H_{14}N_2O_3$) calcd: C, 63.40; H, 5.73; N, 11.38; found: C, 62.39; H, 5.94; N, 11.12.

10-Methyl-3,4-dihydro-2H,10H-azepino[3,4-b]indole-1,5-dione, 9:

Mp 200-205° C. ^1H NMR: (500MHz, d^6-DMSO) δ 2.77 (t, 2H, J = 5.5 Hz), 3.39 (t, 2H, J = 5.5, 4.5 Hz), 3.99 (s, 3H), 7.27 (t, 1H, J = 7.0, 8.0 Hz), 7.37 (t, 1H, J = 7.5 Hz), 7.62 (d, 1H, J = 8.0 Hz), 8.27 (d, 1H, J = 8.0 Hz), 8.77 (t, 1H, J = 5.0, 6.0 Hz); ^{13}C NMR: (124.1 MHz, d^6-DMSO) δ 32.4, 36.6, 44.9, 110.9, 114.7, 122.6, 123.1, 124.5, 125.0, 134.7,

137.9, 162.3, 195.6. IR (NaCl): 3204, 3000, 2924, 1662, 1641, 1506, 1473, 1371, 724 cm^{-1}; HRMS m/e ($C_{13}H_{12}N_2O_2$)(M) 228.0899, found 228.0887. Anal. ($C_{13}H_{12}N_2O_2$) calcd: C, 68.41; H, 5.30; N, 12.27; found: C, 65.80; H, 5.37; N, 11.42.

10-Methyl-5-(5-oxo-2-phenyl-oxazol-4-ylidene)-3,4,5,10-tetrahydro-2H-azepino[3,4-b]indol-1-one, 11:

Mp: 189-192°C. ^{1}H NMR: (300MHz, $CDCl_3$) δ 3.54 (t, 2H, J =3.3, 3.6 Hz), 3.59 (t, 2H, J = 2.7, 3.3 Hz), 4.09 (s, 3H), 6.94 (s, 1H), 7.24-7.27 (m, 1H), 7.38-7.49 (m, 5H), 7.78 (d, 1H, J = 4.8 Hz), 7.92 (d, 2H, J = 6.0 Hz); ^{13}C NMR: (74.47 MHz, $CDCl_3$) δ 31.9, 38.2, 38.4, 110.1, 115.8, 121.3, 124.3, 125.0, 125.1, 125.8, 127.5, 128.7, 130.0, 131.7, 132.4, 138.5, 144.5, 159.4, 165.3, 165.9. IR: (NaCl) 1784, 1753, 1662, 1633, 1473 cm^{-1}; LRMS (EI): 371.1(M); HRMS m/e ($C_{22}H_{17}N_3O_3$)(M) 371.1270, found 371.1268.

5-(2-Amino-5-oxo-1,5-dihydro-imidazol-4-ylidene)-10-methyl-3,4,5,10-tetrahydro-2H-azepino[3,4-b]indol-1-one, 3:

Mp: >260°C. ^{1}H NMR: (300MHz, d^6-DMSO) δ 2.99-3.48 (m, 4H) 3.92 (s, 3H), 7.19 (t, 1H, J = 7.2Hz), 7.36 (t, 1H, J = 6.6Hz), 7.55 (d, 1H, J = 8.1Hz), 7.65 (d, 1H, J = 8.4Hz), 8.57 (t, 1H, J = 5.1Hz), 9.15-9.25 (br-s,1H), 10.48 (s, 1H); ^{13}C NMR: (74.47 MHz, d^6-DMSO) δ 32.1, 37.1, 38.4, 111.7, 112.6, 122.1, 122.2, 123.5, 123.7, 125.1, 128.1, 132.8, 138.5, 154.6, 163.0, 165.1; IR: (KBr-pellet) 3211, 1699, 1635, 1508, 1477 cm^{-1}; LRMS (EI): 308.7 (M); HRMS m/e ($C_{16}H_{15}N_5O_2$)(M) 309.1304, found 309.1291. **3:** Anal. ($C_{16}H_{15}N_5O_2$) C, H, N. **3.HCl:** Anal. ($C_{16}H_{16}N_5O_2Cl$) C, H, N.

3-[(1H-indole-2-carbonyl)-amino]-propionic acid, 6:

Mp 232°C. ^{1}H NMR: (300 MHz, d^6-DMSO) δ 2.55 (t, 2H, J = 7.2 Hz), 3.47 (q, 2H, J = 6.9 Hz), 7.01(t, 1H, J = 7.2 Hz), 7.04 (s, 1H), 7.13 (t, 1H, J = 7.2 Hz), 7.41 (1H, J = 8.1 Hz), 7.57 (1H, J = 8.1 Hz), 8.52 (s, 1H), 11.55 (s, 1H), 12.25 (s, 1H); ^{13}C NMR: (74.47 MHz) (d^6-DMSO) δ 34.6, 35.9, 103.2, 112..9, 120.3, 122.1, 123.9, 127.8, 132.4, 137.1, 161.8, 173.4; IR (NaCl): 3422, 3273, 1745, 1707, 1643, 1549, 1417, 1341, 1259, 746cm^{-1}; HRMS m/e ($C_{12}H_{12}N_2O_3$)(M) 232.0848, found 232.0844. Anal. ($C_{12}H_{12}N_2O_3$) calcd: C; H, 5.21; N, 12.06; found: H, 5.27; N, 11.79.

3,4-Dihydro-2H,10H-azepino[3,4-b]indole-1,5-dione, 8:

Mp: 257-260°C. ^{1}H NMR (300 MHz, d^6-DMSO) δ 2.80-2.85 (m, 2H), 3.40-3.46 (m, 2H), 7.22-7.38 (m, 2H), 7.51 (d, 1H, *J* = 9.0 Hz), 8.28 (d, 1H, *J* = 9.0 Hz), 8.72 (m, 1H), 12.41 (s, 1H); ^{13}C NMR (74.47 MHz, d^6-DMSO) δ 36.5, 44.0, 112.6, 113.7, 122.6, 122.7, 124.7, 126.0, 134.5, 135.6, 162.3, 195.0; IR: (NaCl) 3163, 1662, 1628, 1523, 1437, 1408 cm^{-1}; LRMS (EI): M^+ = 214.3; HRMS m/e ($C_{12}H_{10}N_2O_2$)(M) 214.0742, found 214.0740. Anal. ($C_{14}H_{16}N_2O_3$) calcd: C, 67.28; H, 4.71; N, 13.08; found: C, 65.36; H, 4.66; N, 12.53.

5-(5-oxo-2-phenyl-oxazol-4-ylidene)-3, 4, 5, 10-tetrahydro-2H-azepino [3,4-b]indol-1-one, 10:

Mp: 247-250°C. ^{1}H NMR (300MHz, d^6-DMSO) δ 3.36-3.47 (m, 4H), 7.14 (t, 1H, J = 8.1 Hz), 7.30 (t, 1H, J = 9.0 Hz), 7.49-7.58 (m, 4H), 7.81-7.87 (m, 3H), 8.45-8.56 (m, 1H), 12.11(s, 1H); ^{13}C NMR (74.47MHz, d^6-DMSO) δ 37.7, 38.6, 112.9, 115.3, 121.0, 125.1, 125.3, 126.5, 127.4, 129.0, 129.8, 133.2, 134.0, 136.9, 146.3, 158.4, 165.0, 166.1; IR: (KBr-pellet) 3358, 3179, 1749, 1653, 1633, 1448 cm^{-1}; HRMS m/e ($C_{21}H_{15}N_3O_3$)(M) 357.1113, found 357.1106.

5-(2-Amino-5-oxo-1, 5-dihydro-imidazol-4-ylidene)-3,4,5,10-tetrahydro-2H-azepino[3,4-b]indol-1-one, 2:

Mp:>260°C; **2**: ^{1}H NMR (300 MHz, d^6-DMSO) δ 3.20-3.40 (br, 4H), 7.16 (t, 1H, J = 12.0 Hz), 7.29 (t, 1H, J = 12.0 Hz), 7.52 (m, 2H), 8.30-8.50 (m, 2H), 9.05-9.25 (br., 1H), 10.30-10.40 (m, 1H), 12.48 (s, 1H); ^{13}C NMR: (124.1 MHz), d^6-DMSO) δ 36.5, 39.2, 112.7, 113.5, 121.9, 122.3, 122.8, 124.5, 125.0, 128.6, 132.8, 137.0, 154.6, 163.4, 165.5; IR: (KBr-pellet) 3294, 1620, 1475, 1251 cm^1; LRMS (EI): 295.1 (M); HRMS (FAB) ($C_{15}H_{14}N_5O_2$)(M+H) 296.1148, found 296.1144. **2.HCl:** ^{1}H NMR (500 MHz, d^6-DMSO) δ 3.27-3.38 (br, 4H), 7.16 (t, 1H, J = 7.5 Hz), 7.28 (t, 1H, J = 7.5Hz), 7.40 (d, 1H, 8 Hz), 7.49 (d, 1H, 8Hz) 8.18 (br, 1H), 8.49 (br, 1H), 12.08 (s, 1H); ^{13}C NMR (148.9MHz, d^6-DMSO) δ 34.4, 40.0, 112.7, 114.2, 116.6, 120.4, 121.5, 123.9, 128.3, 128.6, 130.4, 136.2, 160.1, 166.5, 177.1 LRMS (EI): 295.2 (M); FAB+(LRMS): 296.07 FAB + (HRMS)($C_{15}H_{14}N_5O_2$)(M+H) 296.1148, found 296.1147 FAB-(LRMS): 34.99, 37.0 Anal. ($C_{15}H_{14}N_5O_2Cl$) H, N; C: calcd., 54.30; found, 54.56.

Figure 2. X-ray crystal structure of **3**. MeOH.

Table 1. Crystal data and structure refinement for compound **3**

Identification code	tepe008
Empirical formula	C17 H19 N5 O3
Formula weight	341.37
Temperature	173(2) K
Wavelength	0.71073 A
Crystal system	Monoclinic
Space group	P2(1)/n
Unit cell dimensions	a = 7.3762(15) A
	b = 19.493(4) A
	c = 11.302(2) A
	alpha = 90 deg.
	beta = 94.80(3) deg.
	gamma = 90 deg.
Volume	1619.4(6) A^3
Z	4
Density (calculated)	1.400 Mg/m^3
Absorption coefficient	0.100 mm^-1

F(000)	720
Crystal size	0.5 x 0.3 x 0.1 mm
Theta range for data collection	2.09 to 28.23 deg.
Index ranges	-9<=h<=9, -24<=k<=25, -14<=l<=14
Reflections collected / unique	19046 / 3889 [R(int) = 0.0532]
Completeness to theta = 28.23	97.5%
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3889 / 0 / 246
Goodness-of-fit on F^2	1.228
Final R indices [I>2sigma(I)]	R1 = 0.0598, wR2 = 0.1750
R indices (all data)	R1 = 0.0891, wR2 = 0.1864
Largest diff. peak and hole	0.425 and -0.626 e.A^-3