A facile atom economic one pot multicomponent synthesis of bioactive spiro-
indenoquinoxaline pyrrolizines as potent antioxidant and anti cancer agents

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Single Crystal X-ray Diffraction studies

A colorless plate, measuring 0.25 x 0.20 x 0.05 mm³ was mounted on a loop with oil. Data was collected at 21°C on a Nonius Kappa CCD FR590 single crystal X-ray diffractometer, Mo-radiation. Crystal-to-detector distance was 30 mm and exposure time was 200 seconds per degree for all sets. The scan width was 2°. Data collection was 99.4% complete to 25° in θ. A total of 12177 partial and complete reflections were collected covering the indices, -11<=h<=12, -15<=k<=15, -18<=l<=18. 7275 reflections were symmetry independent and the R\textsubscript{int} = 0.0769 indicated that the data was of average quality. Indexing and unit cell refinement indicated a triclinic P lattice. The space group was found to be P ̅1 (No.2). The data was integrated and scaled using hkl-SCALEPACK.\(^1\) This program applies a multiplicative correction factor (S) to the observed intensities (I) and has the following form:

\[ S = \left( e^{-2B\sin^2θ/λ^2} \right) / \text{scale} \]

S is calculated from the scale and the B factor determined for each frame and is then applied to I to give the corrected intensity (I\textsubscript{corr}). Solution by direct methods (SHELXS, SIR97\(^2\)) produced a complete heavy atom phasing model consistent with the proposed structure. The structure was completed by difference Fourier synthesis with SHELXL97.\(^3\)^4 Scattering factors are from Waasmair and Kirfel.\(^5\) Hydrogen atoms were placed in geometrically idealised positions and constrained to ride on their parent atoms with C---H distances in the range 0.95-1.00 Angstrom. Isotropic thermal parameters U\textsubscript{eq} were fixed such that they were 1.2U\textsubscript{eq} of their parent atom Ueq for CH's and 1.5U\textsubscript{eq} of their parent atom U\textsubscript{eq} in case of methyl groups. All non-hydrogen atoms were refined anisotropically by full-matrix least-squares.
Table 1: Crystallographic data for the structures provided.

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EXPERIMENTAL

1. CHEMISTRY

1.1. GENERAL METHODS

All chemicals and reagents were purchased from Sigma Aldrich and were used as such. Commercial grade solvents were distilled according to literature procedure. IR Spectra were recorded on JASCO FT IR 4100 spectrometer using KBr disc and the absorption frequencies quoted in reciprocal centimeters. $^1$H and $^{13}$C NMR spectra were recorded on Bruker advance (400 MHz for $^1$H and 100 MHz for $^{13}$C) in CDCl$_3$ and DMSO-d$_6$ solvents. The reaction courses were monitored by TLC on silica gel precoated F254 Merck plates. Chemical shifts are reported in δ values (ppm) downfield from tetramethylsilane and coupling constants are reported in Hertz (Hz). The following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m). Elemental analyses were performed on a Perkin Elmer 2400 series II Elemental CHNS analyzer. Mass spectra were obtained on a GC mass spectrometer.

1.2. SYNTHESIS

1.2.1. SYNTHESIS OF SPIRO INDENO-QUINOXALINE PYRROLIZINES (5a-f)

An equimolar mixture of ninhydrin 1 (1mmol) and o-phenylenediamine 2 (1mmol) in 10ml methanol and allow to stir at room temperature for 10min. Then L-proline 3 (1.2mmol) and quinoline derived chalcones 4(a-f) were added and allow the solution to reflux in a water bath. The progresses of the reaction were monitored by TLC, after the completion of reaction the solvent was evaporated and the obtained precipitate were washed with water which afforded a single product (5a-f). The purest form of the product will be obtained through recrystallization using chloroform/methanol in 1:1 ratio.

4.2.1.1. (1'-(2-chloro-6-methylquinolin-3-yl)-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-b]quinoxaline-11,3'-pyrrolizin]-2'-yl)(phenyl)methanone (5a). Brown solid; mp 240-242 °C; Yield 85%; IR(KBr) υ(cm$^{-1}$): 1680, 1588, 759; $^1$H NMR (400MHz, CDCl$_3$) (ppm) δ: 8.49(s, 1H), 8.44(d, $J = 8.4$ Hz, 1H), 8.19-7.44(m, 10H), 6.97(t, $J = 7.6$ Hz, 1H), 6.76(d, $J = 7.2$ Hz, 2H), 6.57(t, $J = 7.6$ Hz, 2H), 5.70(d, $J = 11.6$ Hz, 1H), 4.99(t, $J = 9.6$ Hz, 1H), 4.50-4.44(m, 1H), 2.72-2.65(m, 1H), 2.56-2.54(m, 1H), 2.53(s, 3H), 2.18-2.16(m, 1H), 2.14-2.05(m, 2H), 2.04-
1.97 (m, 1H); $^{13}$C NMR (100MHz, CDCl$_3$) (ppm) δ: 197.340, 164.120, 150.851, 145.208, 143.239, 142.814, 142.209, 137.684, 137.136, 136.651, 136.046, 132.510, 132.238, 131.340, 129.805, 129.742, 129.673, 129.022, 128.982, 128.552, 127.962, 127.690, 127.574, 127.452, 127.249, 126.088, 122.216, 75.127, 73.492, 65.844, 47.949, 47.773, 31.216, 28.027, 21.605; HRMS (TOF MS ES+) m/z (M+H) 593.2092; Anal. Calcd. for C$_{38}$H$_{29}$ClN$_4$O: C, 76.95; H, 4.93; N, 9.45; Found: C, 76.90; H, 4.95; N, 9.42.

4.2.1.2. (1'-(2-chloro-7-methylquinolin-3-yl)-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-b]quinoxaline-11,3'-pyrrolizin]-2'-yl)(phenyl)methanone (5b): Brown solid; mp 200-204 °C; Yield 80%; IR (KBr) υ (cm$^{-1}$): 1664, 1587, 762; $^1$H NMR (400MHz, CDCl$_3$) (ppm) δ: 8.55 (s, 1H), 8.44 (d, $J = 8.0$ Hz, 1H), 8.02-7.36 (m, 10H), 6.96 (t, $J = 7.2$ Hz, 1H), 6.77 (d, $J = 7.2$ Hz, 2H), 5.70 (d, $J = 11.6$ Hz, 1H), 4.99 (t, $J = 10.0$ Hz, 1H), 4.51-4.45 (m, 1H), 2.72-2.66 (m, 1H), 2.56-2.54 (m, 1H), 2.53 (s, 3H), 2.20-2.16 (m, 1H), 2.14-2.08 (m, 2H), 2.07-1.94 (m, 1H); $^{13}$C NMR (100MHz, DMSO-d$_6$) (ppm) δ: 197.434, 164.112, 152.715, 151.642, 146.830, 143.217, 142.746, 142.133, 140.785, 137.626, 136.631, 136.492, 132.235, 131.498, 131.344, 129.810, 129.773, 129.606, 129.372, 129.017, 128.972, 127.667, 127.434, 127.240, 126.859, 125.596, 122.217, 75.104, 73.426, 65.792, 47.777, 31.193, 28.001, 21.914; Anal. Calcd. for C$_{38}$H$_{29}$ClN$_4$O: C, 76.95; H, 4.93; N, 9.45; Found: C, 76.94; H, 4.92; N, 9.43.

4.2.1.3. (1'-(2-chloro-8-methylquinolin-3-yl)-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-b]quinoxaline-11,3'-pyrrolizin]-2'-yl)(phenyl)methanone (5c): Brown solid; mp 204-206 °C; Yield 90%; IR (KBr) υ (cm$^{-1}$): 1660, 1579, 762; $^1$H NMR (400MHz, CDCl$_3$) (ppm) δ: 8.56 (s, 1H), 8.44 (d, $J = 8.4$ Hz, 1H), 8.01-7.40 (m, 10H), 6.96 (t, $J = 11.6$ Hz, 1H), 5.72 (d, $J = 7.6$ Hz, 2H), 5.01 (t, $J = 9.6$ Hz, 1H), 4.53-4.48 (m, 1H), 2.76 (s, 3H), 2.73-2.67 (m, 1H), 2.57-2.52 (m, 1H), 2.18-2.14 (m, 1H), 2.12-2.05 (m, 2H), 2.01-1.98 (m, 1H); $^{13}$C NMR (100MHz, CDCl$_3$) (ppm) δ: 197.392, 164.112, 152.717, 150.664, 145.856, 143.246, 142.771, 142.173, 137.661, 137.038, 136.638, 136.485, 132.218, 132.192, 131.327, 130.207, 129.785, 129.743, 129.654, 128.986, 127.692, 127.553, 127.443, 127.235, 126.869, 125.132, 122.201, 75.136, 73.406, 65.779, 47.772, 31.209, 28.014, 17.777; Anal. Calcd. for C$_{38}$H$_{29}$ClN$_4$O: C, 76.95; H, 4.93; N, 9.45; Found: C, 76.91; H, 4.92; N, 9.43.

4.2.1.4. (1'-(2-chloro-6-methoxyquinolin-3-yl)-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-b]quinoxaline-11,3'-pyrrolizin]-2'-yl)(phenyl)methanone (5d): Brown solid; mp 222-224 °C;
Yield 84 %; IR(KBr) v(cm⁻¹): 1680, 1590, 760; ¹H NMR (400MHz, CDCl₃) (ppm) δ: 8.45(s, 1H), 8.43(d, J = 7.2 Hz, 1H), 8.01-7.11(m, 10H), 6.97(t, J = 7.2 Hz, 1H), 6.76(d, J = 7.6 Hz, 2H), 6.57(t, J = 7.6 Hz, 2H), 5.71(d, J = 11.6 Hz, 1H), 4.96(t, J = 9.6 Hz, 1H), 4.50-4.45(m, 1H), 3.94(s, 3H), 2.68-2.65(m, 1H), 2.56-2.51(m, 1H), 2.18-2.14(m, 1H), 2.11-2.08(m, 2H), 2.01-1.96(m, 1H); ¹³C NMR(100MHz, CDCl₃) (ppm)δ: 197.341, 164.133, 158.243, 152.767, 149.108, 143.228, 142.806, 142.661, 142.180, 137.665, 136.648, 135.537, 132.246, 131.353, 129.819, 129.753, 129.695, 129.613, 129.039, 128.970, 128.601, 127.657, 127.455, 127.234, 122.920, 122.226, 104.181, 75.150, 73.346, 65.713, 56.629, 47.775, 31.238, 28.038; Anal. Calcd. for C₃₈H₂₉ClN₄O₂: C, 76.93; H, 4.80; N, 9.20; Found: C, 76.92; H, 4.82; N, 9.21.

4.2.1.5. (1'-(2-chloro-6,8-dimethylquinolin-3-yl)-1',2',5',6',7',7a'-hexahydropyrido[1,2-b]quinolizine-11,3'-pyrrolizin]-2'-yl)(phenyl)methanone (5e): Brown solid; mp 190-192 °C; Yield 92 %; IR(KBr) v(cm⁻¹): 1679, 1580, 761; ¹H NMR (400MHz, CDCl₃) (ppm) δ: 8.64(s, 1H), 8.43(d, J = 8.4 Hz, 1H), 8.00-7.22(m, 9H), 6.97(t, J = 7.6 Hz, 1H), 6.80(d, J = 7.6 Hz, 2H), 6.59(t, J = 7.6 Hz, 2H), 6.59(d, J = 11.6 Hz, 1H), 5.03(t, J = 9.6 Hz, 1H), 4.56-4.50(m, 1H), 2.71(s, 6H), 2.69-2.66(m, 1H), 2.57-2.53(m, 1H), 2.20-2.16(m, 1H), 2.13-2.10(m, 2H), 2.09-2.02(m, 1H); ¹³C NMR(100MHz, CDCl₃) (ppm)δ: 197.217, 163.973, 152.726, 150.270, 146.213, 143.310, 142.778, 142.165, 137.650, 136.696, 134.365, 133.575, 132.247, 131.702, 131.355, 131.302, 129.951, 129.749, 129.696, 128.626, 127.780, 127.427, 127.341, 127.317, 126.933, 122.39, 75.225, 73.281, 65.546, 48.228, 47.681, 31.298, 28.167, 18.806, 17.748; Anal. Calcd. for C₃₉H₃₁ClN₄O: C, 77.15; H, 5.15; N, 9.23; Found: C, 77.12; H, 5.12; N, 9.21.

4.2.1.6. (1'-(2-chloroquinolin-3-yl)-1',2',5',6',7',7a'-hexahydropyrido[1,2-b]quinolizine-11,3'-pyrrolizin]-2'-yl)(phenyl)methanone (5f): Brown solid; mp 220-224 °C; Yield 75 %; IR(KBr) v(cm⁻¹): 1660, 1586, 764; ¹H NMR (400MHz, CDCl₃) (ppm) δ: 8.43(s, 1H), 8.32(dd, J = 8.4 Hz, 1H), 7.90-7.33(m, 11H), 6.80(t, J = 7.6 Hz, 1H), 6.63(d, J = 8.4 Hz, 2H), 6.52(t, J = 7.6 Hz, 2H), 5.60(d, J = 11.6 Hz, 1H), 4.91(t, J = 10.0 Hz, 1H), 4.43(d, J = 9.6 Hz, 1H), 2.64-2.58(m, 2H), 2.10-2.07(m, 1H), 2.02-1.97(m, 2H), 1.93-1.88(m, 1H); ¹³C NMR(100MHz, DMSO-d₆) (ppm)δ: 197.46, 164.56, 151.34, 150.68, 148.23, 144.53, 143.78, 142.13, 140.49, 136.96, 136.77, 136.36, 133.47, 131.92, 130.41, 130.02, 129.77, 129.58, 129.20, 128.60, 127.57, 127.40, 127.19, 127.01, 126.24, 125.84, 122.83, 73.14, 72.69, 65.96, 48.77, 47.56, 46.92, 31.66,
26.90, 21.57; Anal. Calcd. for C_{37}H_{27}ClN_{4}O: C, 76.74; H, 4.70; N, 9.67; Found: C, 76.72; H, 4.69; N, 9.64.

1.2.2. SYNTHESIS OF SPIRO INDENO-QUINOXALINE PYRROLIZINES (6a-f)

An equimolar mixture of ninhydrin 1(1mmol) and 2, 3-diaminobenzophenone 2a (1mmol) in 10ml methanol and allow to stir at room temperature for 10min. Then L-proline 3(1.2mmol) and quinoline derived chalcones 4(a-f) were added and allow the solution to reflux in a water bath. The progresses of the reaction were monitored by TLC, and after the completion of reaction the obtained precipitate were filtered and washed with water to afford a single product (6a-f). The purest form of the product was obtained through recrystallization using chloroform/methanol in 1:1 ratio.

4.2.2.1. (1'- (2-chloro-6-methylquinolin-3-yl)-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-b]quinoxaline-11,3'-pyrrolzine]-2',8-diyl)bis(phenylmethanone) (6a): Brown solid; mp 160-162 °C; Yield 87 %; IR(KBr) \( \nu (\text{cm}^{-1}) \): 1659, 1586, 701; \(^1\)H NMR (400MHz, CDCl\(_3\)) (ppm) \( \delta \): 8.81(d, \( J = 1.6 \) Hz, 1H), 8.40(s, 1H), 8.29(dd, \( J = 1.6 \) Hz, 1H), 8.11(d, \( J = 8.8 \) Hz, 1H), 8.00-7.48(m, 12H), 6.99(t, \( J = 7.2 \) Hz, 1H), 6.73(d, \( J = 7.6 \) Hz, 2H), 6.59(t, \( J = 8.0 \) Hz, 2H), 5.69(d, \( J = 11.6 \) Hz, 1H), 4.96(t, \( J = 9.6 \) Hz, 1H), 4.46-4.41(m, 1H), 2.71-2.65(m, 1H), 2.58-2.54(m, 1H), 2.50(s, 3H), 2.20-2.17(m, 1H), 2.15-2.08(m, 2H), 2.07-1.97(m, 1H); \(^{13}\)C NMR(100MHz, CDCl\(_3\)) (ppm)\( \delta \): 197.116, 195.863, 165.485, 154.414, 150.689, 145.207, 144.940, 143.660, 141.196, 137.403, 137.333, 137.215, 136.607, 135.985, 132.901, 132.674, 132.578, 132.380, 132.108, 132.048, 130.261, 130.045, 129.889, 129.475, 128.637, 127.928, 127.778, 127.501, 127.376, 127.333, 126.054, 122.708, 75.181, 73.425, 65.694, 47.956, 47.859, 31.281, 28.080, 21.574; Anal. Calcd. for C\(_{45}\)H\(_{33}\)ClN\(_4\)O\(_2\): C, 77.52; H, 4.77; N, 8.04; Found: C, 77.50; H, 4.72; N, 8.02.

4.2.2.2. (1’-(2-chloro-7-methylquinolin-3-yl)-1’,2’,5’,6’,7’,7a’-hexahydrospiro[indeno[1,2-b]quinoxaline-11,3’-pyrrolzine]-2’,8-diyl)bis(phenylmethanone) (6b): Brown solid; mp 190-192 °C; Yield 82 %; IR(KBr) \( \nu (\text{cm}^{-1}) \): \(^1\)H NMR (400MHz, CDCl\(_3\)) (ppm) \( \delta \): 8.82(d, \( J = 1.6 \) Hz, 1H), 8.46(s, 1H), 8.29-7.34(m, 14H), 7.00(t, \( J = 7.2 \) Hz, 1H), 6.74(d, \( J = 7.2 \) Hz, 2H), 6.59(t, \( J = 8.0 \) Hz, 2H), 5.67(d, \( J = 11.2 \) Hz, 1H), 4.96-4.90(m, 1H), 4.48-4.42(m, 1H), 2.70-2.67(m, 1H), 2.58-2.56(m, 1H), 2.53(s, 3H), 2.18-2.14(m, 1H), 2.09-2.06(m, 2H), 2.05-1.98(m, 1H); \(^{13}\)C

4.2.2.3. (1'-(2-chloro-8-methylquinolin-3-yl)-1',2',5',6',7',7a'-hexahydropyrido[1,2-b]quinoxaline-11,3'-pyrrolizine]-2',8-diyl)bis(phenylmethanone) (6c): Brown solid; mp 192-194°C; Yield 92 %; IR(KBr) υ(cm⁻¹): 1661, 1585, 717; ¹H NMR (400MHz, CDCl₃) (ppm)δ: 8.73(d, J = 1.6 Hz, 1H), 8.40(s, 1H), 8.22-7.31(m, 14H), 6.92(t, J = 7.2 Hz, 1H), 6.68(d, J = 7.6 Hz, 2H), 6.53(t, J = 7.6 Hz, 2H), 5.62(d, J = 11.6 Hz, 1H), 4.91(t, J = 10.0 Hz, 1H), 4.43(dt, J = 6Hz, J = 9.2 Hz, 1H), 2.69(s, 3H), 2.67-2.60(m, 1H), 2.51-2.46(m, 1H), 2.12-2.09(m, 1H), 2.06-2.01(m, 2H), 2.00-1.90(m, 1H); ¹³C NMR(100MHz, CDCl₃) (ppm)δ: 197.179, 195.843, 165.538, 154.419, 150.526, 145.897, 144.962, 143.768, 141.185, 137.413, 137.373, 137.262, 137.047, 136.658, 136.658, 136.521, 132.857, 132.707, 132.366, 132.071, 131.879, 130.013, 129.863, 129.493, 128.631, 127.833, 127.508, 127.407, 127.354, 126.919, 125.100, 122.699, 75.171, 73.309, 65.613, 55.137, 48.114, 47.862, 31.208, 28.012, 17.735; HRMS (TOF MS ES+) m/z 697.2476 Anal. Calcd. for C₄₅H₃₃ClN₄O₂: C, 77.52; H, 4.77; N, 8.04; Found: C, 77.51; H, 4.74; N, 8.02.

4.2.2.4. (1'-(2-chloro-6-methoxyquinolin-3-yl)-1',2',5',6',7',7a'-hexahydropyrido[1,2-b]quinoxaline-11,3'-pyrrolizine]-2',8-diyl)bis(phenylmethanone) (6d): Brown solid; mp 208-210°C; Yield 88 %; IR(KBr) υ(cm⁻¹): 1662, 1587, 700; ¹H NMR (400MHz, CDCl₃) (ppm)δ: 8.74(d, J = 2.0 Hz, 1H), 8.31(s, 1H), 8.19(dd, J = 2.0 Hz, 1H), 8.04(d, J = 8.8 Hz, 1H), 7.92-6.99(m, 12H), 6.92(t, J = 7.6 Hz, 1H), 6.67(d, J = 7.2 Hz, 2H), 6.52(t, J = 7.6 Hz, 2H), 5.61(d, J = 11.6 Hz, 1H), 4.87(t, J = 10 Hz, 1H), 4.40-4.35(m, 1H), 3.83(s, 3H), 2.62-2.59(m, 1H), 2.51-2.46(m, 1H), 2.11-2.07(m, 1H), 2.03-2.00(m, 2H), 1.94-1.90(m, 1H); ¹³C NMR(100MHz, CDCl₃) (ppm)δ: 197.127, 195.830, 165.571, 158.293, 154.434, 148.954, 144.930, 143.678, 142.681, 141.231, 137.453, 137.317, 137.227, 136.643, 135.470, 132.904, 132.533, 132.378, 132.305, 132.096, 130.247, 130.247, 129.899, 129.675, 129.450, 128.632, 128.565, 127.783, 127.390, 127.333, 123.060, 122.709, 104.716, 75.150, 73.312, 65.585, 55.648, 48.078, 47.882, 31.209,
28.018; Anal. Calcd. for C_{45}H_{33}ClN_{4}O_{3} : C, 75.78; H, 4.66; N, 7.86; Found: C, 75.75; H, 4.62; N, 7.84.

4.2.2.5.(1'-(2-chloro-6,8-dimethylquinolin-3-yl)-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-b]quinoxaline-11,3'-pyrroline]-2',8-diyl)bis(phenylmethanone)(6e): Brown solid; mp 170-174 °C; Yield 92 %; IR(KBr) υ(cm⁻¹): 1660, 1587, 718; ¹H NMR (400MHz, CDCl₃) (ppm) δ: 8.81(d, J = 1.6 Hz, 1H), 8.35(s, 1H), 8.29(dd, J = 2.0 Hz, 1.6 Hz, 1H), 8.10(d, J = 8.8 Hz, 1H), 8.00-7.48(m, 9H), 7.41(s, 1H), 7.35(s, 1H), 6.98(t, J = 7.2 Hz, 1H), 6.73(d, J = 7.2 Hz, 2H), 6.59(t, J = 7.6 Hz, 2H), 5.70(d, J = 11.6 Hz, 1H), 4.95(t, J = 10 Hz, 1H), 4.47-4.41(m, 1H), 2.70(s, 3H), 2.68-2.66(m, 1H), 2.58-2.54(m, 1H), 2.44(s, 3H), 2.18-2.14(m, 1H), 2.13-2.07(m, 2H), 2.05-1.98(m, 1H); ¹³C NMR(100MHz, CDCl₃) (ppm)δ:197.087, 195.841, 154.416, 149.626, 144.942, 144.530, 143.758, 141.210, 137.367, 137.230, 136.812, 136.641, 136.209, 136.031, 132.861, 132.683, 132.596, 132.309, 132.069, 131.654, 130.240, 130.005, 129.831, 129.466, 128.630, 127.829, 127.609, 127.386, 127.313, 123.941, 122.681, 75.202, 73.357, 65.599, 47.834, 31.286, 28.083, 21.522, 17.606; HRMS (TOF MS ES+) m/z 711.2644; Anal. Calcd. for C_{46}H_{35}ClN_{4}O_{3}: C, 75.97; H, 4.85; N, 7.70; Found: C, 75.94; H, 4.82; N, 7.68.

4.2.2.6.(1'-(2-chloroquinolin-3-yl)-1',2',5',6',7',7a'-hexahydrospiro[indeno[1,2-b]quinoxaline-11,3'-pyrroline]-2',8-diyl)bis(phenylmethanone)(6f): Brown solid; mp 195-198 °C; Yield 78 %; IR(KBr) υ(cm⁻¹): 1664, 1590, 723; ¹H NMR (400MHz, CDCl₃) (ppm) δ: 8.64(s, 1H), 8.42(d, J = 1.6 Hz, 1H), 8.32(d, J = 9.4 Hz, 1H), 8.10-7.36(m, 14H), 7.32(t, J = 7.6 Hz, 1H), 6.92(d, J = 9.2 Hz, 2H), 6.62(t, J = 7.6 Hz, 2H), 5.64(d, J = 11.6 Hz, 1H), 4.92(t, J = 10.0 Hz, 1H), 4.45(dt, J = 6Hz, J = 9.2 Hz, 1H), 2.62-2.61(m, 1H), 2.50-2.48(m, 1H), 2.11-2.09(m, 1H), 2.07-2.01(m, 2H), 2.00-1.91(m, 1H); ¹³C NMR(100MHz, CDCl₃) (ppm)δ: 197.229, 195.123, 164.538, 155.149, 149.426, 145.577, 144.742, 142.741, 141.195, 138.463, 137.353, 137.262, 137.078, 136.658, 135.658, 136.521, 133.747, 132.707, 132.256, 132.055, 131.749, 130.124, 129.743, 129.593, 128.631, 127.933, 127.558, 127.427, 127.344, 126.149, 124.147, 122.479, 74.411, 72.269, 66.158, 54.787, 49.744, 47.242, 31.247, 27.146; Anal. Calcd. for C_{44}H_{35}ClN_{4}O_{2}: C, 77.35; H, 4.57; N, 8.20; Found: C, 77.32; H, 4.54; N, 8.19.
1.3. BIOLOGICAL EVALUATION

1.3.1. INVITRO ANTIOXIDANT ACTIVITY

INSTRUMENTATION

Spectrophotometer: UV1800, Shimadzu

1.3.1.1. DPPH RADICAL SCAVENGING ACTIVITY

The antioxidant activity of the extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the method of Braca et al. (2001). Samples were taken in various concentrations and the volume was adjusted to 100 µL with methanol. About 3 mL of a 0.1 mM methanolic solution of DPPH• was added to the aliquots of samples and standards (BHT and Rutin) and vortexed well. Negative control was prepared by adding 100 µL of methanol in 3 mL of 0.1 mM methanolic solution DPPH•. The tubes were allowed to stand in dark for 30 minutes at room temperature. The absorbance of the sample was measured at 517 nm against the blank. Radical scavenging activity of the samples was expressed as IC\textsubscript{50} which is the concentration of the sample required to inhibit 50% of DPPH• concentration.

1.3.1.2. NITRIC OXIDE RADICAL SCAVENGING ACTIVITY

The procedure is based on the method of Sreejayan and Rao (1997), where sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside 2 mL (5 mM) in 0.2 M phosphate buffered saline of pH-7.4 (10 mM dibasic sodium phosphate, 2 mM KH\textsubscript{2}PO\textsubscript{4}, 137 mM NaCl, 2.7 mM KCl) was mixed with 100 µL sample solution of various extracts or standard (Rutin) and incubated at room temperature for 150 minutes. The same reaction mixture containing distilled water in place of extract or standard was used as the negative control. After the incubation period, 2 mL of Griess reagent (1% sulfanilamide, 2% H\textsubscript{3}PO\textsubscript{4} and 0.1% N- (1-naphthyl) ethylene diamine dihydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm against the blank. The scavenging activity on superoxide anion generation was calculated as IC\textsubscript{50} which is the concentration of the sample required to inhibit 50% of radical concentration.
1.3.1.3. SUPEROXIDE RADICAL SCAVENGING ACTIVITY

The assay was based on the capacity of various extracts to inhibit formazan formation by scavenging the superoxide radicals generated in the riboflavin-light-NBT system (Beuchamp and Fridovich, 1971). Each 3 mL reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 µg riboflavin, 12 mM EDTA, 0.1 mg NBT and 25-100 µL of the of sample solution and standard (BHT). Reaction was started by illuminating the reaction mixture with sample extract for 90 sec. After illumination instantly the absorbance was measured at 590 nm against the reagent blank. Identical tubes with reaction mixture kept in the dark served as blank. The scavenging activity on superoxide anion generation was calculated as IC$_{50}$ which is the concentration of the sample required to inhibit 50% of radical concentration.

1.3.2. CYTOTOXIC ACTIVITY

1.3.2.1. METHODOLOGY

CELL CULTURE

The Human lung and breast cancer cells were purchased from the National Center for Cell Sciences (NCCS), Pune, India. The cancer cells were maintained in Dulbecco’s modified eagles medium (DMEM) supplemented with 2mM l-glutamine and balanced salt solution (BSS) adjusted to contain 1.5 g/L Na$_2$CO$_3$, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM l-glutamine, 1.5 g/L glucose, 10 mM (4-(2-hydroxyethyl)-1-piperazinemethane sulfonic acid) (HEPES) and 10% fetal bovine serum (GIBCO, USA). Penicillin and streptomycin (100 IU/100µg) were adjusted to 1mL/L. The cells were maintained at 37 °C with 5% CO$_2$ in a humidified CO$_2$ incubator.

EVALUATION OF CYTOTOXICITY

The inhibitory concentration (IC$_{50}$) value was evaluated using an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cancer cells were grown (1×10$^5$cells/well) in a 96-well plate for 48 h in to 75% confluence. The medium was replaced with fresh medium containing serially diluted synthesized compounds, and the cells were further incubated for 48 h. The culture medium was removed, and 100µL of the MTT [3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl tetrazolium bromide] (Hi-Media) solution was added to each well and incubated at 37°C for 4 hrs. After removal of the supernatant,
50 µL of DMSO was added to each of the wells and incubated for 10 min to solubilize the formazan crystals. The optical density was measured at 620 nm in an ELISA multiwell plate reader (Thermo Multiskan EX, USA). The OD value was used to calculate the percentage of viability using the following formula.

\[
\% \text{ of viability} = \frac{\text{OD value of experimental sample}}{\text{OD value of experimental control}} \times 100
\]

**FLUORESCENCE MICROSCOPIC ANALYSIS OF APOPTOTIC CELL DEATH**

Approximately 1µL of a dye mixture (100 mg/mL acridine orange (AO) and 100 mg/mL ethidium bromide (EtBr) in distilled water) was mixed with 9 mL of cell suspension (1×10⁵ cells/mL) on clean microscope cover slips. The selected cancer cells were collected, washed with phosphate buffered saline (PBS) (pH 7.2) and stained with 1 mL of AO/EtBr. After incubation for 2 min, the cells were washed twice with PBS (5 min each) and visualized under a fluorescence microscope (Nikon Eclipse, Inc, Japan) at 400× magnification with an excitation filter at 480 nm. Likewise the cells were plated on glass coverslip in a 24-well plate and treated with complex for 24 hrs. The fixed cells were permeabilised with 0.2% triton X-100 (50µl) for 10min at room temperature and incubated for 3min with 10µl of DAPI by placing a coverslip over the cells to enable uniform spreading of the stain. The cells were observed under (Nikon Eclipse, Inc, Japan) fluorescent microscope.

**MOLECULAR DOCKING**

Docking analysis was performed by Autodock4.2 tool.6 Human epidermal growth factor receptor (PDB ID: 1M17)7 and the ligand were prepared for docking analysis with MGL Tool 1.5.6. The grid box was prepared as X=56, Y=58, Z=56, spacing at 0.586Å and grid point value of X=27.296, Y= -1.972, Z=61.717 were constructed enveloping the active site of the human epidermal growth factor receptor. The given input parameters were analyzed using a genetic algorithm and set 100 runs for each docking procedure. The complex structure showing lowest binding energy, ligand efficiency, with more number of hydrogen bonds was selected for competent results. Finally, docked complex structure has been visualized using PyMol software.
REFERENCES


Fig. S1. IR spectrum of compound 5a

Fig. S2. $^1$H NMR spectrum of compound 5a
Fig. S3. $^{13}$C NMR spectrum of compound 5a

Fig. S4. HRMS spectrum of compound 5a
Fig. S5. $^1$H NMR spectrum of compound 5b

Fig. S6. $^{13}$C NMR spectrum of compound 5b
Fig. S7. IR spectrum of compound 5c

Fig. S8. $^1$H NMR spectrum of compound 5c
Fig. S8(1). $^1$H NMR expansion spectrum of compound 5c

Fig. S9. $^{13}$C NMR spectrum of compound 5c
Fig. S10. DEPT-135 NMR spectrum of compound 5c

Fig. S11. IR spectrum of compound 5d
Fig. S12. $^1$H NMR spectrum of compound 5d

Fig. S13. $^{13}$C NMR spectrum of compound 5d
Fig. S14. IR spectrum of compound 5e

Fig. S15. \textsuperscript{1}H NMR spectrum of compound 5e
Fig. S16. $^{13}$C NMR spectrum of compound 5e

Fig. S17. IR spectrum of compound 6a
Fig. S18. $^1$H NMR spectrum of compound 6a

Fig. S18(1). $^1$H NMR expansion spectrum of compound 6a
Fig. S19. $^{13}$C NMR spectrum of compound 6a

Fig. S20. IR spectrum of compound 6b
Fig. S21. $^1$H NMR spectrum of compound 6b

Fig. S21(1). $^1$H NMR expansion spectrum of compound 6b
Fig. S22. $^{13}$C NMR spectrum of compound 6b

Fig. S23. HRMS spectrum of compound 6b
Fig. S24. IR spectrum of compound 6c

Fig. S25. $^1$H NMR spectrum of compound 6c
Fig. S25(1). $^1$H NMR expansion spectrum of compound 6c

Fig. S26. $^{13}$C NMR spectrum of compound 6c
Fig. S27. HRMS spectrum of compound 6c

Fig. S28. IR spectrum of compound 6d
Fig. S29. $^1$H NMR spectrum of compound 6d

Fig. S29(1). $^1$H NMR expansion spectrum of compound 6d
Fig. S30. $^{13}$C NMR spectrum of compound 6d

Fig. S31. IR spectrum of compound 6e
Fig. S32. $^1$H NMR spectrum of compound 6e

Fig. S32(1). $^1$H NMR expansion spectrum of compound 6e
Fig. S33. $^{13}$C NMR spectrum of compound 6e

Fig. S34. HRMS spectrum of compound 6e