Supporting Information

Novel [(N-alkyl-3-indolylmethylene)hydrazono]oxindoles arrest cell cycle and induce cell apoptosis by inhibiting CDK2 and Bcl-2: Synthesis, biological evaluation and in silico studies

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$^{13}$C NMR

Chemical shifts:
- 137.08
- 131.21
- 125.80
- 123.07
- 122.76
- 121.30
- 112.02
- 110.95

Structure:

- Compound 5c
- 137.08 ppm
- 131.21 ppm
- 125.80 ppm
- 123.07 ppm
- 122.76 ppm
- 121.30 ppm
- 112.02 ppm
- 110.95 ppm

Instruments:
- AVG400

Groups:
- MGM
1H NMR
Aromatic Zoom
$^1$H NMR
Aliphatic Zoom
MEE-5Me.1.fid —

1H NMR
Aliphatic Zoom
$^1$H NMR
$^{1}H$ NMR
Aliphatic Zoom
NL: 6.71E5
ESI7.1802 #14-27 RT: 0.17-0.31 AV: 7 NL: 2.55E+007
T. FTMS {1,1} + p ESI Full ms
[80.00-1600.00]

Measured Spectrum
MEE-NO2.1.fid

7d

1H NMR
MEE-NO2.1.fid

$^1$H NMR

Aromatic Zoom
$^1\text{H NMR}$
$^1$H NMR
Aromatic Zoom
NL: 7.58E5
ESI71804 #14-27 RT: 0.17-0.31 AV: 7 NL: 2.72E+007
T: FTMS {1,1} + p ESI Full ms
[80.00-1600.00]

Measured Spectrum
$^1$H NMR
Aromatic Zoom
| Panel/Cell Line | Mean Optical Densities | Percent Growth |
|----------------|------------------------|----------------|
| CCRF-CEM       | 1.440                  | 0.073          |
| MOLT-4         | 1.619                  | 0.049          |
| K-562          | 1.060                  | 0.009          |
| MOLT-4         | 1.619                  | 0.049          |
| RPMI-2656      | 1.440                  | 0.073          |
| SR             | 1.370                  | 0.017          |
| Non-Small Cell Lung Cancer |        |                |
| A549/ATCC      | 1.298                  | 0.067          |
| COLO 205       | 1.494                  | 0.069          |
| HCC-2998       | 1.321                  | 0.049          |
| HCT-116        | 1.261                  | 0.038          |
| HCT-15         | 1.400                  | 0.067          |
| HT29           | 1.272                  | 0.049          |
| SW-620         | 1.298                  | 0.067          |
| CNS Cancer     |                        |                |
| SF-268         | 1.272                  | 0.049          |
| SF-298         | 1.360                  | 0.073          |
| SE-530         | 1.272                  | 0.049          |
| SNB-18         | 1.440                  | 0.073          |
| SNB-75         | 1.370                  | 0.017          |
| U251           | 1.272                  | 0.049          |
| Melanoma       |                        |                |
| LOX IMVI       | 1.298                  | 0.067          |
| MALME-3M       | 1.261                  | 0.038          |
| M14            | 1.272                  | 0.049          |
| MDA-MB-435     | 1.360                  | 0.073          |
| SK-MEL-2       | 1.272                  | 0.049          |
| SK-MEL-8       | 1.272                  | 0.049          |
| SK-MEL-5       | 1.272                  | 0.049          |
| UACC-257       | 1.272                  | 0.049          |
| UACC-82       | 1.272                  | 0.049          |
| Ovarian Cancer |                        |                |
| IGROV1         | 1.298                  | 0.067          |
| OVCAR-3        | 1.298                  | 0.067          |
| OVCAR-4        | 1.298                  | 0.067          |
| OVCAR-5        | 1.298                  | 0.067          |
| OVCAR-8        | 1.298                  | 0.067          |
| NCI/ADR-RES    | 1.298                  | 0.067          |
| Renal Cancer   |                        |                |
| 786-0          | 1.298                  | 0.067          |
| A498           | 1.298                  | 0.067          |
| ACHN           | 1.298                  | 0.067          |
| RXF 393        | 1.298                  | 0.067          |
| SN12C          | 1.298                  | 0.067          |
| TK-10          | 1.298                  | 0.067          |
| UO-31          | 1.298                  | 0.067          |
| Prostate Cancer|                        |                |
| PC-3           | 1.298                  | 0.067          |
| DU-145         | 1.298                  | 0.067          |
| Breast Cancer  |                        |                |
| MCF7           | 1.298                  | 0.067          |
| MDA-MB-231/ATCC| 1.298                  | 0.067          |

**Note:** The data provided includes optical densities measured for various cancer cell lines under different conditions, as well as growth percentages. The values are presented in a tabular format with columns for Panel/Cell Line, Mean Optical Densities, Percent Growth, and additional columns for specific measurements such as GI50 and TGI.
## Mean Graphs

| Panel/Cell Line | Log$_{10}$ GI50 | GI50 | Log$_{10}$ TGI | TGI | Log$_{10}$ LC50 | LC50 |
|----------------|----------------|------|----------------|-----|----------------|------|
| **Leukemia**   |                |      |                |     |                |      |
| CCRF-CEM       | -5.71          |      | > -4.00        |     | > -4.00        |      |
| HL-60(TB)      | -5.88          |      | > -4.00        |     | > -4.00        |      |
| K-562          | -5.65          |      | > -4.00        |     | > -4.00        |      |
| MOLT-4         | -6.17          |      | > -4.00        |     | > -4.00        |      |
| RPMI-8226      | -5.88          |      | > -4.00        |     | > -4.00        |      |
| SR             | -6.28          |      | > -4.00        |     | > -4.00        |      |
| **Non-Small Cell Lung Cancer** | | | | | | |
| A549/ATCC      | -5.44          |      | > -4.00        |     | > -4.00        |      |
| EKVX           | -5.63          |      | > -4.00        |     | > -4.00        |      |
| HOP-29         | -5.26          |      | > -4.00        |     | > -4.00        |      |
| HOP-R9         | -5.18          |      | > -4.00        |     | > -4.00        |      |
| NCI-H226       | -5.53          |      | > -4.00        |     | > -4.00        |      |
| NCI-H232       | -5.60          |      | > -4.00        |     | > -4.00        |      |
| NCI-H322M      | -5.47          |      | > -4.00        |     | > -4.00        |      |
| NCI-H460       | -5.66          |      | > -4.00        |     | > -4.00        |      |
| NCI-H692        | -5.18          |      | > -4.00        |     | > -4.00        |      |
| **Colon Cancer** |                |      |                |     |                |      |
| COLO 205       | -5.91          |      | > -4.00        |     | > -4.00        |      |
| HCC-2998       | -5.68          |      | > -4.00        |     | > -4.00        |      |
| HCT-116        | -5.76          |      | > -4.00        |     | > -4.00        |      |
| HCT-15         | -5.58          |      | > -4.00        |     | > -4.00        |      |
| HT29           | -5.47          |      | > -4.00        |     | > -4.00        |      |
| KM12           | -5.49          |      | > -4.00        |     | > -4.00        |      |
| SW-620         | -5.53          |      | > -4.00        |     | > -4.00        |      |
| **CNS Cancer** |                |      |                |     |                |      |
| SF-268         | -5.48          |      | > -4.00        |     | > -4.00        |      |
| SF-295         | -5.08          |      | > -4.00        |     | > -4.00        |      |
| SF-539         | -5.57          |      | > -4.00        |     | > -4.00        |      |
| SNB-19         | -5.37          |      | > -4.00        |     | > -4.00        |      |
| SNB-75         | -5.64          |      | > -4.00        |     | > -4.00        |      |
| U251           | -5.45          |      | > -4.00        |     | > -4.00        |      |
| **Melanoma**   |                |      |                |     |                |      |
| LOX IMVI       | -5.90          |      | > -4.00        |     | > -4.00        |      |
| MALME-3M       | -5.73          |      | > -4.00        |     | > -4.00        |      |
| M14            | -5.72          |      | > -4.00        |     | > -4.00        |      |
| MDA-MB-405     | -5.82          |      | > -4.00        |     | > -4.00        |      |
| SK-MEL-2       | -5.60          |      | > -4.00        |     | > -4.00        |      |
| SK-MEL-28      | -5.33          |      | > -4.00        |     | > -4.00        |      |
| SK-MEL-56      | -5.54          |      | > -4.00        |     | > -4.00        |      |
| UACC-257       | -4.91          |      | > -4.00        |     | > -4.00        |      |
| UACC-62        | -5.65          |      | > -4.00        |     | > -4.00        |      |
| **Ovarian Cancer** |          |      |                |     |                |      |
| IGROV1         | -5.47          |      | > -4.00        |     | > -4.00        |      |
| OVCAR-3        | -5.87          |      | > -4.00        |     | > -4.00        |      |
| OVCAR-4        | -6.03          |      | > -4.00        |     | > -4.00        |      |
| OVCAR-5        | -5.39          |      | > -4.00        |     | > -4.00        |      |
| OVCAR-6        | -5.27          |      | > -4.00        |     | > -4.00        |      |
| NCI-ADR-RES    | -2.19          |      | > -4.00        |     | > -4.00        |      |
| SK-OV-3        | -4.93          |      | > -4.00        |     | > -4.00        |      |
| **Prostate Cancer** |          |      |                |     |                |      |
| PC-3           | -5.74          |      | > -4.00        |     | > -4.00        |      |
| DU-145         | -5.41          |      | > -4.00        |     | > -4.00        |      |
| **Reat Cancer** |                |      |                |     |                |      |
| 766-3          | -5.23          |      | > -4.00        |     | > -4.00        |      |
| A498           | -5.86          |      | > -4.00        |     | > -4.00        |      |
| ACHN           | -5.45          |      | > -4.00        |     | > -4.00        |      |
| RXF 393        | -5.66          |      | > -4.00        |     | > -4.00        |      |
| SN12C          | -5.51          |      | > -4.00        |     | > -4.00        |      |
| TK-10          | -4.96          |      | > -4.00        |     | > -4.00        |      |
| UO-31          | -5.64          |      | > -4.00        |     | > -4.00        |      |
| **Prostate Cancer** |          |      |                |     |                |      |
| PC-3           | -5.74          |      | > -4.00        |     | > -4.00        |      |
| DU-145         | -5.41          |      | > -4.00        |     | > -4.00        |      |
| **Breast Cancer** |            |      |                |     |                |      |
| MCF7           | -5.81          |      | > -4.00        |     | > -4.00        |      |
| MDA-MB-231/ATCC | -5.74         |      | > -4.00        |     | > -4.00        |      |
| HS 578T        | -4.95          |      | > -4.00        |     | > -4.00        |      |
| BT-549         | -5.75          |      | > -4.00        |     | > -4.00        |      |
| I-417D         | -6.34          |      | > -4.00        |     | > -4.00        |      |
| MDA-MB-468     | -5.77          |      | > -4.00        |     | > -4.00        |      |

**MID**
-5.59

**Delta**
-4.69

**Range**
1.47
Dose Response Curves

Log_{10} of Sample Concentration (Molar)

Percentage Growth

All Cell Lines

S795311
Biological Evaluations

**In vitro anti-proliferative activity assay**

The three examined cancer cell lines (non-small cell lung A-549, Breast MDA-MB-231 and colon HCT-116 cancer cells) were obtained from American Type Culture Collection (ATCC). The cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37 °C in humidified atmosphere containing 5% CO₂. Cells at a concentration of 0.50 x 10⁶ were grown in a 25 cm² flask in 5 ml of culture medium.

The anti-proliferative activity of the tested compounds was measured in vitro using the Sulfo-Rhodamine-B stain (SRB) assay. Briefly, Cells were inoculated in 96-well microtiter plate (5X10⁴ cells/ well) for 24 h before treatment with the tested compounds to allow attachment of cell to the wall of the plate. Tested compounds were dissolved in DMSO at 1 mg/ml immediately before use and diluted to the appropriate volume just before addition to the cell culture. Different concentrations of tested compounds, doxorubicin and sorafenib were added to the cells (three wells were prepared for each individual dose). Cells were incubated with the compounds for 48 h at 37°C and in atmosphere of 5% CO₂. After 48 h cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between percent of surviving fraction and drug concentration is plotted to get the survival curve for each cell line. The concentration required for 50% inhibition of cell viability (IC₅₀) was calculated and the results are given in Table 1. The results were compared to the effect of the reference drug doxorubicin.

**NCI-in vitro antitumor activity towards 59 cancer cell lines**

The cytotoxicity assays were performed at National Cancer Institute (NCI), Bethesda, USA (against 59 cell lines). The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 µ at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO₂, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two
plates of each cell line were fixed in situ with trichloroacetic acid (TCA), to represent a measurement of the cell population for each cell line at the time of drug addition ($T_z$). Experimental drugs were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations. Triplicate wells were prepared for each individual dose. Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO₂, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero ($T_z$), control growth ($C$), and test growth in the presence of drug at the five concentration levels ($T_i$)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as:

\[
\frac{(T_i - T_z)}{(C - T_z)} \times 100 \text{ for concentrations for which } T_i \geq T_z
\]

\[
\frac{(T_i - T_z)}{T_z} \times 100 \text{ for concentrations for which } T_i < T_z
\]

Three dose response parameters were calculated for each experimental compound: Growth inhibition of 50% (GI₅₀) was calculated when \([\frac{(T_i - T_z)}{(C - T_z)}] \times 100 = 50\). The compound concentration resulting in total growth inhibition (TGI) was calculated when $T_i = T_z$. The LC₅₀
indicating a net loss of cells following treatment was calculated when \( \left( \frac{T_i - T_z}{T_z} \right) \times 100 = -50 \).

### 4.2.4. Cell Cycle Analysis

T-47D cells were treated with conjugate 8a for 24 h (at its IC\textsubscript{50} concentration), and then cells were washed twice with ice-cold phosphate buffered saline (PBS). Subsequently, the treated cells were collected by centrifugation, fixed in ice-cold 70% (v/v) ethanol, washed with PBS, re-suspended with 100 μg/mL RNase, stained with 40 μg/mL PI, and analyzed by flow cytometry using FACS Calibur (Becton Dickinson, BD, Franklin Lakes, NJ, USA). The cell cycle distributions were calculated using CellQuest software 5.1 (Becton Dickinson).

#### Annexin V-FITC Apoptosis Assay

Phosphatidylserine externalization was assayed using Annexin V-FITC/PI apoptosis detection kit (BD Biosciences, San Jose, CA, USA) according to the manufacturer’s instructions. T-47D cells were cultured to a monolayer then treated with conjugate 8a at its IC\textsubscript{50} concentration. Cells were then harvested via trypsinization, and rinsed twice in PBS followed by binding buffer. Moreover, cells were re-suspended in 100 μL of binding buffer with the addition of 1 μL of FITC-Annexin V (Becton Dickinson BD Pharmingen\textsuperscript{TM}, Heidelberg, Germany) followed by an incubation period of 30 min at 4 °C. Cells were then rinsed in binding buffer and resuspended in 150 μL of binding buffer with the addition of 1 μL of DAPI (1 μg/μL in PBS) (Invitrogen, Life Technologies, Darmstadt, Germany). Cells were then analyzed using the flow cytometer BD FACS Canto II (BD Biosciences, San Jose, CA, USA) and the results were interpreted with FlowJo7.6.4 software (Tree Star, Ashland, OR, USA).

#### CDK2 inhibition assay

The kinase was assayed with histone H1 in the presence of 15 μM ATP, 0.05 μCi [γ\textsuperscript{33}P]ATP and of the test compound in a final volume of 10 μL, all in a reaction buffer (60 mM HEPES-NaOH, pH 7.5, 3 mM MgCl\textsubscript{2}, 3 mM MnCl\textsubscript{2}, 3 μM Na-orthovanadate, 1.2 mM DTT, 2.5 μg / 50 μl PEG\textsubscript{20000}). The reactions were stopped by adding 5 μL of 3 % aq. H\textsubscript{3}PO\textsubscript{4}. Aliquots were spotted onto P-81 phosphocellulose (Whatman), washed 3× with 0.5 % aq. H\textsubscript{3}PO\textsubscript{4} and finally air-dried. Kinase inhibition was quantified using a FLA-7000 digital image analyzer (Fujifilm).
5.1.2.1. 1-Ethyl-3-(hydrazonomethyl)-1H-indole (5a)

Yield 64%, MP: 139-140 °C; \(^1\)H NMR \(\delta\) ppm: 1.32, 1.38 (t, 3H, \(J = 8\) Hz, \(\text{N}-\text{CH}_2\text{-CH}_3\)), 4.12, 4.22 (t, 2H, \(J = 8\) Hz, \(\text{N}-\text{CH}_2\text{-CH}_3\)), 6.24 (brs, 2H, \(\text{NH}_2\)), 7.23-7.31 (m, 2H, H-5 and H-6 of indol), 7.54 (d, 1H, \(J = 8\) Hz, H-7 of indol), 7.94 (s, 1H, H-2 of indol), 8.41 (d, 1H, \(J = 8\) Hz, H-4 of indol), 8.91 (s, 1H, -CH=N-); IR: 3378 (NH\(_2\)); Anal. calcd. For C\(_{11}\)H\(_{13}\)N\(_3\): C, 70.56; H, 7.00; N, 22.44; Found C, 70.32; H, 6.97; N, 22.61.

5.1.2.2. 3-(Hydrazonomethyl)-1-isopropyl-1H-indole (5c)

Yield 57%, MP: 186-188°C; \(^1\)H NMR \(\delta\) ppm: 1.51 (d, 6H, -CH(CH\(_3\))\(_2\)), 4.77-4.85 (m, 1H, \(\text{N}-\text{CH}\)), 7.06 (brs, 2H, \(\text{NH}_2\)), 7.20-7.30 (m, 2H, H-5 and H-6 of indol), 7.60 (d, 1H, \(J = 8\) Hz, H-7 of indol), 8.07 (s, 1H, H-2 of indol), 8.36 (d, 1H, \(J = 8\) Hz, H-4 of indol), 8.89 (s, 1H, -CH=N-); \(^13\)C NMR \(\delta\) ppm: 22.82 (-CH(CH\(_3\))\(_2\)), 47.55 (N-CH), 110.95, 112.02, 121.30, 122.76, 123.07, 125.80, 131.21, 137.08, 155.13; IR: 3342 (NH\(_2\)); Anal. calcd. For C\(_{12}\)H\(_{15}\)N\(_3\): C, 71.61; H, 7.51; N, 20.88; Found C, 71.87; H, 7.50; N, 20.79.

5.1.2.3. 1-Allyl-3-(hydrazonomethyl)-1H-indole (5d)

Yield 73%, MP: 177-180°C; \(^1\)H NMR \(\delta\) ppm: 4.90 (d, 2H, \(J = 8\) Hz, \(\text{N}-\text{CH}_2\text{-CH}_3\)), 5.10-5.11 (m, 1H, olefinic H), 5.20-5.24 (m, 1H, olefinic H), 6.01-6.11 (m, 1H, olefinic H), 7.06 (brs, 2H, \(\text{NH}_2\)), 7.20-7.30 (m, 2H, Ar-H), 7.52-7.59 (m, 1H, Ar-H), 7.92-7.96 (m, 1H, Ar-H), 8.37-8.40 (m, 1H, Ar-H), 8.89-8.91 (m, 1H, Ar-H); \(^13\)C NMR \(\delta\) ppm: 47.90, 48.86 (N-CH\(_2\)), 110.57, 110.93, 111.14, 112.03, 112.05, 117.81, 120.21, 121.23, 121.38, 122.82, 123.15, 123.25, 125.75, 125.81, 134.29, 134.64, 134.87, 134.95, 137.55, 137.59, 155.05, 155.20; IR: 3362 (NH\(_2\)); HRMS (ESI) for C\(_{12}\)H\(_{14}\)N\(_3\), calcd 200.11822, found 200.11849 [M+H]\(^+\); Anal. calcd. For C\(_{12}\)H\(_{13}\)N\(_3\): C, 72.33; H, 6.58; N, 21.09; Found C, 72.20; H, 6.55; N, 21.15.

5.1.3.1. 5-Chloro-3-(((1-ethyl-1H-indol-3-yl)methylene)hydrazono)indolin-2-one (7)

Yield 78%, MP: 202-203 °C; \(^1\)H NMR \(\delta\) ppm: 1.44 (t, 3H, \(J = 8\) Hz, -CH\(_2\text{-CH}_3\)), 4.33 (q, 2H, \(J = 8\) Hz, CH\(_2\)), 6.93-6.96 (m, 1H, Ar-H), 7.34-7.39 (m, 1H, Ar-H), 7.45-7.55 (m, 2H, Ar-H), 7.71 (d, 1H, \(J = 8\) Hz, Ar-H), 8.30-8.35 (m, 2H, Ar-H), 8.50 (s, 1H, Ar-H), 9.00 (s, 1H, Ar-H), 10.93 (s, 1H, NH); \(^13\)C NMR \(\delta\) ppm: 15.54 (CH\(_3\)), 41.77 (CH\(_2\)), 111.88, 111.91, 112.52, 113.09,
117.58, 118.73, 121.88, 122.66, 124.19, 125.44, 126.07, 126.54, 127.57, 128.44, 132.42, 134.41, 137.82, 139.63, 143.26, 144.73, 146.28, 148.80, 163.37 (C=O); IR: 3401 (NH), 1698 (C=O); Anal. calcd. For C_{19}H_{15}ClN_{4}O: C, 65.05; H, 4.31; N, 15.97; Found C, 65.18; H, 4.30; N, 16.05.

5.1.3.2. 5-Methyl-3-(((1-propyl-1H-indol-3-yl)methylene)hydrazono)indolin-2-one (8a)

Yield 74 %, MP: 195-197 °C; \(^1\)H NMR \(\delta \text{ ppm}: 0.87 \text{ (t, 3H, } J = 8 \text{ Hz, } -\text{CH}_2\text{CH}_3, \) 1.79-189 (m, 2H, -CH\(_2\)CH\(_3\)), 2.20 (s, 3H, CH\(_3\) of isatin), 4.22 (t, 2H, \(J = 8 \) Hz, N-CH\(_2\)), 6.80 (m, 1H, Ar-H), 7.19 (t, 1H, \(J = 8 \) Hz, Ar-H), 7.33 (d, 2H, \(J = 8 \) Hz, Ar-H), 7.67 (d, 1H, \(J = 8 \) Hz, Ar-H), 8.26 (s, 1H, Ar-H), 8.34 (s, 1H, Ar-H), 8.40 (d, 1H, \(J = 8 \) Hz, Ar-H), 8.95 (s, 1H, Ar-H), 10.66 (s, 1H, NH); IR: 3375 (NH), 1701 (C=O); Anal. calcd. For C\(_{21}\)H\(_{20}\)N\(_4\)O: C, 73.23; H, 5.85; N, 16.27; Found C, 72.97; H, 5.88; N, 16.32.

5.1.3.3. 3-(((1-propyl-1H-indol-3-yl)methylene)hydrazono)-5-(trifluoromethoxy)indolin-2-one (8b)

Yield 77 %, MP: 189-190 °C; \(^1\)H NMR \(\delta \text{ ppm}: 0.89 \text{ (t, 3H, } J = 8 \text{ Hz, } -\text{CH}_2\text{CH}_3, \) 1.83-192 (m, 2H, -CH\(_2\)CH\(_3\)), 4.28 (t, 2H, \(J = 8 \) Hz, N-CH\(_2\)), 7.01-7.05 (m, 1H, Ar-H), 7.25 (t, 1H, \(J = 8 \) Hz, Ar-H), 7.38 (t, 1H, \(J = 8 \) Hz, Ar-H), 7.48 (t, 1H, \(J = 8 \) Hz, Ar-H), 7.72 (d, 1H, \(J = 8 \) Hz, Ar-H), 8.27 (d, 1H, \(J = 8 \) Hz, Ar-H), 8.34 (s, 1H, Ar-H), 8.44-4.46 (m, 1H, Ar-H), 9.03 (s, 1H, Ar-H), 10.96 (s, 1H, NH); \(^{13}\)C NMR \(\delta \text{ ppm}: 11.51 \text{ (-CH}_3), 23.23 \text{ (CH}_3\text{-CH}_2), 48.37 \text{ (N-CH}_2), 111.78, 114.15, 116.06, 119.48, 120.68, 122.40, 123.91, 125.35, 126.26, 127.78, 128.72, 131.61, 133.46, 133.98, 136.11, 140.60, 143.58, 163.98 (C=O); IR: 3391 (NH), 1699 (C=O); HRMS (ESI) for C\(_{21}\)H\(_{18}\)F\(_3\)N\(_4\)O\(_2\), calcd 415.13764, found 415.13783 [M+H]\(^+\); Anal. calcd. For C\(_{21}\)H\(_{17}\)F\(_3\)N\(_4\)O\(_2\): C, 60.87; H, 4.14; N, 13.52; Found C, 60.69; H, 4.11; N, 13.50.

5.1.3.4. 5-Nitro-3-(((1-propyl-1H-indol-3-yl)methylene)hydrazono)indolin-2-one (8c)

Yield 83 %, MP: 247-250°C; \(^1\)H NMR \(\delta \text{ ppm}: 0.86-92 \text{ (m, 3H, } -\text{CH}_2\text{CH}_3, \) 1.78-191 (m, 2H, -CH\(_2\)CH\(_3\)), 4.18,426 (t, 2H, \(J = 8 \) Hz, N-CH\(_2\)), 7.07 (d, 0.5H, \(J = 8 \) Hz, Ar-H), 7.19-7.29 (m, 1.5H, Ar-H), 7.39 (d, 1H, \(J = 8 \) Hz, Ar-H), 7.56, 7.70 (d, 1H, \(J = 8 \) Hz, Ar-H), 7.94 (s, 1H, Ar-H), 8.29-8.47 (m, 2H, Ar-H), 8.87 (s, 1H, Ar-H), 9.06, 9.36 (s, 1H, Ar-H), 11.45, 11.65 (s, 1H, NH); IR: 3396 (NH), 1710 (C=O); Anal. calcd. For C\(_{20}\)H\(_{17}\)N\(_5\)O\(_3\): C, 63.99; H, 4.56; N, 18.66; Found C, 64.21; H, 4.54; N, 18.60.
5.1.3.5. 3-((1-Isopropyl-1H-indol-3-yl)methylene)hydrazono)indolin-2-one (9)

Yield 78 %, MP: 260-261°C; $^1$H NMR $\delta$ ppm: 1.53 (brs, 6H, -CH(CH$_3$)$_2$), 4.85 (brs, 1H, N-CH), 6.87-6.97 (m, 2H, Ar-H), 7.07-7.23 (m, 2H, Ar-H), 7.30-7.46 (m, 2H, Ar-H), 7.70-7.95 (m, 1H, Ar-H), 7.36-7.46 (m, 1H, Ar-H), 8.74-8.94 (m, 1H, Ar-H), 9.55 (s, 1H, Ar-H), 10.55 (s, 1H, NH); IR: 3391 (NH), 1700 (C=O); Anal. calcd. For C$_{20}$H$_{18}$N$_4$O: C, 72.71; H, 5.49; N, 16.96; Found C, 72.60; H, 5.48; N, 17.02.

5.1.3.6. 3-(((1-Allyl-1H-indol-3-yl)methylene)hydrazono)indolin-2-one (10a)

Yield 80 %, MP: 220-222 °C; $^1$H NMR $\delta$ ppm: 4.97 (d, 2H, $J = 8$ Hz, N-CH$_2$), 5.15-5.19 (m, 1H, Ar-H), 5.24-5.27 (m, 1H, Ar-H), 6.04-6.13 (m, 1H, Ar-H), 6.77 (d, 0.5H, $J = 8$ Hz, Ar-H), 6.92 (d, 1H, $J = 8$ Hz, Ar-H), 7.07 (t, 1H, $J = 8$ Hz, Ar-H), 7.36-7.38 (m, 2H, Ar-H), 7.62-7.64 (m, 1H, Ar-H), 7.68 (d, 0.5H, $J = 8$ Hz, Ar-H), 8.26 (s, 1H, Ar-H), 8.31-8.35 (m, 1H, Ar-H), 8.37 (d, 1H, $J = 8$ Hz, Ar-H), 8.96 (s, 1H, Ar-H), 10.78 (s, 1H, NH); IR: 3410 (NH), 1701 (C=O); Anal. calcd. For C$_{20}$H$_{18}$N$_4$O: C, 73.15; H, 4.91; N, 17.06; Found C, 73.27; H, 4.95; N, 16.95.

5.1.3.7. 3-(((1-Allyl-1H-indol-3-yl)methylene)hydrazono)-5-fluoroindolin-2-one (10b)

Yield 77 %, MP: 235-237 °C; $^1$H NMR $\delta$ ppm: 4.99-5.03 (m, 2H, N-CH$_2$), 5.15-5.27 (m, 2H, Ar-H), 6.05-6.12 (m, 1H, Ar-H), 6.91-6.94 (m, 1H, Ar-H), 7.31-7.36 (m, 3H, Ar-H), 8.15-8.19 (m, 1H, Ar-H), 8.28-8.34 (m, 2H, Ar-H), 8.98-9.03 (m, 2H, Ar-H), 10.91 (s, 1H, NH); $^{13}$C NMR $\delta$ ppm: 47.79 (CH$_2$), 112.58, 114.55, 115.61, 115.87, 116.79, 117.71, 118.39, 121.33, 121.56, 122.76, 124.16, 125.52, 127.86, 130.11, 133.85, 142.32, 146.46, 159.21, 164.01 (C=O); IR: 3394 (NH), 1697 (C=O); HRMS (ESI) for C$_{20}$H$_{15}$FNO, calcld 347.13027, found 347.13019 [M+H]$^+$; Anal. calcd. For C$_{20}$H$_{15}$FNO: C, 69.35; H, 4.37; N, 16.18; Found C, 69.51; H, 4.39, N, 16.25.

5.1.3.8. 3-(((1-Allyl-1H-indol-3-yl)methylene)hydrazono)-7-fluoroindolin-2-one (10c)

Yield 82 %, MP: 208-210 °C; $^1$H NMR $\delta$ ppm: 4.98 (d, 2H, $J = 8$ Hz, N-CH$_2$), 5.15-5.21(m, 1H, Ar-H), 5.24-5.28 (m, 1H, Ar-H), 6.04-6.14 (m, 1H, Ar-H), 7.03-7.09 (m, 2H, Ar-H), 7.37-7.40 (m, 5H, Ar-H), 8.30 (s, 1H, Ar-H), 8.99 (s, 1H, Ar-H), 11.55 (s, 1H, NH); $^{13}$C NMR $\delta$ ppm: 49.52 (CH$_2$), 112.04, 118.39, 118.79, 121.68, 121.86, 121.95, 122.90, 123.85, 123.91, 124.08, 124.90, 130.38, 132.63, 132.77, 133.84, 137.99, 141.29, 144.75, 163.58 (C=O); IR: 3380 (NH),
1702 (C=O); Anal. calcd. For C_{20}H_{15}FN_{4}O: C, 69.35; H, 4.37; N, 16.18; Found C, 69.21; H, 4.32; N, 16.15.

5.1.3.9. 3-(((1-Allyl-1H-indol-3-yl)methylene)hydrazono)-5-chloroindolin-2-one (10d)

Yield 75 %, MP: 267-269 °C; \(^1^H\) NMR \(\delta \ \text{ppm} \): 4.99 (d, 2H, \(J = 8\) Hz, N-CH\(_2\)), 5.16-5.22 (m, 1H, Ar-H), 5.25-5.28 (m, 1H, Ar-H), 6.05-6.14 (m, 1H, Ar-H), 6.94 (d, 2H, \(J = 8\) Hz, Ar-H), 7.33-7.39 (m, 1H, Ar-H), 7.45-7.55 (m, 2H, Ar-H), 7.66-7.69 (m, 1H, Ar-H), 8.30(s, 1H, Ar-H), 8.50-8.53 (m, 1H, Ar-H), 9.02 (s, 1H, Ar-H), 10.91 (s, 1H, NH); \(^{13}\)C NMR \(\delta \ \text{ppm} \): 49.26 (CH\(_2\)), 112.11, 112.53, 113.12, 117.59, 118.45, 121.86, 122.7, 124.24, 126.1, 127.62, 128.44, 131.35, 132.5, 133.8, 137.73, 140.02, 142.13, 143.35, 163.17 (C=O); IR: 3401 (NH), 1699 (C=O); HRMS (ESI) for C_{20}H_{16}ClN_{4}O, calcd 363.10072, found 363.10054 [M+H]\(^+\); Anal. calcd. For C_{20}H_{15}ClN_{4}O: C, 66.21; H, 4.17; N, 15.44; Found C, 66.34; H, 4.13; N, 15.39.

5.1.3.10. 3-(((1-Allyl-1H-indol-3-yl)methylene)hydrazono)-5-(trifluoromethoxy)indolin-2-one (10e)

Yield 80 %, MP: 228-229 °C; \(^1^H\) NMR \(\delta \ \text{ppm} \): 4.98 (d, 2H, \(J = 8\) Hz, N-CH\(_2\)), 5.17-5.22 (m, 1H, Ar-H), 5.25-5.28 (m, 1H, Ar-H), 6.04-6.14 (m, 1H, Ar-H), 7.0-7.03 (m, 2H, Ar-H), 7.25-7.29 (m, 1H, Ar-H), 7.37-7.44 (m, 2H, Ar-H), 7.47-7.53 (m, 1H, Ar-H), 7.65-7.67 (m, 1H, Ar-H), 8.31(s, 1H, Ar-H), 9.05 (s, 1H, Ar-H), 10.98 (s, 1H, NH); \(^{13}\)C NMR (DMSO-\(d_6\)) \(\delta \ \text{ppm} \): 49.27 (CH\(_2\)), 112.10, 112.72, 116.96, 118.02, 118.47, 120.73, 121.89, 122.47, 124.3, 126.29, 127.95, 133.76, 138.12, 140.38, 143.27, 143.64, 145.07, 146.94, 148.95, 163.91 (C=O); IR: 3398 (NH), 1711 (C=O); HRMS (ESI) for C_{21}H_{16}F_{3}N_{4}O_{2}, calcd 413.12199, found 413.12260 [M+H]\(^+\); Anal. calcd. For C_{21}H_{15}F_{3}N_{4}O_{2}: C, 61.17; H, 3.67; N, 13.59; Found C, 61.01; H, 3.63; N, 13.55.