Supporting Information

Design and Synthesis of Benzimidazole-Chalcone Derivatives as Potential Anticancer Agents

Cheng-Ying Hsieh 1, Pi-Wen Ko 2, Yu-Jui Chang 1, Mohit Kapoor 3, Yu-Chuan Liang 4, Hsueh-Liang Chu 5, Hui-Hsien Lin 6, Jia-Cherng Horng 1,* and Ming-Hua Hsu 7,*

1 Department of Chemistry, National Tsing Hua University, Hsinchu 30013, Taiwan; futariwhisper@gmail.com (C.-Y.H.); rick5569268@gmail.com (Y.-J.C.)
2 Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Hsinchu 30013, Taiwan; koko37bebe@hotmail.com (P.-W.K.)
3 Chitkara University Institute of Engineering and Technology, Chitkara University, Punjab 140 401, India; mohitkapoor.chemistry@gmail.com (M.K.)
4 Agricultural Biotechnology Research Center, Academia Sinica, Taipei 11529, Taiwan; ycliang@sinica.edu.tw (Y.-C.L.)
5 Graduate Institute of Translational Medicine, College of Medicine and Technology, Taipei Medical University, Taipei 11031, Taiwan; szxchu@gmail.com (H.-L.C.)
6 Division of Radiotherapy, Department of Oncology, Taipei Veterans General Hospital, Taipei 11217, Taiwan; twwarcgogo@gmail.com (H.-H.L.)
7 Department of Chemistry, National Changhua University of Education, Changhua 50007, Taiwan
* Correspondence: jchorng@mx.nthu.edu.tw (J.-C.H.); minghuahsu@cc.ncue.edu.tw (M.-H.H.); Tel.: +886-3-5715131 (ext. 35635) (J.-C.H.); +886-4-7232105 (ext. 3547) (M.-H.H.) Fax: +886-4-7211190 (M.-H.H.)

These authors contributed equally to this work.

Contents

(1) Characterization Data:
  1H-NMR, 13C-NMR, IR, HPLC

(2) Flow Cytometry Data
(1) Characterization Data

$^1$H-NMR spectrum of compound 19d
$^{13}$C-NMR spectrum of compound 19d

IR spectrum of compound 19d
HPLC spectrum of compound 19d

| Peak | RetTime [min] | Width [min] | Area mAU *s | Area % |
|------|---------------|-------------|-------------|-------|
| 1    | 8.05          | 0.2178      | 56.94883    | 0.5562|
| 2    | 8.235         | 0.2043      | 36.42011    | 0.3557|
| 3    | 14.017        | 0.2913      | 94.06382    | 0.9187|
| 4    | 16.825        | 0.3131      | 10051.4     | 98.169|

1H-NMR spectrum of compound 20a
\textbf{13C-NMR spectrum of compound 20a}

\textbf{IR spectrum of compound 20a}
HPLC spectrum of compound 20a

| Peak | RetTime [min] | Width [min] | Area mAU *s | Area % |
|------|---------------|-------------|-------------|--------|
| 1    | 11.453        | 0.3000      | 71.80160    | 3.8726 |
| 2    | 19.691        | 0.6477      | 1782.26953  | 96.1274|

\(^1\)H-NMR spectrum of compound 20b
$^{13}$C-NMR spectrum of compound 20b

IR spectrum of compound 20b
### HPLC spectrum of compound 20b

| Peak | RetTime [min] | Width [min] | Area mAU *s | Area % |
|------|---------------|-------------|-------------|--------|
| 1    | 5.323         | 0.0522      | 36.09950    | 0.2756 |
| 2    | 9.270         | 0.1684      | 180.59157   | 1.3785 |
| 3    | 9.783         | 0.1675      | 287.45895   | 2.1943 |
| 4    | 22.407        | 1.3899      | 12596.0     | 96.1516|

### 'H-NMR spectrum of compound 20c
$^{13}$C-NMR spectrum of compound 20c

IR spectrum of compound 20c
HPLC spectrum of compound 20c

| Peak | RetTime [min] | Width [min] | Area mAU *s | Area % |
|------|---------------|-------------|-------------|-------|
| 1    | 18.052        | 0.8788      | 16437.4     | 95.0057 |
| 2    | 21.588        | 0.9133      | 864.08667   | 4.9943  |

$^1$H-NMR spectrum of compound 20d
$^{13}$C-NMR spectrum of compound 20d

IR spectrum of compound 20d
### HPLC spectrum of compound 20d

| Peak | RetTime [min] | Width [min] | Area mAU *s | Area % |
|------|---------------|-------------|-------------|--------|
| 1    | 12.129        | 0.4295      | 491.38254   | 3.5048 |
| 2    | 22.660        | 1.0394      | 13528.9     | 96.4953|

### H-NMR spectrum of compound 21a
$^{13}$C-NMR spectrum of compound 21a

IR spectrum of compound 21a
### HPLC spectrum of compound 21a

| Peak # | RetTime [min] | Width [min] | Area mAU *s | Area % |
|-------|---------------|-------------|-------------|-------|
| 1     | 18.471        | 0.4193      | 3837.38745  | 95.0169 |
| 2     | 21.337        | 0.9008      | 201.24811   | 4.9831 |

### 'H-NMR spectrum of compound 21b
$^{13}$C-NMR spectrum of compound 21b

IR spectrum of compound 21b
| Peak | RetTime [min] | Width [min] | Area   | Area % |
|------|--------------|-------------|--------|--------|
| 1    | 4.951        | 0.0699      | 68.29758 | 1.0947 |
| 2    | 7.661        | 0.1966      | 38.23409 | 0.6129 |
| 3    | 10.939       | 0.1666      | 64.38687 | 1.0320 |
| 4    | 11.152       | 0.2067      | 91.49991 | 1.4666 |
| 5    | 27.933       | 0.6205      | 5976.29102 | 95.7937 |

^H-NMR spectrum of compound 21c
$^{13}$C-NMR spectrum of compound 21c

IR spectrum of compound 21c
HPLC spectrum of compound 21c

| Peak | RetTime [min] | Width [min] | Area mAU *s | Area % |
|------|---------------|-------------|-------------|--------|
| 1    | 5.150         | 0.0654      | 120.31086   | 0.6396 |
| 2    | 8.468         | 0.1586      | 143.72900   | 0.7641 |
| 3    | 8.756         | 0.1657      | 182.23738   | 0.9688 |
| 4    | 9.117         | 0.2214      | 113.33360   | 0.6025 |
| 5    | 9.815         | 0.2669      | 299.16333   | 1.5904 |
| 6    | 18.698        | 0.4951      | 17951.6     | 95.4346 |
$^1$H-NMR spectrum of compound 21d

$^{13}$C-NMR spectrum of compound 21d
IR spectrum of compound 21d

HPLC spectrum of compound 21d

| Peak | RetTime [min] | Width [min] | Area mAU *s | Area % |
|------|---------------|-------------|-------------|-------|
| 1    | 11.298        | 0.3362      | 439.36765   | 2.4527|
| 2    | 22.672        | 0.5824      | 17474.1     | 97.5473|
$^1$H-NMR spectrum of compound 22a

$^1$C-NMR spectrum of compound 22a
IR spectrum of compound 22a

HPLC spectrum of compound 22a

| Peak # | RetTime [min] | Width [min] | Area mAU  *s | Area % |
|-------|--------------|-------------|--------------|--------|
| 1     | 13.081       | 0.263       | 131.5152     | 3.5487 |
| 2     | 15.72        | 0.2962      | 3574.548     | 96.4513 |
$^1$H-NMR spectrum of compound 22b

$^{13}$C-NMR spectrum of compound 22b
| Peak # | RetTime [min] | Width [min] | Area mAU *s | Area % |
|-------|---------------|-------------|-------------|--------|
| 1     | 11.128        | 0.2204      | 51.86035    | 18.558 |
| 2     | 11.419        | 0.2363      | 68.21777    | 2.4412 |
| 3     | 12.963        | 0.2307      | 2674.29761  | 95.7029|
$^1$H-NMR spectrum of compound 22c

$^{13}$C-NMR spectrum of compound 22c
IR spectrum of compound 22c

HPLC spectrum of compound 22c

| Peak | RetTime [min] | Width [min] | Area mAU *s | Area % |
|------|--------------|-------------|-------------|--------|
| 1    | 6.949        | 0.1349      | 25.79159    | 0.5232 |
| 2    | 7.162        | 0.1196      | 32.28178    | 0.6549 |
| 3    | 7.25         | 0.1134      | 27.91112    | 0.5662 |
| 4    | 9.808        | 0.1719      | 153.38657   | 3.1118 |
| 5    | 10.166       | 0.1891      | 4689.81201  | 95.1438 |
$^1$H-NMR spectrum of compound 22d

$^{13}$C-NMR spectrum of compound 22d
IR spectrum of compound 22d

HPLC spectrum of compound 22d

| Peak | RetTime [min] | Width [min] | Area    mAU *s | Area %  |
|------|--------------|-------------|--------------|--------|
| 1    | 7.409        | 0.1596      | 80.65369     | 1.5400 |
| 2    | 7.797        | 0.1728      | 40.02195     | 0.7642 |
| 3    | 8.326        | 0.1800      | 26.33945     | 0.5029 |
| 4    | 11.771       | 0.2311      | 50.84013     | 0.9708 |
| 5    | 13.598       | 0.2397      | 5039.33447   | 96.2221 |
\textbf{H-NMR spectrum of compound 23a}

\textbf{\textsuperscript{13}C-NMR spectrum of compound 23a}
IR spectrum of compound 23a

HPLC spectrum of compound 23a

| Peak | RetTime [min] | Width [min] | Area mAU *s | Area % |
|------|---------------|-------------|-------------|--------|
| 1    | 10.908        | 0.1868      | 105.0548    | 2.6781 |
| 2    | 12.627        | 0.2189      | 3817.72192  | 97.3219|
$^1$H-NMR spectrum of compound 23b

$^{13}$C-NMR spectrum of compound 23b
IR spectrum of compound 23b

HPLC spectrum of compound 23b

| Peak # | RetTime [min] | Width [min] | Area mAU *s | Area % |
|-------|---------------|-------------|-------------|--------|
| 1     | 12.806        | 0.2203      | 278.77322   | 2.0574 |
| 2     | 14.789        | 0.2576      | 13271.3     | 97.9426|
$^1$H-NMR spectrum of compound 23c

$^{13}$C-NMR spectrum of compound 23c
IR spectrum of compound 23c

HPLC spectrum of compound 23c

| Peak | RetTime [min] | Width [min] | Area mAU *s | Area % |
|------|--------------|-------------|-------------|-------|
| 1    | 11.105       | 0.1937      | 513.34729   | 3.631 |
| 2    | 11.619       | 0.2083      | 13623.2     | 96.369 |
$^1$H-NMR spectrum of compound 23d

$^{13}$C-NMR spectrum of compound 23d
IR spectrum of compound 23d

HPLC spectrum of compound 23d

| Peak | RetTime [min] | Width [min] | Area mAU *s | Area % |
|------|---------------|-------------|-------------|--------|
| 1    | 7.621         | 0.1547      | 187.74409   | 2.1252 |
| 2    | 7.905         | 0.1755      | 161.00226   | 1.8225 |
| 3    | 16.250        | 0.2775      | 8485.20020  | 96.052 |
Figure S1. DNA histograms of exponentially growing OVCAR-3 cells with different treatments: (a) cell only; (b) compound 20a; (c) compound 21a; (d) compound 22a; (e) compound 23a.