Novel Cytotoxic Oxopyridoindolizines: *iso*-Propyl-7,8,9-trichloro-6,7,8,9-tetrahydro-5-oxopyrido[2,3-a]-indolizine-10-carboxylates (OPIC)

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Abstract: A series of eight new alkyl-7,8,9-trichloro-6,7,8,9-tetrahydro-5-oxopyrido[2,3-a]-indolizine-10-carboxylates (OPIC), analogues of camptothecin (CPT), were prepared in a one-pot reaction of 2,2'-bipyridine-3,3'-dicarboxylic acid (BPA) with a mixture of thionyl chloride/chlorine, followed by addition of the appropriate alcohol. This led to a mixture of OPIC compounds 3a-d, 4a-d and 3,3'-dialkoxyxycarbonyl-2,2'-bipyridines (BPE, 2a-d). The isopropyl OPIC 3c and its corresponding diastereoisomer 4c showed marked activity against three cancer cell lines compared to other analogs. These same diastereoisomers also displayed high cytotoxic activity against five leukemia cell lines, thus the presence of an isopropyl substituent on the carboxylic ester, as opposed to other alkyl substituents, appears to play a key role in the cytotoxic potency of this new class of compounds.
Keywords: Oxopyridopyndolizine carboxylate (OPIC), Cycloacylation, Camptothecin, Cancer, Chlorination, N-ligands.

Introduction

The natural alkaloid camptothecin (CPT, Scheme 1), extracted from Camptotheca acuminata by Wall and Wani [1], has demonstrated efficacy in the treatment of several cancers. However, interest in its application as an anti-tumour agent has declined due to its toxic secondary effects. The cellular target of the alkaloid has been shown to be topoisomerase I [2], an enzyme essential for religation of DNA during a number of critical cellular processes, including replication, transcription and repair [3]. Camptothecin, topoisomerase I and DNA form a so-called "cleavable complex" [4] that results in topoisomerase I-mediated DNA breaks by preventing DNA religation. These results have prompted the synthesis of a variety of derivatives and analogues of camptothecin. Topotecan (Hycamtin) and irinotecan (Camptosar) are two of the leading examples [5-7]. Much effort, including that leading to the two drugs above cited, has been devoted towards increasing the water solubility of the camptothecin analogues in order to obtain compounds with an improved pharmacological profile and enhanced efficacy against human tumors [8-10].

Scheme 1.

From structure-activity relationship studies [11] it appears that the E-ring lactone and the natural 20S-configuration are essential for anti-tumour activity. While the activity of compounds with substitutions in rings C and D is critically dependent on the size and type of the substituents [12], most structural modifications have concerned rings A and B where wide possibilities of variation exist, especially at positions 7, 9, 10 and 11. Recently the structure of topoisomerase I covalent and non-covalent complexes with a 22-base pair DNA duplex has been solved by X-ray analysis. Based on the crystal structure and structure-activity relationships, a mode of binding for camptothecin has been proposed [13]. In this and in the analogous compound [14], there is plenty of room for substitution at position 7 without steric interference.

A number of camptothecin derivatives have been developed and tested against various cancer cell lines in the past few years and have indicated the importance of lipophilic groups at position 7 of
camptothecin for potent cytotoxic activity. The low solubility in water of these compounds does not amount to a grave disadvantage due to the possibility of successful administration *per os* of camptothecin derivatives [15,16].

We report the synthesis and *in vitro* anti-tumour activity of a new series of OPIC compounds (Scheme 1) that are readily separated into their pure diastereoisomers (3a-d and 4a-d). These new OPIC compounds, deriving from a pyridyl-oxo cyclisation, can be obtained through a simpler and more economical synthetic method than CPT. The starting material, 2,2'-bipyridine-3,3'-dicarboxylic acid (I), was obtained in good yield from commercial 1,10-phenanthroline by oxidation using the KMnO₄ procedure described by Ben-Hadda et al [17]. The reaction of I with thionyl chloride under an inert atmosphere provides an unstable chloro-addition intermediate, the 3,3'-dichlorocarbonyl-2,2'-bipyridine, which is converted into the 3,3'-dialkoxycarbonyl-2,2'-bipyridine (BPE) upon addition of the desired alcohol. The same method for the synthesis of BPE could be slightly modified and usefully exploited to prepare the new family of OPIC compounds.

**Results and Discussion**

**Chemistry**

Scheme 2 depicts the general reaction. In our first attempt, oxopyridoindolizine-carboxylates (OPIC 3, 4) were prepared from BPA (I) and freshly distilled thionyl chloride. After gentle reflux for 5-24 h, followed by subsequent addition of the alcohol, the title compounds were isolated in very poor yield (2-5%). The majority of products (62-93%) were the 3,3'-dialkoxycarbonyl-2,2'-bipyridines (BPE).

**Scheme 2.**

\[
\begin{align*}
\text{BPA : 1} & \quad \text{BPE : 2a-d} & \quad \text{OPI : 3a-d, 4a-d} \\
[\text{R = Me (a), R = Et (b), R = i-Pr (c), R = i-Bu (d)}].
\end{align*}
\]

\[
\begin{align*}
2a: (R = \text{Me}) & \quad 52\% \\
2b: (R = \text{Et}) & \quad 81\% \\
2c: (R = \text{i-Pr}) & \quad 73\% \\
2d: (R = \text{i-Bu}) & \quad 78\% \\
3a: (R = \text{Me}) & \quad 7\% \\
3b: (R = \text{Et}) & \quad 4\% \\
3c: (R = \text{i-Pr}) & \quad 6\% \\
3d: (R = \text{i-Bu}) & \quad 3\% \\
4a: (R = \text{Me}) & \quad 31\% \\
4b: (R = \text{Et}) & \quad 12\% \\
4c: (R = \text{i-Pr}) & \quad 12\% \\
4d: (R = \text{i-Bu}) & \quad 16\%
\end{align*}
\]
The use of technical grade thionyl chloride (which contains small amounts of chlorine), under the same conditions as above led to higher, but still modest, yields of the OPIC compounds: 3-7% for 3a-d and 12-31% for 4a-d. The thionyl chloride contained a small amount of gaseous chlorine, most likely produced in situ by thermo and/or photodecomposition of the reagent. This observation led us to believe that the deliberate addition of chlorine would greatly improve the yield of OPIC. We found that in addition to improving the yield of OPIC, the addition of gaseous chlorine to the reaction decreased the reaction time from 5h to 2h. The optimisation and mechanism of the formation of OPIC has been reported recently in more detail [20].

Evaluation of in vitro Anti-tumour Activity

A series of substituted OPIC (3 and 4) and BPE (2) differing in the substituents on the carboxylate groups were selected by the National Cancer Institute for evaluation of their in vitro anticancer activity (Table 1).

Table 1. In vitro cytotoxic activity of selected OPIC derivatives against three cell lines.

| Compound | R | Growth Percentages 20 |
|----------|---|-----------------------|
|          |   | (Lung) NCI-H460 | (Breast) MCF7 | (CNS) SF-268 |
| 2b       | Et | 97      | 89      | 90      | Inactive |
| 2c       | i-Pr | 105     | 90      | 114     | Inactive |
| 2d       | i-Bu | 81      | 82      | 74      | Inactive |
| 3c       | i-Pr | 96      | 20      | 72      | Active   |
| 4b       | Et | 99      | 67      | 96      | Inactive |
| 4c       | i-Pr | 105     | 19      | 62      | Active   |
| 4d       | i-Bu | 100     | 62      | 100     | Inactive |

From the data in Table 1 it appears that only the oxopyridoindolizines 3c and 4c show any marked cytotoxic activity compared to the rest of series (3 and 4). In this case, the degree of lipophilicity of the carboxylate substituent does not correlate positively with the cytotoxic activity. A direct influence of the lipophilic group from the pyridyl nucleus appears to be more important for activity. Compounds 3c and 4c were further tested for their in vitro activity against 60 cell lines (Tables 2-4).
Scheme 3.

Table 2. Comparative study of *in vitro* anti-tumour activity of diastereoisomers 3c and 4c (Scheme 3) against leukemia and non-small cell lung cancer cell lines.

| Panel                  | Cell line    |GI50 3c |GI50 4c |
|------------------------|--------------|--------|--------|
| Leukemia               | HL-60(TB)    |2.58x10^{-5} |3.82x10^{-5} |
|                        | K-563        |3.34x10^{-5} |3.06x10^{-5} |
|                        | MOLT-4       |3.19x10^{-5} |2.25x10^{-5} |
|                        | RPMI-8226    |2.98x10^{-5} |2.18x10^{-5} |
|                        | SR           |4.96x10^{-5} |2.65x10^{-5} |
| Non-small cell lung    | A549/ATCC    |> 10^{-4}  |> 10^{-4}  |
|                        | EKVX         |> 10^{-4}  |> 10^{-4}  |
|                        | HOP-62       |> 10^{-4}  |> 10^{-4}  |
|                        | HOP-92       |> 10^{-4}  |4.63x10^{-5} |
|                        | NCI-H226     |> 10^{-4}  |> 10^{-4}  |
|                        | NCI-H23      |> 10^{-4}  |4.62x10^{-5} |
|                        | NCI-H322M    |> 10^{-4}  |> 10^{-4}  |
|                        | NCI-H460     |> 10^{-4}  |> 10^{-4}  |
|                        | NCI-H522     |3.57x10^{-5} |2.62x10^{-5} |
Table 3. Comparative *in vitro* anti-tumour activity of diastereoisomers 3c and 4c against CNS, melanoma, ovarian and renal cancer cell lines.

| Panel         | Cell line        | GI\(_{50}\) 3c | GI\(_{50}\) 4c |
|---------------|------------------|----------------|----------------|
| CNS cancer    | SF-268           | 8.21x10\(^{-5}\) | 4.79x10\(^{-5}\) |
|               | SF-295           | > 10\(^{-4}\)     | > 10\(^{-4}\)     |
|               | SF-539           | > 10\(^{-4}\)     | > 10\(^{-4}\)     |
|               | SNB-19           | > 10\(^{-4}\)     | > 10\(^{-4}\)     |
|               | U251             | > 10\(^{-4}\)     | 5.68x10\(^{-5}\)  |
| Melanoma      | LOX IMVI         | > 10\(^{-4}\)     | 6.88x10\(^{-5}\)  |
|               | MALME-3M         | > 10\(^{-4}\)     | 2.88x10\(^{-5}\)  |
|               | M14              | > 10\(^{-4}\)     | > 10\(^{-4}\)     |
|               | SK-MEL-2         | > 10\(^{-4}\)     | > 10\(^{-4}\)     |
|               | SK-MEL-28        | > 10\(^{-4}\)     | > 10\(^{-4}\)     |
|               | SK-MEL-5         | > 10\(^{-4}\)     | 8.30x10\(^{-5}\)  |
|               | UACC-257         | > 10\(^{-4}\)     | 3.41x10\(^{-5}\)  |
|               | UACC-62          | > 10\(^{-4}\)     | > 10\(^{-4}\)     |
| Ovarian cancer | IGROV1           | 9.63x10\(^{-5}\)  | 3.11x10\(^{-5}\)  |
|               | OVCAR-3          | > 10\(^{-4}\)     | > 10\(^{-4}\)     |
|               | OVCAR-4          | > 10\(^{-4}\)     | > 10\(^{-4}\)     |
|               | OVCAR-5          | > 10\(^{-4}\)     | > 10\(^{-4}\)     |
|               | OVCAR-8          | 3.98x10\(^{-5}\)  | 3.17x10\(^{-5}\)  |
| Renal cancer  | 786-0            | 7.39x10\(^{-5}\)  | 5.52x10\(^{-5}\)  |
|               | A498             | > 10\(^{-4}\)     | > 10\(^{-4}\)     |
|               | ACHIN            | > 10\(^{-4}\)     | > 10\(^{-4}\)     |
|               | CAKI-1           | > 10\(^{-4}\)     | > 10\(^{-4}\)     |
|               | RXF 393          | > 10\(^{-4}\)     | > 10\(^{-4}\)     |
|               | SN12C            | 2.12x10\(^{-5}\)  | 2.18x10\(^{-5}\)  |
|               | TK-10            | > 10\(^{-4}\)     | > 10\(^{-4}\)     |
|               | UO-31            | > 10\(^{-4}\)     | > 10\(^{-4}\)     |
Table 4. Comparative in vitro anti-tumour activity of diastereoisomers 3c and 4c against prostate, breast and colon cancer cell lines.

| Panel          | Cell line         | GI50 3c       | GI50 4c       |
|----------------|-------------------|---------------|---------------|
| Prostate Cancer| PC-3              | > 10^{-4}     | 5.66x10^{-5}  |
|                | DU-145            | > 10^{-4}     | > 10^{-4}     |
|                | MCF7              | 8.44x10^{-5}  | 3.91x10^{-5}  |
|                | NCI/ADR-RES       | > 10^{-4}     | > 10^{-4}     |
| Breast Cancer  | DU-145            | > 10^{-4}     | > 10^{-4}     |
|                | MDA-MB-231/ATCC   | 8.15x10^{-5}  | > 10^{-4}     |
|                | HS 578T           | 8.15x10^{-5}  | > 10^{-4}     |
|                | MDA-MB-435        | > 10^{-4}     | 8.10x10^{-5}  |
|                | MDA-N             | 4.41x10^{-5}  | 5.10x10^{-5}  |
|                | BT-549            | > 10^{-4}     | 3.69x10^{-5}  |
| Colon Cancer   | COLO 205          | > 10^{-4}     | 4.39x10^{-5}  |
|                | HCC-2998          | > 10^{-4}     | > 10^{-4}     |
|                | HCT-116           | > 10^{-4}     | 5.06x10^{-5}  |
|                | HCT-15            | > 10^{-4}     | 7.03x10^{-5}  |
|                | HT29              | > 10^{-4}     | > 10^{-4}     |
|                | KM12              | > 10^{-4}     | > 10^{-4}     |
|                | SW-620            | 3.49x10^{-5}  | 3.46x10^{-5}  |

Conclusions

Chlorination of BPA with thionyl chloride in the presence of gaseous chlorine has been demonstrated to be a powerful and optimal method for pyridyl-oxo cyclisation in the preparation of a new heterocyclic family; the oxopyridoindolizine carboxylates (OPIC). This work provides for the first time a simple one-pot synthetic methodology for the preparation of a wide range of OPIC compounds which are analogues or hybrids of the natural alkaloid Camptothecin (CPT). The cytotoxic activity of this new family suggests a promising novel approach to the design of prospective compounds for the treatment of cancer.

As a guide for future work, the data reported here indicate that the OPIC compounds have a definite potential efficacy that merits development through modification to both the pyridyl and the chlorinated rings. We note, however, that although the compounds are nominally camptothecin analogues there is no necessary presumption that they do in fact resemble camptothecin and thereby target topoisomerase I. It might be possible to address this question by testing their effect(s) on two cell lines, one of which has normal topoisomerase I and the other has a mutant (camptothecin-resistant) enzyme. If there were a difference in GI50 value this would indicate that topoisomerase I is a critical target for the drugs. Given
the rather low GI$_{50}$ values of the present compounds, around 200 µM, it would be prudent first to seek new derivatives having GI$_{50}$ values around 1 micromolar or less so that the assay can be conducted more efficiently, though the interest in compounds 3c and 4c because of their particular stereochemistry remains undiminished. A further consideration relates to the poor solubility of the chloro derivatives in water; for the cell line assays to work it is important to prepare derivatives that are more soluble. Substituting the chloro atoms with some other groups such as alkyloxy or amino is possible and might produce the desired effect.

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Experimental

General

Melting points are determined by using a Buchi 510 apparatus and are uncorrected. $^1$H- and $^{13}$C-NMR spectra were recorded on a Bruker WP-80 operating at 200.131 MHz and AC 250 at 250.14 MHz or an AM 300 at 300.134 MHz spectrometer. The numbering used in the assignment of the NMR spectra is shown in Scheme 4. Mass spectra were recorded on a Platform II Micro Mass spectrometer, and FTIR spectra on a Nicolet 205 instrument.

General procedure for the synthesis of 3,3'-di-alkyloxy carbonyl-2,2'-bipyridines (BPE: 2) and alkyl-7,8,9-trichloro-6,7,8,9-tetrahydro-5-oxopyrido[2,3-a]indolizine-10-carboxylates (OPIC: 3 and 4).

The 3,3'-dihydroxycarbonyl-2,2'-bipyridine (1) used in this work was prepared from 1,10-phenanthroline 1 by a procedure described previously [17]. 2,2'-bipyridine-3,3'-dicarboxylic acid (BPA, 600 mg, 2.5 mmol) was added to technical grade thionyl chloride (10 mL) and the mixture was refluxed for 1 h. The excess thionyl chloride was removed under vacuum to leave a yellow residue. Toluene (20 mL) and an alcohol (ROH, 1 mL) were added and the solution was heated under reflux for 3 h. Chloroform (40 mL) was added and the organic phase was washed with a cooled solution of sodium bicarbonate (2.5%). The organic layer was dried over sodium sulfate and the chloroform was removed under reduced pressure. The crude product was purified on a silica gel column (l = 30 cm, θ = 3 cm). Three white solids were successively obtained. Compound 4 was eluted first using a (10:90)
mixture of petroleum ether/dichloromethane. Derivative 3 eluted next using a 5:95 ratio of ether/dichloromethane. Finally, the diester 2 was recovered by elution with (40:60) ether/acetone.

**Scheme 4.** Labelling used for NMR assignments [a (R = Me), b (R = Et), c (R = i-Pr), d (R = i-Bu)]

3,3'-di-Methoxycarbonyl-2,2'-bipyridine (2a): Yield 52%; white powder; m.p. 135-136 °C; $^1$H-NMR (250.14 MHz, CDCl$_3$) δ: 8.74 (dd, 2 H, H$_6$/H$_6'$, $^3$J$_{H6-H5}$ = 4.83 Hz, $^4$J$_{H6-H4}$ = 1.62 Hz), 8.37 (dd, 2 H, H$_4$/H$_4'$, $^3$J$_{H4-H5}$ = 7.94 Hz, $^4$J$_{H4-H6}$ = 1.62 Hz), 7.44 (dd, 2 H, H$_3$/H$_3'$, $^3$J$_{H3-H5}$ = 7.94 Hz, $^3$J$_{H3-H6}$ = 4.83 Hz), 3.66 (s, 6 H, 2 CH$_3$); Analysis, Calcd. (Found) for C$_{14}$H$_{12}$N$_2$O$_4$: C 61.76 (60.13), H 4.42 (4.45), N 10.29 (9.82); MS m/z: 273.0 (Calcd for C$_{14}$H$_{12}$N$_2$O$_4$ : 272.26). IR (KBr) ν cm$^{-1}$: 1720 (C=O, s), 1582, 1582 (C=C, m), 1440 (C=N, m), 1307, 1299 (C-O, m).

3,3'-di-Ethoxycarbonyl-2,2'-bipyridine (2b): Yield 81%; white powder; m.p. 89-90 °C; $^1$H-NMR (250.14 MHz, CDCl$_3$) δ ppm: 8.74 (dd, 2 H, H$_6$/H$_6'$, $^3$J$_{H6-H5}$ = 4.80 Hz, $^4$J$_{H6-H4}$ = 1.56 Hz), 8.36 (dd, 2 H, H$_4$/H$_4'$, $^3$J$_{H4-H5}$ = 7.93 Hz, $^4$J$_{H4-H6}$ = 1.56 Hz), 7.42 (dd, 2 H, H$_3$/H$_3'$, $^3$J$_{H3-H5}$ = 7.93 Hz, $^3$J$_{H3-H6}$ = 4.80 Hz), 4.08 (qu, 4 H, 2 CH$_2$, $^3$J$_{CH2-CH3}$ = 7.15 Hz), 1.02 (t, 6 H, 2 CH$_3$, $^3$J$_{CH3-CH2}$ = 7.15 Hz); Analysis, Calcd. (Found) for C$_{16}$H$_{16}$N$_2$O$_4$: C 64.01 (63.56.), H 5.33 (5.62), N 9.33 (9.13); MS m/z : 301.10 (Calcd. for C$_{16}$H$_{16}$N$_2$O$_4$ : 300.316). IR (KBr) ν cm$^{-1}$: 1724 (C=O, s), 1578, 1565 (C=C, w), 1423 (C=N, m), 1277 (C-O, w).

3,3'-di-iso-Propyloxycarbonyl-2,2'-bipyridine (2c): Yield 73%; white powder; m.p. 81-82 °C; $^1$H-NMR (250.14 MHz, CDCl$_3$) δ ppm: 8.73 (dd, 2 H, H$_6$/H$_6'$, $^3$J$_{H6-H5}$ = 4.80 Hz, $^4$J$_{H6-H4}$ = 1.70 Hz), 8.37 (dd, 2 H, H$_4$/H$_4'$, $^3$J$_{H4-H5}$ = 7.93 Hz, $^4$J$_{H4-H6}$ = 1.70 Hz), 7.42 (dd, 2 H, H$_3$/H$_3'$, $^3$J$_{H3-H5}$ = 7.93 Hz, $^3$J$_{H3-H6}$ = 4.80 Hz), 4.95 (qu, 2 H, CH, $^3$J$_{CH-CH3}$ = 6.31 Hz), 0.97 (d, 12 H, 4 CH$_3$, $^3$J$_{CH3-CH}$ = 6.31 Hz); Analysis Calcd. (Found) for C$_{18}$H$_{20}$N$_2$O$_4$: C 65.85 (65.78.), H 6.10 (6.22), N 8.53 (8.38); MS m/z : 329.10 (Calcd. for C$_{18}$H$_{20}$N$_2$O$_4$ : 328.371); IR (KBr) ν cm$^{-1}$: 1695 (C=O, s), 1535, 1565 (C=C, w), 1415 (C=N, m), 1260 (C-O, w).
3,3’-di-iso-Butyloxy carbonyl-2,2’-bipyridine (2d): Yield 78%; white powder; m.p. 88-89 °C; 1H-NMR (250.14 MHz, CDCl3) δ ppm: 8.74 (dd, 2 H, H6/H6’, 3JH6-H5 = 4.83 Hz, 4JH6-H4 = 1.66 Hz), 8.37 (dd, 2 H, H4/H4’, 3JH4-H5 = 7.93 Hz, 3JH4-H6 = 4.83 Hz), 3.84 (d, 2 H, CH2, 3JCH2-CH2 = 6.6 Hz), 1.68 (m, 2 H, 2 CH), 0.76 (d, 12 H, 4 CH3, 3JCH3-CH = 6.6 Hz); Analysis, Calcd. (Found) for C20H24N2O4: C 67.42 (67.55), H 6.74 (6.82), N 7.86 (7.87); MS m/z: 357.10 (Calc. for C20H24N2O4 : 356.425); IR (KBr) ν cm⁻¹: 1713 (C=O, s), 1589/1561 (C=C, w), 1470 (C=N, m), 1289 (C-O, w).

(7R,8R,9S)-Methyl-7,8,9-trichloro-6,7,8,9-tetrahydro-5-oxopyrido[2,3-a]indolizine-10-carboxylate (3a): Yield 7%; white powder; m.p. 125-126 °C; 1H-NMR (200,131 MHz; CDCl3) δ ppm: 8.86 (dd, 1 H, H2, 3JH2-H3 = 4.9 Hz, 4JH2-H4 = 1.6 Hz), 8.14 (dd, 1 H, H4, 3JH4-H3 = 7.8 Hz, 4JH4-H2 = 1.6 Hz), 7.48 (dd, 1 H, H3, 3JH3-H4 = 7.8 Hz, 3JH3-H2 = 4.9 Hz), 6.47 (d, 1 H, H8, 3JH8-H9 = 3 Hz), 5.26 (d, 1 H, H10, 3JH10-H9 = 9.8 Hz), 4.52 (dd, 1 H, H9, 3JH9-H8 = 3.05 Hz, 3JH9-H10 = 9.75 Hz), 3.96 (s, 3 H, CH3); MS m/z : 347.0 (Calcd. for C13H9N2O3Cl3: 346.60). IR (KBr) ν cm⁻¹: 1744 (C=O, s), 1715 (C=O, s), 1604, 1584 (C=C, w), 1437 (C=N, m).

(7R,8R,9S)-Ethyl-7,8,9-trichloro-6,7,8,9-tetrahydro-5-oxopyrido[2,3-a]indolizine-10-carboxylate (3b): Yield 4%; white powder; m.p. 104-106 °C; 1H-NMR (200.131 MHz, CDCl3) δ ppm: 8.86 (dd, 1 H, H2, 3JH2-H3 = 4.8 Hz, 4JH2-H4 = 1.6 Hz), 8.14 (dd, 1 H, H4, 3JH4-H3 = 7.8 Hz, 4JH4-H2 = 1.6 Hz), 7.48 (dd, 1 H, H3, 3JH3-H4 = 7.8 Hz, 3JH3-H2 = 4.8 Hz), 6.46 (d, 1 H, H8, 3JH8-H9 = 3.2 Hz), 5.305 (d, 1 H, H10, 3JH10-H9 = 9.8 Hz), 4.53 (dd, 1 H, H9, 3JH9-H8 = 3.2 Hz, 3JH9-H10 = 9.8 Hz), 4.46 (m, 2 H, CH2, 3J = 7.12 Hz), 1.37 (t, 3 H, CH3, 3J = 7.12 Hz); MS m/z : 360.9  (Calcd. for C14H11N2O3Cl3 : 360.63); IR (KBr) ν cm⁻¹: 1752 (C=O, s), 1718 (C=O, s), 1604, 1583 (C=C, w), 1392 (C=N, m).  The crystallographic structure of 3c has been recently reported [22].

(7R,8R,9S)-iso-Propyl-7,8,9-trichloro-6,7,8,9-tetrahydro-5-oxopyrido[2,3-a]indolizine-10-carboxylate (3c): Yield 6%; white powder; m.p. 99-102 °C; 1H-NMR (200.131 MHz, CDCl3) δ ppm: 8.83 (dd, 1 H, H2, 3JH2-H3 = 4.8 Hz, 4JH2-H4 = 1.4 Hz), 8.12 (dd, 1 H, H4, 3JH4-H3 = 7.7 Hz, 4JH4-H2 = 1.4 Hz), 7.51 (dd, 1 H, H3, 3JH3-H4 = 7.7 Hz, 3JH3-H2 = 4.8 Hz), 6.47 (d, 1 H, H8, 3JH8-H9 = 3.03 Hz), 5.33 (m, 1 H, H13), 5.26 (d, 1 H, H10, 3JH10-H9 = 9.7 Hz), 4.52 (dd, 1 H, H9, 3JH9-H8 = 3.03 Hz, 3JH9-H10 = 9.76 Hz), 1.39 (d, 3 H, CH3, 3J = 6.2 Hz), 1.34 (d, 3 H, H14, 3J = 6.2 Hz); MS m/z : 374.90  (Calcd. for C15H13N2O3Cl3 : 374.653); IR (KBr) ν cm⁻¹: 1752 (C=O, s), 1718 (C=O, s), 1604, 1583 (C=C, w), 1457 (C=N, m).
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(C=O, s), 1715 (C=O, s), 1582 (C=C, w), 1473 (C=N, m); MS m/z : 388.90 (Calc. for C_{16}H_{15}N_{2}O_{3}Cl_{3}: 388.68); IR (KBr) v cm^{-1} : 1751 (C=O, s), 1713 (C=O, s), 1584 (C=C, w).

(7S,8R,9S)-Methyl-7,8,9-trichloro-6,7,8,9-tetrahydro-5-oxopyrido[2,3-a]indolizine-10-carboxylate (4a): Yield 31%; white powder; m.p. 121-122 °C; 1H-NMR (250.14 MHz; CDCl3) δ ppm: 8.9 (dd, H2, 3JH2-H3 = 4.9 Hz, 4JH2-H4 = 1.6 Hz), 8.21 (dd, 1 H, H4, 3JH4-H3 = 7.8 Hz, 4JH4-H2 = 1.6 Hz), 7.53 (dd, 1 H, H3, 3JH3-H4 = 7.8 Hz, 3JH3-H2 = 4.9 Hz), 6.49 (dd, 1 H, H8, 3JH8-H9 = 1.33 Hz, 4JH8-H10 = 2.1 Hz), 5.35 (t, 1 H, H9, 3JH9-H8 = 1.33 Hz, 3JH9-H10 = 1.33 Hz), 5.03 (dd, 1 H, H10, 3JH10-H9 = 1.33 Hz, 4JH10-H8 = 2.1 Hz), 4.0 (s, 3 H, CH3); Analysis Calcd. (Found) for C_{16}H_{15}N_{2}O_{3}Cl_{3}: C 44.94 (44.98), H 2.59 (2.63), N 8.06 (7.99); MS m/z: 347.0 (Calcd. for C_{16}H_{15}N_{2}O_{3}Cl_{3}: 346.60); IR (KBr) ν cm^{-1}: 1744 (C=O, s), 1717 (C=O, s), 1601-1581 (C=C, w), 1434 (C=N, m), 1297 (C-O, w).

(7S,8R,9S)-Ethyl-7,8,9-trichloro-6,7,8,9-tetrahydro-5-oxopyrido[2,3-a]indolizine-10-carboxylate (4b): Yield 12%; white powder; m.p. 102-103 °C; 1H-NMR (300.134 MHz, CDCl3) δ ppm: 8.90 (dd, H2, 3JH2-H3 = 4.8 Hz, 4JH2-H4 = 1.6 Hz), 8.21 (dd, 1 H, H4, 3JH4-H3 = 7.8 Hz, 4JH4-H2 = 1.6 Hz), 7.53 (dd, 1 H, H3, 3JH3-H4 = 7.8 Hz, 3JH3-H2 = 4.8 Hz), 6.49 (dd, 1 H, H8, 3JH8-H9 = 1.45 Hz, 4JH8-H10 = 2.04 Hz), 5.35 (t, 1 H, H9, 3JH9-H8 = 1.42 Hz, 3JH9-H10 = 1.45 Hz), 5.00 (dd, 1 H, H10, 3JH10-H9 = 1.45 Hz, 4JH10-H8 = 2.04 Hz), 4.48 (m, 2 H, CH2, 3J = 7.12 Hz), 1.39 (t, 3 H, CH3, 3J = 7.12 Hz). {1H} 13C NMR (MHz, CDCl3) δ ppm: 154.5 (C 2), 132.2 (C 4), 125.2 (C3), 62.5 (C13), 60.8 (C8), 52.8 (C10), 57.1 (C9), 14.5 (C14); Analysis, Calcd. (Found) for C_{14}H_{11}N_{2}O_{3}Cl_{3}: C 46.97 (47.44), H 3.05 (3.33), N 7.74 (7.35); MS m/z: 360.9 (Calcd. for C_{14}H_{11}N_{2}O_{3}Cl_{3}: 360.63); IR (KBr) ν cm^{-1}: 1750 (C=O, s), 1716 (C=O, s), 1609 (C=O, s), 1477 (C=O, w). The crystallographic structure of 4b has been reported [20].

(7S,8R,9S)-iso-Propyl-7,8,9-trichloro-6,7,8,9-tetrahydro-5-oxopyrido[2,3-a]indolizine-10-carboxylate (4c): Yield 12%; white powder; m.p. 97-99 °C; 1H-NMR (300.134 MHz, CDCl3) δ ppm: 8.90 (dd, 1 H, H2, 3JH2-H3 = 4.8 Hz, 4JH2-H4 = 1.6 Hz), 8.21 (dd, 1 H, H4, 3JH4-H3 = 7.8 Hz, 4JH4-H2 = 1.6 Hz), 7.53 (dd, 1 H, H3, 3JH3-H4 = 7.8 Hz, 3JH3-H2 = 4.8 Hz), 6.48 (dd, 1 H, H8, 3JH8-H9 = 1.4 Hz, 4JH8-H10 = 2.1 Hz), 5.34 (m, 1 H, H9, 3JH9-H8 = 1.4 Hz, 3JH9-H10 = 1.4 Hz), 1.38 (d, 3 H, CH3, 3J = 6.7 Hz), 1.32 (d, 3 H, CH3, 3J = 6.3 Hz); Analysis Calcd. (Found) for C_{15}H_{13}N_{2}O_{3}Cl_{3}: C 47.97 (47.96), H 3.46 (3.52), N 7.46 (7.39); MS m/z: 374.90 (Calcd. for C_{15}H_{13}N_{2}O_{3}Cl_{3}: 374.653); IR (KBr) ν cm^{-1}: 1741 (C=O, s), 1711 (C=O, s), 1600, 1582 (C=C, w), 1396 (C=N, m).

(7S,8R,9S)-iso-Butyl-7,8,9-trichloro-6,7,8,9-tetrahydro-5-oxopyrido[2,3-a]indolizine-10-carboxylate (4d): Yield 16%; white powder; m.p. 96-97 °C; 1H-NMR (200.131 MHz, DCCl3) δ ppm: 8.88 (dd, 1 H, H2, 3JH2-H3 = 4.86 Hz, 4JH2-H4 = 1.6 Hz), 8.21 (dd, 1 H, H4, 3JH4-H3 = 7.8 Hz, 4JH4-H2 = 1.6 Hz), 7.52 (dd, 1 H, H3, 3JH3-H4 = 7.8 Hz, 3JH3-H2 = 4.86 Hz), 6.50 (dd, 1 H, H8, 3JH8-H9 = 1.39 Hz, 4JH8-H10 = 2.04 Hz), 5.36 (t, 1 H, H9, 3JH9-H8 = 1.39 Hz, 3JH9-H10 = 1.39 Hz), 5.03 (dd, 1 H, H10, 3JH10-H9 = 1.39 Hz, 4JH10-H8 = 2.04 Hz), 4.22 (m, 2 H, CH2, 3J = 6.7 Hz), 2.09 (m, 1 H, H14, 3J = 6.7 Hz), 1.0 (d, 6 H, 2 CH3, 3J = 6.7 Hz); Analysis Calcd. (Found) for C_{16}H_{15}N_{2}O_{3}Cl_{3}: C 49.32 (49.23), H 3.85(3.89), N 7.19 (6.99);
MS m/z: 388.90 (Calc. for C_{16}H_{15}N_{2}O_{3}Cl_{3} : 388.68); IR (KBr) ν cm\(^{-1}\) : 1750 (C=O, s), 1715 (C=O, s), 1582 (C=C, w), 1473 (C=N, m).

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21. In the current protocol, each cell line is inoculated and preincubated on a microtiter plate. Test agents are then added at a single concentration and the culture incubated for 48 hours. End-point determinations are made with Alamar blue (*Biotechniques* 1996, 21, 780). Results for each test agent are reported as the percent growth of the treated cells compared to the untreated controls. Compounds which reduce the growth of any one of the cell lines to approximately 32% or less
(negative numbers indicate cell kill) are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range. All compounds were tested at a final concentration of $10^{-4}$ M.

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*Sample Availability:* Not available.

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