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# Preliminary biological evaluation and mechanism of action studies of selected 2-arylindoles against glioblastoma

Saurabh Prabhu [a], Zaheer Akbar [a], Frederick Harris [a], Katherine Karakoula [b], Robert Lea [a], Farzana Rowther [b], Tracy Warr [b], Timothy Snape [a,*]

[a] School of Pharmacy and Biomedical Sciences, Maudland Building, University of Central Lancashire, Preston PR1 2HE, UK
[b] Brain Tumour UK Neuro-Oncology Research Centre, University of Wolverhampton, Wulfruna Street, Wolverhampton WV1 1LY, UK

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ABSTRACT

A series of related 2-arylindoles have been evaluated for their anticancer activity against a range of glioblastoma cell lines using a number of different cell-based assays to determine cell viability after treatment with the compounds. The best indoles, which showed comparable activity to cisplatin against a U87MG cell line in the MTS assay, were taken forward and initial studies suggest that their mechanism of action is consistent with the generation of reactive oxygen species followed by autophagic cell death. Furthermore, activity was also observed in glioblastoma short-term cell cultures for the best lead compound and in some cases gave low micromolar $IC_{50}$s.



## 1. Introduction

Indole-3-carbinol (I3C (**1**), Fig. 1) has received a great deal of attention over the past circa 30 years for the chemoprevention and treatment of a number of cancers, including: breast, prostate, keratinocyte, endometrial and non-small cell lung adenocarcinoma cells,[1–3] but not, to our knowledge, for the treatment of glioma. Similarly, it has been demonstrated that other indoles, so-called privileged structures,[4] such as 2-arylindoles (Fig. 1), possess biological activity in a number of therapeutic areas, including cancer.[5]

Indole-3-carbinol, a natural product obtained from cruciferous vegetables, has been identified as a potential treatment/preventative for breast cancer, where it has been shown to act through various mechanisms, such as: affecting DNA repair and nuclear regulatory factors, induction of cell cycle arrest and induction of apoptosis, effects which have been observed in both mice and human volunteers.[2] Additionally, a substantial amount of research has been undertaken studying structure activity relationships of more heavily substituted indoles for the treatment of breast cancer,[6–13] where it has been established that the 2-phenyl group appears to be important for biological activity in certain cases. For example, in studies to identify new 3-formyl-2-phenylindoles as inhibitors of tubulin polymerization, it was found that the parent indole (**5**, R = CHO, $R^1$ = H) inhibits cell growth, but that 3-formylindole, which lacks the 2-phenyl group, did not.[6]

## 2. Results and discussion

We are currently interested in developing new and improved chemotherapeutic agents for the treatment of glioma and also have an interest in the development of new methods for the synthesis of substituted 2-arylindoles,[14] therefore a study was embarked upon in order to examine what effects indoles would have on various glioma cell lines to see if new lead compounds could be identified.

Both I3C (**1**) and 2-arylindoles (such as **2**) have established activity against cancer cells and thus the initial investigation began by ascertaining what kind of activity these two compounds have against the established glioma cell lines 1321N1 and U87MG. The results (Table 1, entries 1 and 2) reveal that whilst I3C (**1**) has modest activity [$IC_{50}$ = 309 ± 2 μM (1321N1) and 526 ± 2 μM (U87MG)], the 2-arylindole (**2**) does not reach its $IC_{50}$ over the same time frame (48 h) and only reaches a maximum growth inhibition of 7% (U87MG) at 400 μM, however, **2** is not able to inhibit the growth of the 1321N1 cell line.

Based on these results, whilst bearing in mind the observed importance of both I3C and the 2-phenyl group in indoles,[6] it was of interest to know that if making hybrid structures of **1** and **2**, thus generating **3** and **4** (Figs. 1 and 2), would result in improved activity against these cell lines when compared to **1** and **2**. In the event, indoles **3**[15] and **4**[14] were prepared as previously reported

* Corresponding author. Tel.: +44 (0)1772 895805.
*E-mail address:* tjsnape@uclan.ac.uk (T. Snape).

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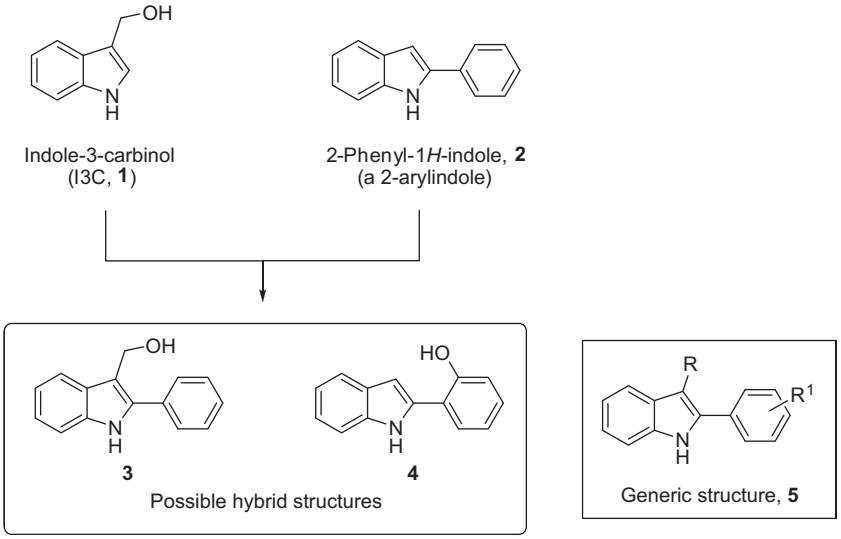


**Figure 1.** Chemical structures of indole-3-carbinol (I3C, **1**), a 2-arylindole (**2**) and their structural hybrids (**3** and **4**), and their generic structure, **5**.

**Table 1**
A table to show the $IC_{50}$ values of the indoles against two different cell lines using the MTS assay

| Entry | Compounds | MTS assay $IC_{50}$ values[a] | |
|---|---|---|---|
| | | 1321N1 (μM) | U87MG (μM) |
| 1 | **1** (I3C) | 309 ± 2 | 526 ± 2 |
| 2 | **2**[b] | – | – |
| 3 | **3** | 111 ± 1 | 176 ± 1 |
| 4 | **4** | 445 ± 4 | 379 ± 4 |
| 5 | **6**[b] | – | – |
| 6 | **7**[b] | – | – |
| 7 | Cisplatin | 11 ± 2 | 310 ± 4 |

The values shown are the average and standard deviations of four repeats.
[a] $IC_{50}$ values reached after 48 h.
[b] $IC_{50}$ value not reached.

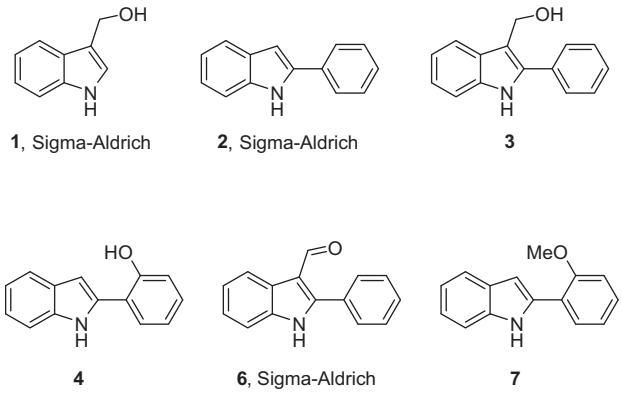


**Figure 2.** The compounds screened. Compounds **1**, **2**, and **6** were purchased from Sigma–Aldrich, UK, whilst **3**,[15] **4**,[14] and **7**[16] were prepared as referenced here.

and both were subjected to the same assay as indoles **1** and **2**; the activities (Table 1, entries 3 and 4) demonstrate that both hybrid compounds were more active than **2**, in both cell lines, and both were more active than **1** in the malignant U87MG glioma cell line. Indoles **6** and **7**[16] (Fig. 2) were also prepared or purchased, and evaluated in the same assay (Table 1, entries 5 and 6) as a test to determine the influence of the hydroxyl group in these compounds. Interestingly, these two indoles (**6** and **7**) which lack the hydroxyl group are both inactive, which in addition to the result with indole **2** (Table 1, entry 2), which also lacks this group, highlights the hydroxyl's apparent importance for activity against these cell lines. All indoles assessed (Fig. 2 and Table 1) were screened alongside the known anticancer agent—cisplatin—to gain a rudimentary measure of their therapeutic efficacy. Cisplatin was chosen because, in previous studies, it was shown to possess the best cytotoxic effects against these cell lines when compared to other known anticancer agents (temozolomide, etoposide and carmustine).[17]

Only **1**, **3**, **4** and cisplatin were active against the cell lines, with cisplatin showing an order of magnitude better activity than the indoles in the 1321N1 cell line. However, it is of note that, against the U87MG cell line, compound **4** has an $IC_{50}$ value of a similar order of magnitude to the established anticancer drug cisplatin, whilst indole **3** possesses better activity than cisplatin. A result which adds weight to the potential of these indoles as lead compounds, however, it is not suggested that indoles **3** and **4** have a mechanism of action similar to the DNA-alkylation behavior of the platinum drug, more that, since a potent anticancer agent such as cisplatin also gives high $IC_{50}$ values with these cell lines, it is promising that activity is observed at all with these simple indoles, and thus they are worthy of further investigation. Evidently, the 2-Ph group is having a large influence on the activity of these indoles (compare entry 1 to entry 3 in Table 1), as has been reported against breast cancer cell lines.[6]

Having discovered potential new lead cytotoxic hybrid indoles against these glioma cell lines, the mechanism of action was investigated of the more active indoles (**3** and **4**) in the hope that the biological target(s) identified could be used to help with future rounds of lead development by incorporating in silico modelling.

Preliminary insights into their mechanism of action were gleaned from the structure of the active compounds. It was insightful that compounds **1**, **3** and **4** were active, yet analogues such as **2**, **6** and **7** were not; the hydroxyl group was evidently having an effect in addition to the 2-phenyl ring. It was assumed that such a difference in activity could not be due merely to differences in hydrogen-bonding, and so attention was turned to the hydroxy or phenoxy groups in these compounds to investigate if they were responsible for the observed cytotoxic effects, as has been observed for other hydroxy- and phenoxy-containing compounds.[18–24]

The hypothesis was that the active indoles were acting through a radical-based mechanism, which was most likely related to the generation of reactive oxygen species (ROS).[19] As such, the

Image-iT™ LIVE Green Reactive Oxygen Species Detection Kit from Invitrogen Ltd, UK, was chosen, which is able to detect ROS in live cells, to assess the selected indoles. Oxidatively stressed cells, when labeled with the carboxy-$H_2$DCFDA dye provided, should generate fluorescence which is detectable using confocal microscopy and flow cytometry.

In the event, to confirm and quantify the amount of fluorescence produced the dye was used to detect the presence of ROS in glioma cell lines 1321N1 and U87MG in the presence of indoles **2**, **3**, **4** and **7** (500 μM). The results (Fig. 3) suggest that the cells were under oxidative stress when in the presence of indoles **3** and **4** (Fig. 3D and E), as identified by the shift of the fluorescence signal in these cases compared to the control signal (Fig. 3A), but that oxidative stress was not observed with **2** and **7** (Fig. 3C and F) in which no shift was observed compared to the control signal. Presumably, only **3** and **4** generated ROS as a result of the hydroxyl group in their structures being able to form radical species in vitro upon reaction with redox enzymes. Indole **7** was used as a comparison (which has the hydroxyl group masked as a methoxy group) and it was found that ROS was not induced in this case, as would be expected if the free hydroxyl group was important for ROS generation. Nor was it induced by indole **2**, which lacks any oxygenation on the 2-phenyl group.

The positive control used in this experiment was *tert*-butyl hydroperoxide (TBHP) (100 μM) which is a common inducer of ROS and was suggested and provided by Invitrogen Ltd. Importantly, the indoles inducing ROS (**3** and **4**) appeared to induce it to a greater level than the positive control (TBHP), suggesting that these compounds are powerful inducers of reactive oxygen species and that this presumably is intrinsic in their ability to affect the viability and hence proliferation of the cell lines tested. In particular, the results suggest that the fluorescence generated in the

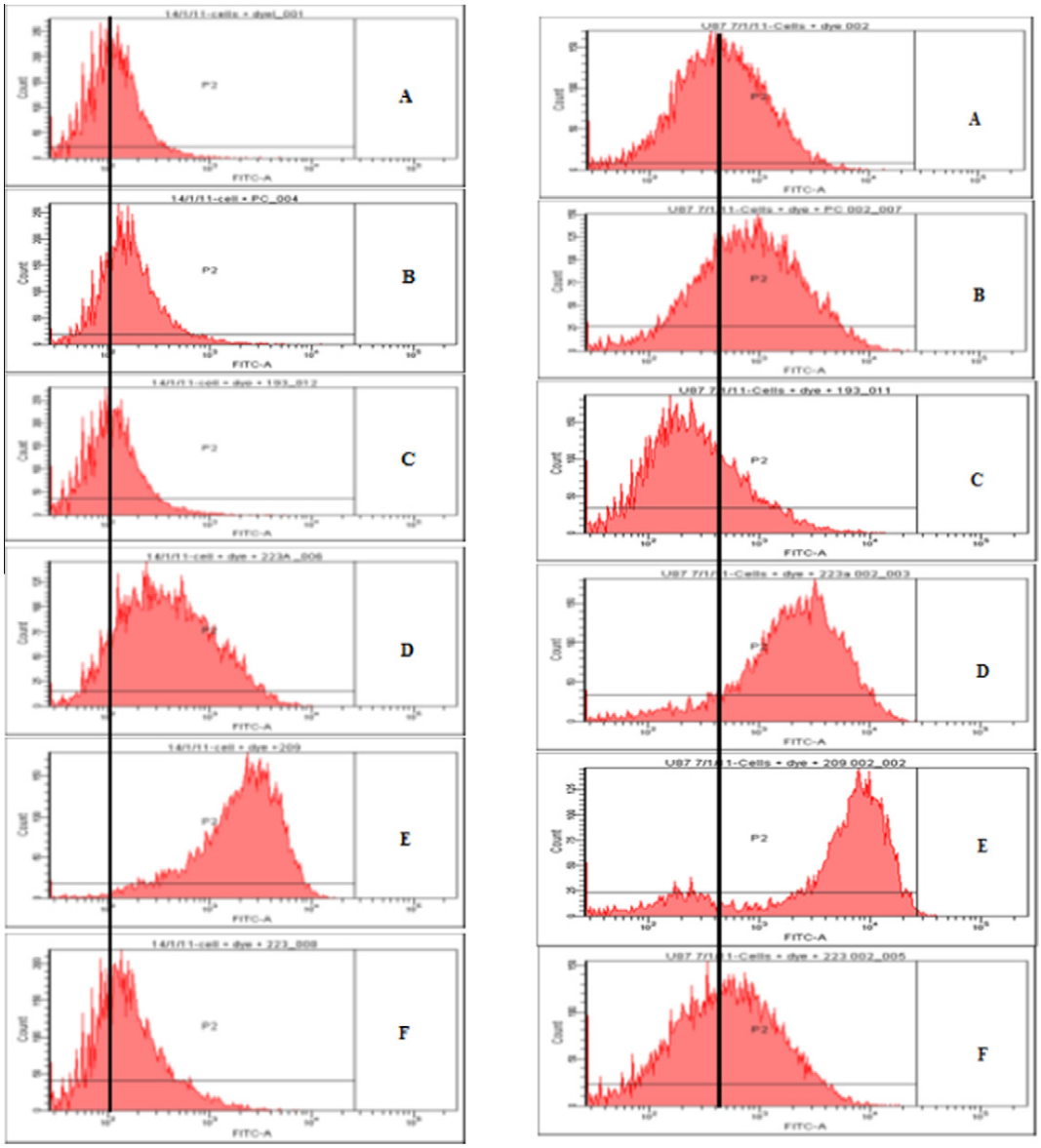


**Figure 3.** (Left) 1321N1 cells were treated with analogues of indole **4** for 1 h.[25] (A) Cells + dye. (B) Cells treated with positive control [100 μM *tert*-butyl hydroperoxide] for 1 h. (C) Cells treated with indole **2** (500 μM) for 1 h. (D) Cells treated with indole **3** (500 μM) for 1 h. (E) Cells treated with indole **4** (500 μM) for 1 h. (F) Cells treated with indole **7** (500 μM) for 1 h. (Right) U87MG cells were treated with analogues of indole **4** for 1 h. (A) Cells + dye. (B) Cells treated with positive control [100 μM *tert*-butyl hydroperoxide (TBHP)] for 1 h. (C) Cells treated with indole **2** (500 μM) for 1 h. (D) Cells treated with indole **3** (500 μM) for 1 h. (E) Cells treated with indole **4** (500 μM) for 1 h. (F) Cells treated with indole **7** (500 μM) for 1 h. Carboxy-$H_2$DCFDA staining intensity was quantified by flow cytometry. Representative data from one of three experiments yielding similar results is shown.

1321N1 and U87MG cell lines treated with indole **4** were significantly higher when compared to the controls (Fig. 3), suggesting that the lack of cell viability observed is mediated through the formation of excessive ROS placing the cells under oxidative stress. As a control experiment, the fluorescence of indole **4** itself plus the cells was measured, and was found to be comparable to the cells alone (data not shown), confirming that it is not the indole itself that is generating false positive results, but that the results shown in Figure 3 are due to the formation of ROS in the presence of **3** and **4**.

Ascorbic acid is a naturally occurring organic compound with antioxidant properties and is a scavenger of hydroxyl radicals, reacting with reactive oxygen species, such as the hydroxyl radical, and neutralizing it.[26] Recent studies have found that ascorbic acid inhibits ROS production by neutralizing the radicals formed,[27] and therefore ascorbic acid was chosen here to validate the ROS production induced by indole **4** (500 μM) in the 1321N1 and U87MG cell lines following the assumption that reduced amounts of ROS production would be seen after the co-treatment of the cells with ascorbic acid (500 μM) and indole **4**. At this stage it was decided to concentrate on the phenol derivative **4**, since in our hands, carbinol **3** had proved to be slightly unstable under acidic conditions, as is well documented for similar structures,[1–3] and thus did not justify further investigation here since its stability would be an issue in the presence of an additional acid (ascorbic acid). Moreover, it is possible that the addition of the 2-aryl ring in **3** may cause further instability through increasing the conjugation of the vinylogous hemiaminal of I3C (**1**), and thus increasing the stability of the acid degradation products, Figure 4.[3] Nevertheless related indole-3-carbinol derivatives have recently demonstrated activity against prostate[28] and breast[29] cancers where the structures have been modified on nitrogen to reduce its inherent acid-lability.

The results of the ascorbic acid treatment reveals that the level of fluorescence produced by both the cell lines treated with a combination of indole **4** and ascorbic acid was shifted back towards the levels shown by the vehicle control cells alone (data not shown), thus further validating that indole **4** expresses its cytotoxic behaviour through ROS generation, but that this cannot happen in the presence appropriate levels of radical scavengers.[30] This was also supported by the MTS assay on both cell lines, which showed that cell viability was unaffected by indole **4** when in the presence of ascorbic acid.

To further understand the effects that such ROS generation was having on the cells the examination of whether indole **4** was responsible for cell death through the induction of autophagy, as has been observed for other small molecules,[25,31–36] was investigated.

Oxidative stress is known to induce autophagy,[31,33,35–37] and cell death associated with autophagy in tumour cell lines treated with chemotherapeutic agents has been proposed recently.[38] Such research has also shown that cell death is associated with autophagy in malignant gliomas,[25] ovarian carcinomas[39] and mammary carcinomas,[40] and that hydrogen peroxide ($H_2O_2$) and 2-methoxyestradiol (2-ME) induce oxidative stress, causing autophagic cell death, in the transformed cell line HEK293 and cancer cell lines U87MG and HeLa cells.[37] Furthermore, β-lapachone induces ROS generation which mediates autophagic cell death in U87MG cells.[25] The phenolic compound, resveratrol, has also been shown to induce autophagy in human U251MG glioma cells.[41]

Based on such precedent it was important to see if autophagy was being induced in the 1321N1 and U87MG cells upon treatment with the most active, stable compound, indole **4**. The process of autophagy is in-part characterized by the formation of acidic vesicular organelles (AVOs) which can be detected and measured by staining with acridine orange. Acridine orange moves freely across biological membranes and accumulates in acidic compartments upon protonation where it can be observed and quantified by fluorescence at a distinct wavelength. Therefore, in view of the finding that AVOs accumulate in cells undergoing autophagy, an investigation into whether indole **4** induced AVO formation by treating the cells with indole **4** was carried out, staining them with acridine orange and analyzing the results by flow cytometry.

The results (Fig. 5) indicated the formation of AVOs had occurred, as concluded by the increased amount of red fluorescence in the cell lines studied.

The results were confirmed on both cell lines by repeating the experiments in the presence of an autophagy inhibitor, 3-methyladenine (3-MA, 5 mM).[42] The results (Fig. 5) indicate that there is a reduction in the amount of fluorescence produced in the experiments with 3-MA, indicating that autophagy is inhibited, and that indole **4** induces AVO formation as a result of the cells being in an autophagic process, which may be mediated by reactive oxygen species generation in these particular cell lines. This potentially reveals a new class of compound which could possibly be optimized further for glioma treatment through analogue preparation.

Based on these preliminary results it was decided to ascertain if the effects were general against other glioblastoma cells and thus set out to screen indole **4** against the short term cell cultures IN1472, IN1265, IN1760 and IN859, and another established cell line—U251MG. The $IC_{50}$ values obtained were better for U251MG, IN1472 and IN1265 (Table 2) compared to 1321N1 and U87MG, possibly suggesting that indole **4** is even more potent against these cells. In contrast however, it was completely inactive against the highly resistant cell culture IN1760, a cell culture which has proved to be highly resistant to a number of commercially available anticancer drugs (CCNU, vincristine and doxorubicin).[43] Furthermore, the $IC_{50}$ value against the short-term cell cultures (except IN1760) are comparable, if not better, than that for U87MG suggesting that indole **4** possesses the ability to kill short-term cell cultures as well as established cancer cell lines.

In conclusion, these combined results suggest that indole **4** (and possibly indole **3**) possess promising anticancer activity against a range of glioblastoma cells and that further development and analogue preparation is warranted in order to improve activity further.

## 3. Materials and methods

### 3.1. MTS assay

The 1321N1 cells (obtained from ECACC, UK) were maintained in Dulbeccos's Modified Eagle's Medium (DMEM) supplemented

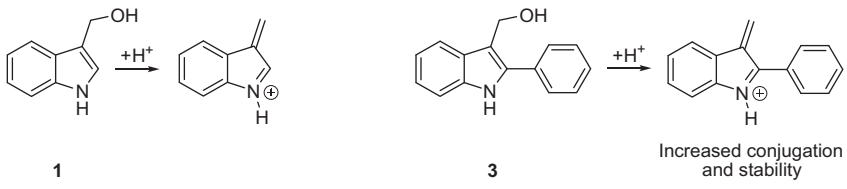


**Figure 4.** Figure depicting the acid instability of I3C (**1**) and the analogous structure for the presumably more stable acid-degradation product from **3**.[3]

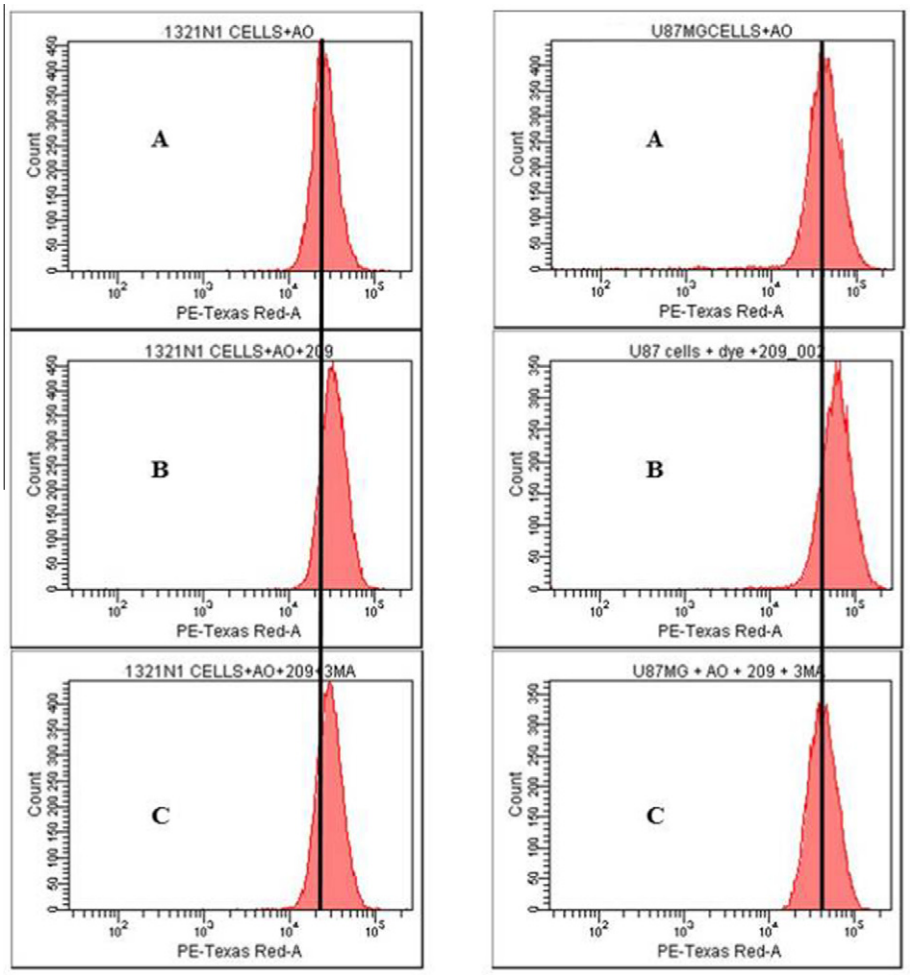


**Figure 5.** Acidic vesicular organelle (AVO) formation in 1321N1 cells (Left) and U87MG cells (Right). (A) Cells + acridine orange. (B) Cells treated with indole **4** (500 μM) for 1 h + acridine orange. (C) Cells treated with indole **4** (500 μM) for 1 h, acridine orange + 3-MA (5 mM for 1 h). Staining intensity was quantified by flow cytometry. Representative data from one of two experiments yielding similar results is shown.

**Table 2**
A table to show the $IC_{50}$ values of indole **4** against different cells using the MTS and MTT assays.

| Compound | MTS assay $IC_{50}$ values[a] | | | MTT assay $IC_{50}$ values[b] | | | |
|---|---|---|---|---|---|---|---|
| | 1321N1 (μM) | U87MG (μM) | IN859[c] (μM) | IN1472 (μM) | IN1265 (μM) | U251MG (μM) | IN1760 (μM) |
| **4** | 445 ± 4 | 379 ± 4 | 400 ± 1 | 48.0 ± 0.2 | 90.0 ± 0.5 | 92.0 ± 0.6 | >1000 |

The values shown are the average and standard deviations of at least two repeats.
[a] $IC_{50}$ values reached after 48 h.
[b] $IC_{50}$ values reached after 72 h.
[c] $IC_{50}$ value reached after 2 h.

with 10% (v/v) foetal bovine serum (FBS) and 2 mM L-glutamine, while the U87MG cell line (obtained from ECACC, UK) was maintained in Essential Minimum Eagle Medium (EMEM) supplemented with 10% (v/v) FBS, 2 mM L-glutamine, 1 mM sodium pyruvate and 1% (v/v) non-essential amino acids (NEAA) (Lonza, UK) in a 37 °C humidified incubator supplied with 5% $CO_2$. The 1321N1 and the U87MG cells were trypsinized and cultured overnight at a cell density of 3500 and 4000 cells/200 μl respectively in 96-well microtitre plates (Sarstedt, UK). The following day when the cells were 50–60% confluent, each concentration of the commercial drugs and novel compounds (see text) were added in quadruplicates and left for incubation for 48 h. After 48 h, 20 μl of the pre-warmed MTS reagent (Promega, UK) was added to each of the wells and the 96-well plates were incubated at 37 °C in a humidified incubator supplied with 5% $CO_2$ for 60 min. At the end of 60 min, the absorbance was recorded at 490 nm using a Tecan GENios Pro® microplate reader. The concentration of compound that inhibits 50% of cell growth ($IC_{50}$) compared to untreated vehicle controls containing ⩽0.5% absolute ethanol was calculated by nonlinear regression analysis. The untreated vehicle controls contained ⩽0.5% absolute ethanol or DMSO, as indole **4** was dissolved in absolute ethanol while indole **2** was dissolved in DMSO.

### 3.2. MTT assay

Three short-term glioblastoma multiforme (GBM) cell cultures (IN1472, IN1265 and IN1760) derived in our laboratory from tumour biopsies, as described by Lewandowicz et al.,[44] and the

established GBM cell line U251MG (obtained from ECACC, UK) were used in this study. Cells were maintained in Hams F10 nutrient mix (Invitrogen Ltd., UK) with 10% foetal calf serum in a 37 °C non-$CO_2$ incubator. The anti-proliferative effects of the compounds were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) assay as previously described.[45] Cells were seeded at a density of $10^3$ cells/well into 96-well plates and incubated in growth media for 48 h prior to treatment with indole **4** at concentrations between 0.01 and 1000 μM. The compound was renewed at 24 h intervals and each dose was tested in quadruplicate. Cells were then allowed a 24-h recovery period at 37 °C in compound-free media before media was aspirated and 100 μl of MTT solution (1 mg/mL in Ham's F10 medium without FCS) was added to each well. Four hours later, formazan crystals were dissolved in DMSO (100 μl/well) and the plates were agitated for 2–3 min before the optical density at 570 nm was determined on a plate reader (Fluoroskan Ascent, Thermo Labsystems, Cheshire, UK). The concentration of compound that inhibits 50% of cell growth ($IC_{50}$) compared to untreated controls was calculated by nonlinear regression analysis.

### 3.3. Flow cytometry

The 1321N1 cells were maintained in Dulbeccos's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) foetal bovine serum (FBS) and 2 mM L-glutamine, while the U87MG cell line was maintained in Essential Minimum Eagle Medium (EMEM) supplemented with 10% (v/v) FBS, 2 mM L-glutamine, 1 mM sodium pyruvate and 1% (v/v) non-essential amino acids (NEAA) (Lonza, UK) in a 37 °C humidified incubator supplied with 5% $CO_2$. The 1321N1 and the U87MG cells were trypsinized and cultured overnight at a cell density of $10^4$ cells/well/mL and were seeded in six well plates (BD Biosciences, UK) and were allowed to attach overnight; 2 mL of this cell suspension was added into each well resulting in a final concentration of 20,000 cells/well. Cells were labelled with sufficient amount of 25 μM carboxy-$H_2$DCFDA dye and were incubated for 30 min at 37 °C, protected from light. Cells were then treated with 500 μM of the test compounds and positive control [*tert*-butyl hydroperoxide (TBHP, 100 μM)] and negative control (indole **2**, 500 μM) for 60 min. Cells were gently washed with Hanks balanced salt solution (HBSS) warm buffer three times and then trypsinized, centrifuged and resuspended in 300 μl PBS. Cells were filtered through cell strainer caps (35 μm mesh) to obtain a single cell suspension and analyzed using a FACSAria flow cytometer using FITC fluorochrome at an excitation/emission of 488/519 nm (BD Biosciences). This same procedure and time point was used for ascorbic acid (500 μM) studies. The untreated vehicle controls contained ⩽0.5% absolute ethanol and DMSO as indole **4** was dissolved in absolute ethanol while TBHP and indole **2** were dissolved in DMSO.

### 3.4. Acridine orange assay

The same procedure was used to prepare the cells as in the flow cytometry experiments above with the carboxy-$H_2$DCFDA dye, up to the point of trypsinization and attaching overnight. After 24 h of incubation, cells were treated with 500 μM of indole **4** or indole **4** (500 μM) + 3-methyladenine (5 mM) for 60 min. After 60 min incubation with the compounds, the cells were stained with 3 μM acridine orange and incubated for 15 min at 37 °C, protected from light. Cells were gently washed with PBS, trypsinised, centrifuged and were resuspended in 300 μl PBS. Cells were filtered through cell strainer caps (35 μm mesh) to obtain a single cell suspension and analysed using a FACSAria flow cytometer using a PE-Texas Red fluorochrome at an excitation/emission of 488/615 nm (BD Biosciences). The untreated vehicle controls contained ⩽0.5% absolute ethanol as indole **4** was dissolved in absolute ethanol, while 3-MA was directly soluble in the cell culture medium.

### 3.5. Synthesis and characterization of indoles

#### 3.5.1. General synthetic procedures

Commercially available reagents were used as received without purification. Analytical thin layer chromatography (TLC) were performed with plastic-backed TLC plates coated with silica G/$UV_{254}$, in a variety of solvents. The plates were visualised by UV light (254 nm). Flash column chromatography was conducted with Davisil silica 60 Å (40–63 μm) under bellows pressure. Low resolution mass spectra were recorded on a Thermo Finnigan LCQ Advantage MAX using chemical ionisation (CI). $^1$H and $^{13}$C NMR spectra were recorded on a Bruker Avance DPX 250 (250 MHz) or a Bruker 400 (400 MHz) spectrometer. All chemical shifts ($\delta$) are quoted in parts per million (ppm) relative to a calibration reference of the residual protic solvent; $CHCl_3$ ($\delta_H$ 7.26, s) or DMSO ($\delta_H$ 2.53, m) was used as the internal standard in $^1$H NMR spectra, and $^{13}$C NMR shifts were referenced using $CDCl_3$ ($\delta_C$ 77.0, t) or DMSO ($\delta_C$ 40.5, sept) with broad band decoupling.

#### 3.5.2. Synthesis of 2-(2′-methoxyphenyl)-1*H*-indole, 7[16]

2′-Methoxyacetophenone (1.38 mL, 10 mmol) was mixed with phenylhydrazine (0.99 mL, 10 mmol) in ethanol (5 mL) and four drops of glacial acetic acid added. The pale yellow solution was heated to 80 °C with stirring for 1 h which produced a red/brown solution. The solvent was evaporated to yield the phenylhydrazone intermediate as red/brown oil. To this oil was added polyphosphoric acid (20 g) and the reaction heated to 120 °C with stirring for 2 h. After completion of the reaction (TLC) the reaction mixture was poured onto crushed ice, followed by the addition of NaOH until a neutral pH was reached. The product was extracted with DCM (3 × 50 mL) and the combined extracts washed with water (50 mL), brine (50 mL), dried ($MgSO_4$), filtered and the solvent evaporated. The product was purified using flash chromatography ($SiO_2$; 50% toluene:50% petroleum ether) to yield the title compound as an off-white solid (532 mg, 24%). $R_f$ 0.64 (100% toluene).

$^1$H NMR (250 MHz, $CDCl_3$) $\delta_H$ 4.03 (s, 3H), 6.93 (br s, 1H). 7.03–7.34 (m, 5H), 7.46 (d, $J$ = 8.0 Hz, 1H), 7.67 (dd, $J$ = 0.5 and 7.5 Hz, 1H), 7.87 (dd, $J$ = 1.5 and 8.0 Hz, 1H), 9.70 (br s, 1H); $^{13}$C NMR (62.5 MHz, $CDCl_3$) $\delta_C$ 55.8, 99.5, 110.9, 111.8, 119.8, 120.2, 120.5, 121.5, 121.8, 128.0, 128.3, 128.6, 135.9, 136.1, 155.7; $\nu_{max}$ (film, $cm^{-1}$) 3443, 1577, 1463, 1435, 1308, 1232; $m/z$ (CI) 224 ($[M+H]^+$, 100%).

#### 3.5.3. Synthesis of (2-phenyl-1*H*-indol-3-yl)methanol, 3[15]

2-Phenylindole-3-carboxaldehyde (300 mg, 1.36 mmol) and $NaBH_4$ (103 mg, 2.72 mmol) were stirred at reflux in ethanol (10 mL) for 1 min followed by stirring at room temperature for 2 h. 1% NaOH (10 mL) was added to the reaction mixture and the product was extracted with $Et_2O$ (3 × 10 mL). The combined extracts were dried ($MgSO_4$), filtered and the solvent evaporated to yield a crude product which was re-crystallised in PhMe:EtOAc:petroleum ether, yielding the title compound as a white solid 133 mg, 44%).

$^1$H NMR (250 MHz, DMSO-$d_6$) $\delta_H$ 4.81 (d, $J$ = 5.0 Hz, 2H), 5.10 (t, $J$ = 5.0 Hz, 1H), 7.14–7.28 (m, 2H), 7.48–7.53 (m, 2H), 7.64 (t, $J$ = 7.5 Hz, 2H), 7.80 (d, $J$ = 7.5 Hz, 1H), 7.92 (d, $J$ = 7.5 Hz, 2H), 11.47 (s, 1H); $^{13}$C NMR (62.5 MHz, DMSO-$d_6$), $\delta_C$ 54.8, 112.1, 113.3, 119.9, 122.6. 128.5, 128.9, 129.67, 129.71, 133.4, 136.8, 136.9; $\nu_{max}$ (film, $cm^{-1}$) 3488, 3183 (br s), 1638, 1491, 1452, 1392; $m/z$ (CI) 206 ($[M-OH]^+$, 100%).

### 3.5.4. Synthesis of 2-(2′-hydroxyphenyl)-1*H*-indole, 4[14]

1-(2-Hydroxyphenyl)-2-(2-nitrophenyl)ethanone,[14] (105 mg, 0.41 mmol) was dissolved in methanol (4.1 mL). Pd/C (10 mg, 10 wt %) was added and the flask evacuated and backfilled with hydrogen (three cycles). The reaction was then stirred under an atmosphere of hydrogen for 18 h. The reaction was filtered through Celite® and eluted with methanol (10 mL) and the solvent removed in vacuo. The crude product was purified by column chromatography on silica gel (10% EtOAc in petroleum ether) to give the title compound as a pale yellow solid (62 mg, 72%). $R_f$ 0.39 (30% ethyl acetate in petroleum ether).Mp (EtOAc:petroleum ether) 170–172 °C; $\nu_{max}/cm^{-1}$ 3500, 3425; $\delta_H$ (300 MHz; $CDCl_3$) 9.22 (1H, br s), 7.70 (1H, dd, $J$ = 1.6 and 7.8 Hz, Ar), 7.66 (1H, d, $J$ = 7.8 Hz, Ar), 7.42 (1H, d, $J$ = 8.1 Hz, Ar), 7.26–7.11 (3H, m, Ar), 7.04 (1H, td, $J$ = 1.1 and 7.6 Hz, Ar), 6.91 (1H, dd, $J$ = 0.9 and 8.1 Hz, Ar), 6.87 (1H, m, Ar), 6.0–5.0 (1H, br s); $\delta_C$ (75 MHz; $CDCl_3$) 152.0, 136.4, 134.8, 128.9, 128.4, 128.3, 122.2, 121.5, 120.4, 120.1, 119.1, 116.6, 111.0, 100.2; $m/z$ (ES+) 210 ($[M+H]^+$, 100%); found 210.0920, $C_{14}H_{12}NO$ ($M+H^+$) requires 210.0919.

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