NJC

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ROYAL SOCIETY OF CHEMISTRY

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DOI: 10.1039/C7NJ04616B

Synthesis of tetrahydro-1H-indolo[2, 3-b]pyrrolo[3, 2-c]quinolones *via* intramolecular oxidative ring rearrangement of tetrahydro-β-carbolines and their biological evaluation

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Abstract

A simple oxidative ring rearrangement of diversely substituted 1-(2-amminoaryl)-tetrahydro-β-carbolines has been developed to generate architecturally interesting tetrahydro-1H-indolo[2, 3-b]pyrrolo[3, 2-c]quinolones. This unique transformation involves four reaction center (aniline, C_1-carboline and C_2/C_3 of indole) and utilizes *tert*-butylhypochlorite as the reagent. The generic nature of the reaction was demonstrated by the synthesis of a wide variety of analogs **9a-j**. A putative reaction mechanism was proposed. Cytotoxicity screening of these compounds against three human cancer cells (A2780 ovarian and HCT116 colorectal carcinoma cell lines and A549 lung adenocarcinoma cell line) revealed selective inhibition of proliferation of A2780 human ovarian carcinoma cell line by one of the molecules **9a** with an IC_{50} of 14 μM. No cytotoxic activity was observed in human normal fibroblasts for concentrations up to 100 μM. Compound **9a** induce hyperpolarization of mitochondrial membrane potential of A2780 cell line leading to an increase of reactive oxygen species (ROS) that trigger cell death *via* apoptosis. Interestingly, compound **9a** was also able to induce cell death *via* autophagy. Compounds that induce apoptosis and autophagy, thus leading to cancer cells' death, are good candidates for cancer therapy.

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Introduction

Spiropyrroloxindole and quinolino indole alkaloids are interesting classes of compounds due to their abundance in nature and their intriguing biological activity.[1] In general they have imparted substantial inhibition against a bevy of cancer cells. For example Spirotryptostatin A and B, **1** and **2** (Scheme 1), isolated from fermentation broth of *Aspergillus fumigatin* inhibit G2/M progression of mammalian tsFT210 cells and synthetic spiroindole, **3**, a novel potent non-peptidic inhibitor of p53-MDM2 protein-protein interaction, critical for regulating p53 protein tumor suppressing activity(Figure 1).[2a-d] Additionally, Perophoramidine, **4** and Communecin B, **5** are structurally concomitant indole alkaloids possessing a unique 6, 5, 6, 6 ring system belong to a class of interesting natural products that elicit inhibitory activity in cancer cells by inducing apoptosis *via* PARP-1 cleavage (a signal irreparable DNA damage) and by disrupting microfilament in cultured mammalian cell, respectively (Figure 1). Interestingly Perophoramidine and dehalo analogs has exhibited inhibitory activity (IC_{50} ~ 60 – 70 μM) against a series of colorectal cancer cell lines (HCT116, HT29 and LoVo).[3a-d] Several procedures have been reported in the literature for the synthesis of spiropyrrolooxindole and 6, 5, 6, 6 ring systems belonging to Perophoramidine and Communecin B. These generally deploy, intramolecular mannich reaction of oxytryptamine hydrochloride, oxidative rearrangement of tetrahydro-β-carbolines, 1, 3-dipolar cycloaddition with azomethine ylides with oxindoylidine 3-ylidene acetate, radical cyclization of 2-bromo-4-methoxyaniline with Cbz-protected glycine ethyl ester and etc. for the synthesis of the synthesis of spiropyrroloxindole. As for the 6,5,6,6-ring system biosynthesis involving inverse electron demand hetero Diels Alder reaction of the ergot alkaloid aurantioclavine with tryptamine derivatives, aza-*o*-xylylene cycloaddition (by Funk and co-workers) and interrupted Fischer indolization based reaction by Garg *et al*. are few of the synthetic approaches.[4-5]

The anticancer legacy of these scaffolds prompted us to develop an efficient intramolecular ring rearrangement of 1-(2-aminoaryl)tetrahydro-β-carbolines for the production of compounds incorporating the two aforementioned heterocycles. The resulting compounds were subjected through phenotypic screening against A2780 ovarian, HCT116 colorectal and A549 lung adenocarcinoma cell lines followed by the phenotypic profiling of the most active compound.

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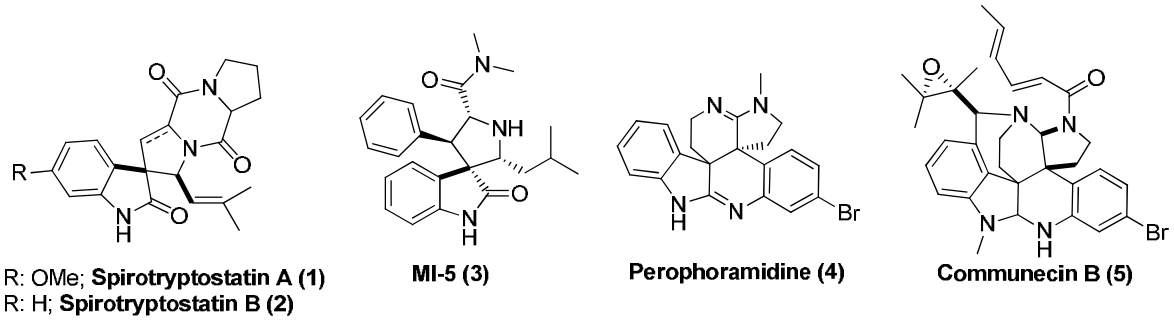

Figure 1. Design and proposed synthesis of hybrids inspired from representative natural products

Results and discussion

Chemistry

In one of our recent works we have demonstrated oxidative ring rearrangement reactions of 1-aryltetrahydro-β-carbolines to provide spiropyrrolooxindole.[6] In the reaction, water acted as the active nucleophile that prompted the ring rearrangement which in turn was initiated by C_2 bromination of carboline. Drawing inspiration from this results and similar oxidative ring rearrangement we explored *tert*-BuOCl mediated intramolecular ring rearrangement of 1-arylamino-tetrahydro-β-carbolines **8a-j** to provide the desired compounds **9a-j** (our initial exploratory effort with NBS, yielded the desired product in moderate yield).[7] *tert*-BuOCl is a versatile and economical oxidizing reagent used in the oxidation of alcohol, aldehydes, sulfides and hydroxylamines.[8] Very recently it was reported to promote C-H functionalization of C_1 of tetrahydro carbazoles.[9] Similar to these reported examples we assume that the initial step of this reaction involved C_3 chlorination of 1-(2-aminophenyl)tetrahydro-β-carboline **8a** to provide **A** (Scheme 1). The aniline present in the moiety prompted an intramolecular attack at C_2 to afford **B**. Subsequent imine formation in **B** promoted the migration of the C_1-C_2 bond to C_3, where the chloride is eliminated to generate the desired product **9a**.

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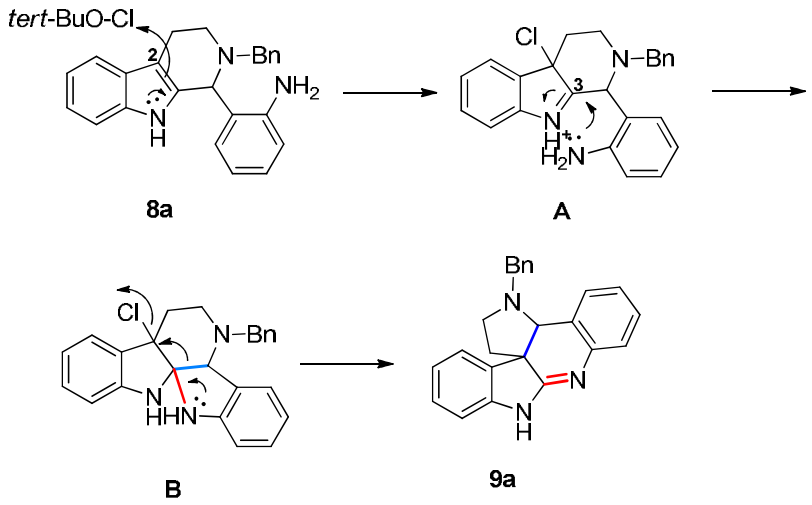
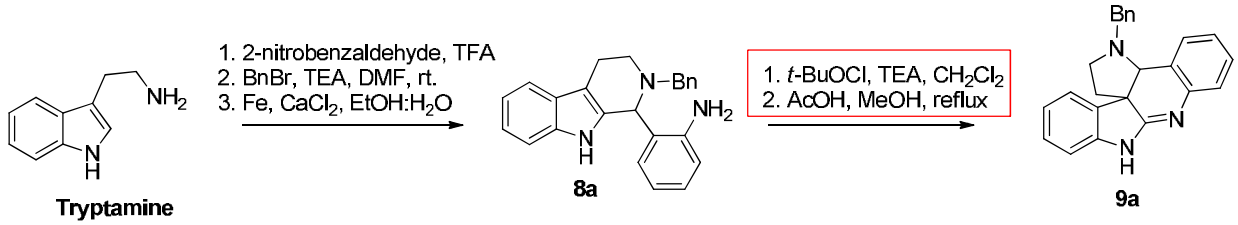


Scheme 1. Putative mechanism of the intramolecular oxidative ring rearrangement

This was demonstrated by reacting 1-arylaminotetrahydro-β-carboline **8a** with 1.1 equiv of *tert*-BuOCl and 1.2 equiv of triethyl amine in dichloromethane. Once the starting material is consumed the solvent was evaporated and the residue was refluxed in a 1:1 mixture of methanol and acetic acid to provide the final compound **9a**, which was isolated by column chromatography. This strategy provided advantages of harnessing inexpensive, air stable reagents like *tert*-BuOCl *via* an operationally simple procedure. Another noteworthy aspect is that the substrate **8a** could be synthesized in a three step sequence from economically viable tryptamine ($ 500/kg) involving Pictet-Spengler condensation with 2-nitrobenzaldehyde, benzyl protection of the carboline nitrogen and iron (Fe) calcium chloride ($CaCl_2$) reduction of the aryl nitro moiety. Interestingly **8a** was obtained clean enough to be used as such in the oxidative ring rearrangement (Scheme 2).



Scheme 2. Intramolecular oxidative rearrangement of **8a** in presence of tert-BuOCl and TEA along with AcOH

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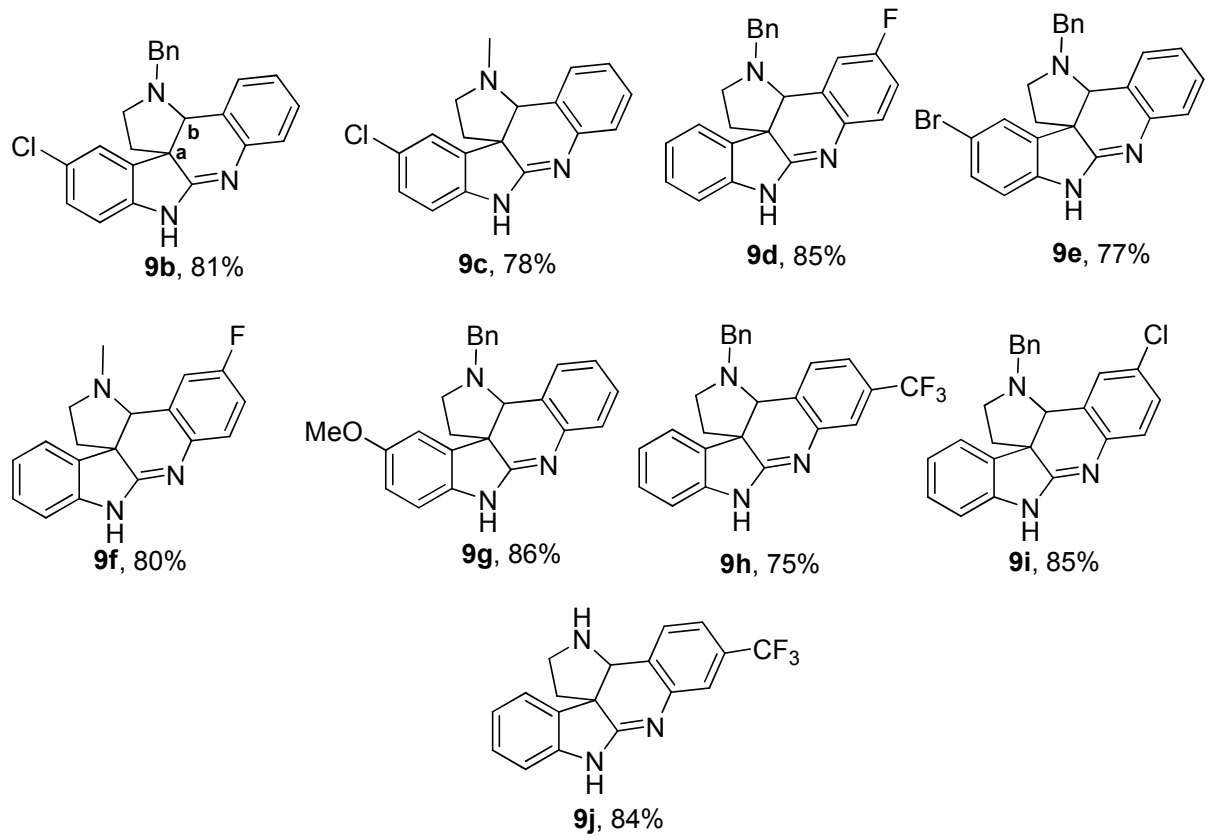
The generic nature of this method was demonstrated by subjecting various 1-aryltetrahydro-β-carbolines **8a-j** to the conditions outlined in Scheme 3. The outcome illustrates that the R groups substituting the aryl moiety as C_1 of the carbolines, R_1 functionality on the carboline nitrogen and R_2 on the indole benzene ring substantiated no significant effect on the yield of the reaction. R included trifluoromethyl, chloro, fluoro and amine functionalities at various position of the C_1 aromatic moiety, R_1 typically encompassed methyl and benzyl functionality and R_2 included bromo, chloro and trifluromethoxy group at C_5 position of the indole benzene ring. The average yield of the desired products **9b-j** ranged from 72-84% (Scheme 3). Interestingly the reaction generates two stereogenic centers, **a** and **b** (refer structure **9b**) within the molecules. The compounds have [3,4,0] bicyclic system at the core, which we envision to favor a cis fusion. The NMR spectra further indicates the presence of only one diastereomer.



Scheme 3. Library of natural product inspired hybrid molecules

***In vitro* screening**

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As discussed in the introduction, both spiropyrrolooxindole and 6, 5, 6, 6, indoloquinolone containing natural products have exhibited substantial inhibitory activity against cancer cells. Consequently, it was pragmatic for us to investigate the inhibition induced by the library of our molecules against the proliferation of several cancer cell lines such as A2780 ovarian cancer cell line, HCT116 human colorectal carcinoma cell line and A549 lung adenocarcinoma cells and normal human fibroblasts using the MTS assay (Table 1).

Compound **9a** exhibited a high cytotoxic effect against A2780 cell line, with a low relative IC_{50} value (14.2 ± 0.3 μM), a moderate cytotoxicity against HCT116 cell line (relative IC_{50} 37.3 ± 0.4 μM) and no cytotoxic activity in A549 ((relative IC_{50} > 100 μM) (Table 1). Of relevance, compound **9a** has no cytotoxic activity in human normal fibroblasts demonstrating its selectivity towards ovarian carcinoma cell line (selectivity index – 7-fold) (Table 1).

All the other compounds did not present any cytotoxic activity in HCT116, A549 cancer cell lines or normal human fibroblasts (Table 1). However, they presented high to median cytotoxicity in A2780 cell line (**9d**>**9e**>**9h**>**9f**>**9g**, **9i-j**) (Table 1). A close scrutiny of the structure activity relationship between the functionalities that encrusted our library of molecules against the antiproliferative effects they impose against the cancer cells indicated not much scope of modification. No functional groups be it electron donating or electron withdrawing at the terminal aromatic rings of the molecules are tolerated, as they lead to the reduction of their antagonistic activity on the cancer cells when compared to the unsubstituted analog **9a**. Additionally, the benzyl functionality at the pyrrolidine nitrogen of our compounds proved to be the best substituent as deprotection of it to NH- or replacement with a methyl functionality proved to be detrimental to the antiproliferative effect of the analogs as illustrated by compounds **9h**/ **j** (30.7 μM *versus* no activity, respectively) and **9d**/ **9f** (23.6 μM *versus* 38.9 μM).

This selective antiproliferative effect of our molecules at low micromolar concentration, accentuates the eminence of our natural product inspired compounds; usually the expected outcome from such unbiased early explorations of molecules are poor (high micromolar). This modest hit further highlights the importance of incorporating natural product scaffolds in discovering novel bioactive molecules.

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Table 1. Relative IC_{50} values for compounds **9a-j** in tumor cell lines (A2780, HCT116 and A549) and normal fibroblasts

Entry	Compounds	IC_{50} (μM)			
		A2780	HCT116	A549	Fibroblast
1	**9a**	14.2	37.3	>100	>100
2	**9b**	59.6	>100	"	"
3	**9c**	72.3	"	"	"
4	**9d**	23.6	"	"	"
5	**9e**	26.4	"	"	"
6	**9f**	38.9	"	"	"
7	**9g**	100	"	"	"
8	**9h**	30.7	"	"	"
9	**9i**	>100	93.3	"	"
10	**9j**	"	>100	"	"

Cells were treated with increasing concentrations of compound **9a** for 48 h and cell viability was determined by MTS assay. Data were normalized against the control treated with 0.1 % (v/v) DMSO. Results are the mean of at least 3 independent biological MTS assays (±SEM).

Since **9a**, was the most potent among our library of molecules we investigated into the mechanism of cytotoxic action induced by compound **9a** against A2780 ovarian carcinoma cell line, apoptosis and autophagy were assessed. Hoechst nuclei staining of A2780 cells in absence (DMSO as control) or presence of compound **9a** at its IC_{50} (Table 1) was assessed (Figure 2).

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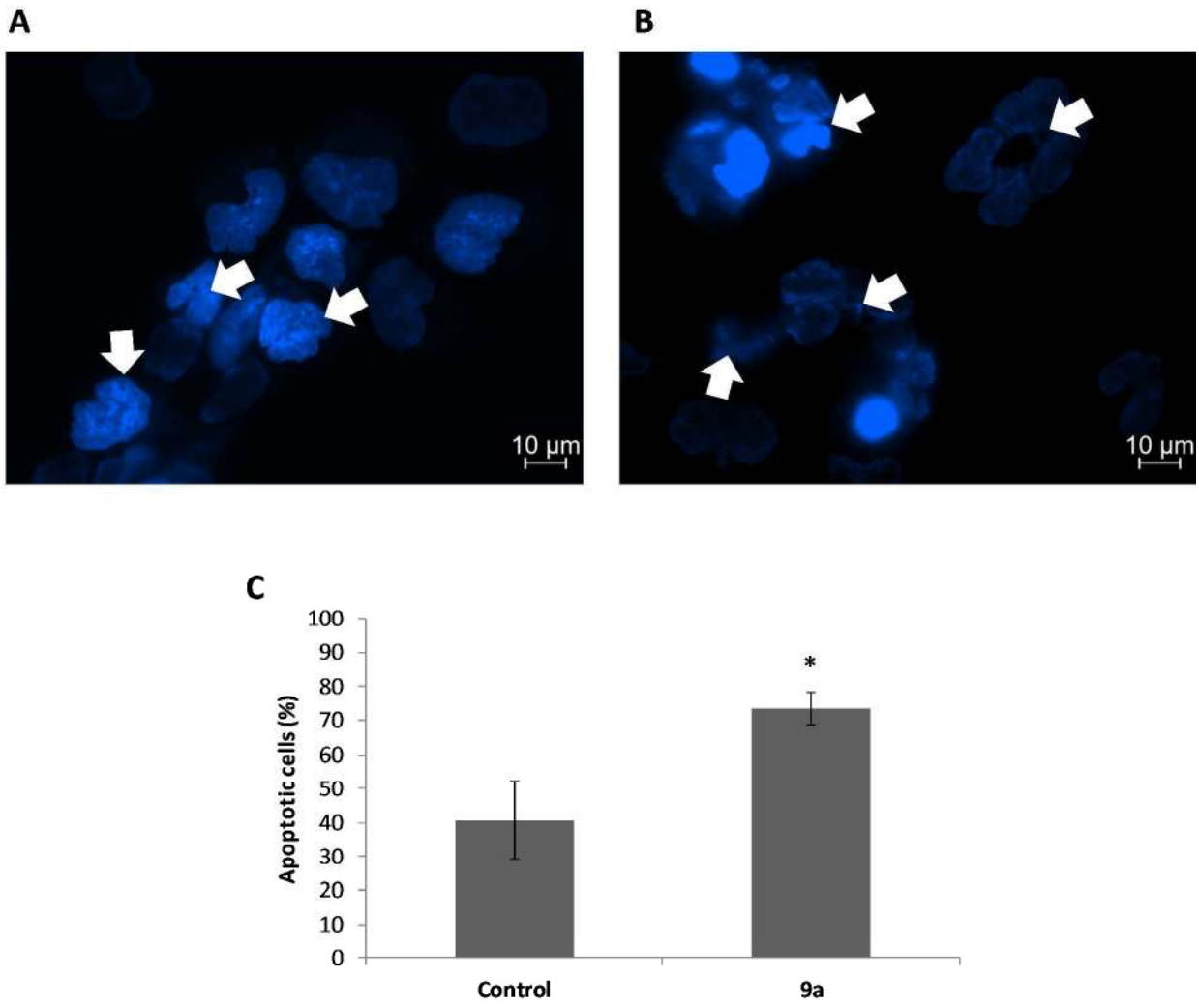

Figure 2. Hoechst staining (excitation and fluorescence emission 352 and 461 nm, respectively) of A2780 cell line for visualization of apoptotic nuclei. Cells were grown in DMEM culture medium supplemented with 10% fetal bovine serum in the presence of: **A)** DMSO control (0.1 % v/v), **B)** compound**9a** (at IC_{50}). Plates were photographed in an AXIO Scope (Carl Zeiss, Oberkochen Germany). Three random microscopic fields per sample with circa 50 nuclei were counted. White arrows indicate chromatin condensation. **C)** % of apoptotic cells after exposition of A2780 cells to control vehicle (DMSO) or the compound **9a**. *p-value < 0.05 relative to apoptosis in cells incubated with DMSO.

Hoechst 33258 (2'-[4-ethoxyphenyl]-5-[4-methyl-1-piperazinyl]-2,5'-bi-1H-benzimidazole trihydrochloride trihydrate) is a cell-permeable DNA stain that binds preferentially to adenine-thymine (A-T) regions of the DNA and it can detect nuclear damage and apoptosis. Normal

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nuclei show non-condensed chromatin uniformly distributed over the entire nucleus, whereas apoptotic nuclei show condensate or fragmented chromatin. Some cells form apoptotic bodies.[10]

Figure 2 shows a clear increase of apoptotic markers, such as chromatin condensation and nuclear structure abnormalities (arrows in Figure 2) in A2780 cells incubated with the compound **9a**. The level of cell death due to apoptosis observed in Figure 1 for compound **9a** is 1.5 x higher that control cells (DMSO).

Despite its ability to induce cell death via apoptosis, we also evaluated if this complex could induce other types of programmed cell death (PCD) such as Type II - autophagic cell death. Autophagy is a catabolic process that digests cellular contents within lysosomes and may be accelerated by a variety of cellular stressors such as nutrient starvation, DNA damage, and organelle damage.[11]

Figure 3 shows that compound **9a** can also induce autophago lysosomes accumulation, a characteristic of autophagy. Rapamycin was used as a positive marker for autophagy (Figure 3).

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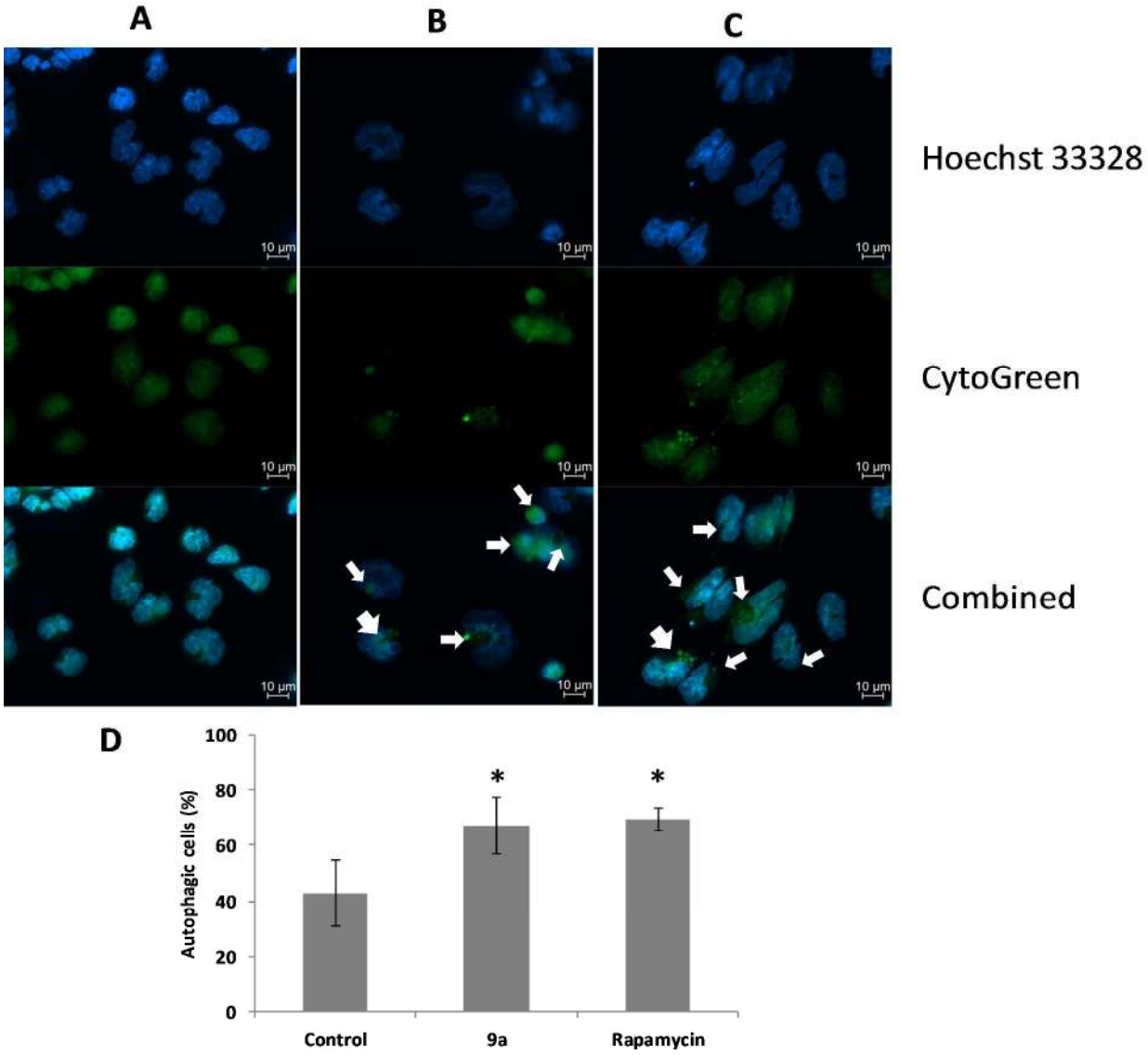

Figure 3. Autophagic cell death evaluation using the CYTO-ID Autophagy detection assay in the presence of DMSO as vehicle control (0.1 % v/v) (**A**), compound **9a** (**B**) andRapamycin as an autophagy marker (**C**) and assessed by fluorescence microscopy. Merge images of nuclei that were stained with DAPI (in blue; excitation and fluorescence emission 358 and 461 nm, respectively) and autophagolysosomes that were stained in green (excitation and fluorescence emission 463 and 534 nm, respectively). White arrows point to accumulation of autophagolysosomes. Plates were photographed in an AXIO Scope (Carl Zeiss, Oberkochen Germany). **D**) % of Autophagic A2780 cells in the presence of compound **9a**. DMSO was used

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as vehicle control and Rapamycin as autophagic positive marker. *p-value < 0.05 relative to DMSO autophagy.

Mitochondrial dysfunction has been demonstrated to participate in the induction of apoptosis and has even been suggested to be central to the apoptotic-signaling pathway.[12] The membrane-permeant JC-1 dye is widely used in apoptosis studies as an indicator of mitochondrial membrane potential in a variety of cell types.[13]

In this regard, the effect of compound **9a** in mitochondrial membrane potential ($\Delta\Psi_M$) of A2780 cell line was evaluated (Figure 4).

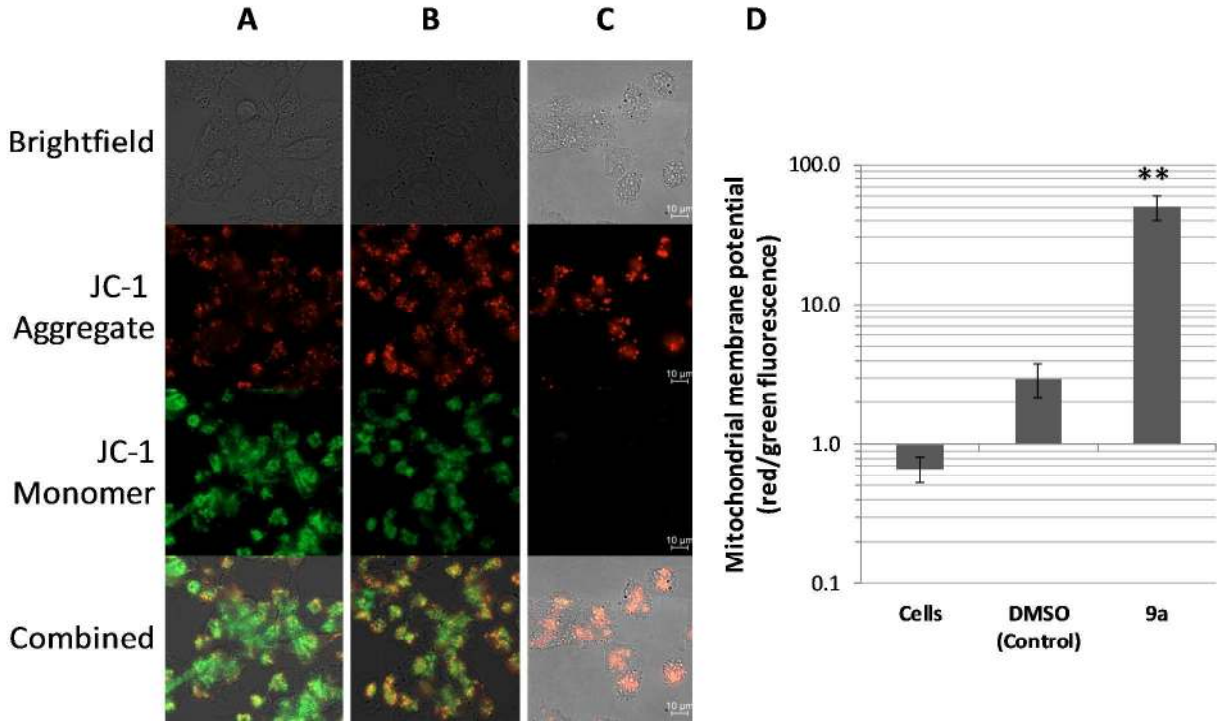

Figure 4. Mitochondrial membrane potential. Cells were incubated in the absence (cells) (**A**) or presence of 0.1% (v/v) DMSO (control) (**B**) or compound **9a** (at IC_{50}) (**C**) for 48 h. **D**) Fluorescence ratio red to green (JC-1 aggregate/JC-1 monomer).Plates were photographed in an AXIO Scope (Carl Zeiss, Oberkochen Germany). Results are the mean ± SEM from three independent biological assays. Statistical analysis was performed by One-Way ANOVA (** $p \leq 0.01$ relative to DMSO).

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As we can observe in Figure 4, a high increase of the red versus green fluorescence in A2780 cells after exposure to compound **9a** can be due to the hyperpolarization of the mitochondrial membrane. Interestingly, mitochondrial hyperpolarization appears to be the earliest change associated with apoptotic pathways and leads to uncoupling of oxidative phosphorylation (i.e. continued reactive oxygen species (ROS) production in the absence of ATP synthesis), which disrupts mitochondrial membrane potential ($\Delta\Psi m$) and damages integrity of the inner mitochondrial membrane. Disruption of $\Delta\Psi m$ has been proposed as the point of no return in cell death signaling.[14-15] These results are in agreement of the apoptosis induction in the presence of compound **9a** (Figure 2).

Taking into consideration these results, the levels of ROS in the absence or presence of compound **9a** was evaluated (Figure 5).

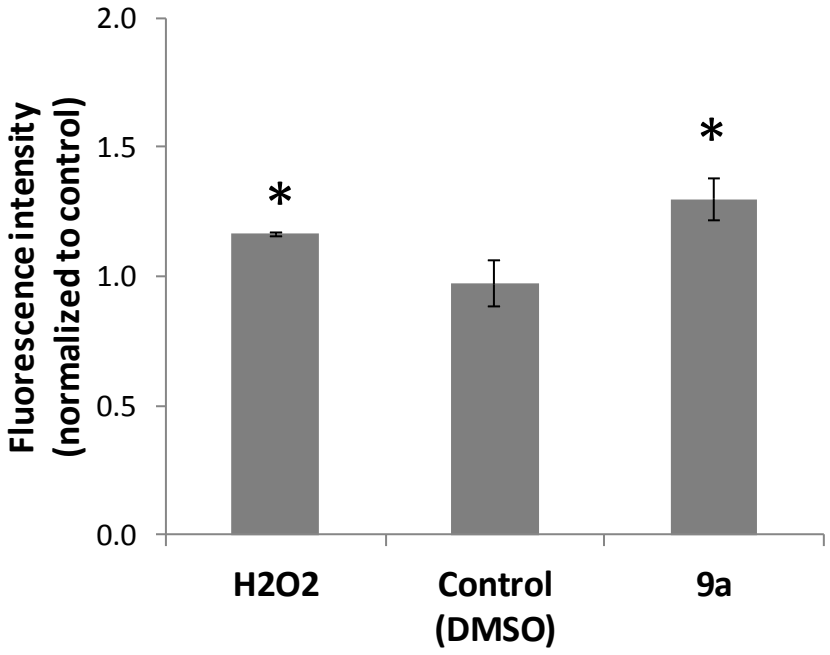

Figure 5. Quantification of the fluorescence intensity of H2DCF-DA dye in A2780 cells after exposition to hydrogen peroxide (H_2O_2; 25 µM), 0.1 % v/v DMSO (control) or compound **9a** (at its IC_{50}) for 48 h. Results are the mean ± SEM of three independent biological experiments and were normalized to control cells (0.1 % v/v DMSO). H_2O_2 was used as a positive control for ROS induction. Statistics analysis was performed by One-Way ANOVA ((* $p \leq 0.05$).

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The cell-permeant 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) (also known as dichlorofluorescin diacetate) is a chemically reduced form of fluorescein used as an indicator for reactive oxygen species (ROS) in cells.[16] This cell-permeable non-fluorescent compound accumulates within cells upon de-acetylation reacting within tracellular ROS, turning highly fluorescent.[17]

As observed in Figure 5 there is a statistical significant increase of ROS production both in the presence of H_2O_2 or compound **9a**. Increase in the rate of ROS production is responsible for the accumulation of ROS-associated damages in biological molecules such as DNA, proteins, and lipids, and may result in progressive cell dysfunctions leading to apoptosis.[18-19]

Taken together these results conforms that compound **9a** induce membrane hyperpolarization and an increase of ROS production that lead to an increase of cell death through apoptosis. Additionally, compound **9a** is also able to induce cell death *via* autophagy.

Compounds that induce apoptosis and autophagy, thus leading to cancer cells' death, are good candidates for cancer therapy.[20] Therefore, compound **9a** is a promising early lead to ultimately identify a suitable candidate for combinatory therapy modalities with standard therapies in ovarian cancer potentiating cancer cell death.

Conclusions

Herein we have reported synthesis of tetrahydro-1H-indolo[2, 3-b]pyrrolo[3, 2-c]quinolones. They are novel natural product inspired molecules, which were accessed through a four step linear sequence starting from tryptamine and its derivatives. Interestingly the intermediates of this sequence were clean enough to be taken forward and only the final compounds were purified. The key step involved atypical intramolecular oxidative ring rearrangement of substituted 1-(2-nitroaryl)-tetrahydro-β-carbolines (**8a-j**). A focussed library of ten compounds (**9a-j**) was prepared based on a putative mechanism of transformation supported by literature evidence. Since the original natural products that inspired this strategy had an anticancer legacy, the antiproliferative potential of the library was examined towards human ovarian carcinoma (A2780), colorectal carcinoma (HCT116) and lung adenocarcinoma (A549) cell lines. Compound **9a** was found to have the highest cytotoxic effect compared to the other compounds particularly in A2780 ovarian carcinoma (IC_{50} of 14 μM). Indeed, compound **9a** exhibits a high

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selectivity (more than 7x) towards this type of cancer cells when compared to normal human fibroblasts (no cytotoxic activity for concentrations up to 100 μM). against colorectal carcinoma cells, with IC_{50} lower than that of cisplatin (10.5 ± 0.3 μM compared to 15.2 ± 0.55 μM, respectively). The viability loss induced by compound **9a** was due to the induction of autophagy and it agrees with Hoechst 33258 staining and the typical morphological apoptotic characteristics like chromatin condensation and nuclear fragmentation. Compound **9a** was shown to hyperpolarize the membrane potential leading to an increase of ROS production and finally a point-of-no-return – cell death via apoptosis.

Taken together these results demonstrate that in comparison to the other compounds, compound **9a** provides several advantages namely its high cytotoxic potential in A2780 cell line with the simultaneously no cytotoxicity in healthy cells, making this a positive feature for further development particularly towards ovarian carcinoma.

We intend to realise the protein target of **9a**, *via* deconvolution technique to develop a much potent candidate through molecular docking and structure activity relationship campaign. This is presently ongoing in our lab.

EXPERIMENTAL SECTION

Chemistry

General. All reactions were carried out in flame-dried glassware with magnetic stirring. Unless otherwise noted, all experiments were performed under argon atmosphere. All reagents were purchased from Sigma Aldrich, Acros organics or Alfa Aesar. Solvents were treated with 4 Å molecular sieves or sodium and distilled prior to use. Purifications of reaction products were carried out by column chromatography using Chem Lab silica gel (230-400 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded with tetramethylsilane (TMS) as internal standard at ambient temperature unless otherwise indicated in Bruker AVHDN at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Chemical shifts are reported in parts per million (ppm) and coupling constants are reported as Hertz (Hz). Splitting patterns are designated as singlet (s), broad singlet (bs), doublet (d), triplet (t). Splitting patterns that could not be interpreted or easily visualized are designated as multiple (m). The Mass Spectrometry analysis was done on the 6540 UHD

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Accurate-Mass Q-TOF LC/MS system (Agilent Technologies) equipped with Agilent 1290 LC system. ^{1}H and ^{13}C NMR data included in supplementary information

General experimental procedure for the synthesis of the final compounds

To a stirred solution of appropriate β-aryl amines (0.5 mmoles, 1 equiv.) and various *o*-nitrobenzaldehydes (0.5 mmoles, 1 equiv.) in dichloromethane (10 mL) was added trifluro acetic acid (0.5 mmoles, 1 equiv.) at ambient temperature. The resulting mixture was then stirred under argon for 10-16h. Once thin layer chromatography (TLC) confirms the complete consumption of the starting materials, the reaction mixture was concentrated under reduced pressure and minimum volume of dichloromethane was added to the resulting solution and stored at 0°C for 30 minutes. The products crystallized from the solution, filtered, washed with dichloromethane and dried to provide the crude compounds which were used in the next step without purification.

The above compound was dissolved in dimethylformamide (DMF) (10 mL) and triethyl amine (TEA) (1.5 mmoles, 3 equiv.) was added. Then the reaction mixture was then cooled to 0°C before adding methyl iodide/benzyl bromide (0.75 mmoles, 1.5 equiv.). The resulting solution was allowed to stir at room temperature for 30 minutes. Once thin layer chromatography (TLC) confirms the complete consumption of the starting materials, the reaction mixture was quenched with water and was extracted with ethyl acetate (2 ×10 mL). The organic layer was separated, dried over anhydrous sodium sulphate (Na_2SO_4) and was evaporated to obtain the desired crude compound. This crude compound was further used for next step without purification.

The crude intermediate from the previous step was dissolved in EtOH : Water (9.5 : 0.5 mL) mixture and treated with Fe powder (0.75 mmoles, 1.5 equiv.) and $CaCl_2$ (0.75 mmoles, 1.5 equiv.) at room temperature. The resulting mixture was warmed to 60°C and stirred for 45 minutes. Once thin layer chromatography (TLC) confirms the complete consumption of the starting materials, the reaction was cooled to room temperature and concentrated under reduced pressure to obtain the desired crude compounds **8a-j**.

8a-j were dissolved in dichloromethane at room temperature and was further treated with triethyl amine (1.2 equiv.) and *tert*-butylhypochlorite (1.1 equiv). The resulting mixture was further stirred at same temperature for about 4h. Once thin layer chromatography (TLC) confirms the complete consumption of the starting materials, the reaction was quenched with water and the

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organic layer was isolated and evaporated. The crude reaction mixture was dissolved in 1:1 acetic acid: methanol (10 mL) mixture and was refluxed for ~ 3h. Then the reaction was quenched with solid $NaHCO_3$, filtered and evaporated to obtain the desired crude compounds. The crude solids were purified by flash column chromatography (FCC) using ethyl acetate-hexane as the mobile phase, to provide the final compounds **9a-j** as solid or semi solid form.

1-benzyl-2,3,8,13b-tetrahydro-1H-indolo[2,3-b]pyrrolo[3,2-c]quinoline (9a):

Following the general procedure tryptamine, o-nitrobenzaldehyde and benzyl bromide provided **9a** (84% yield). ^{1}H NMR (400 MHz; $CDCl_3$): 7.57 (s, 1H), 7.55 (s, 1H), 7.47 (d, *J* = 4 Hz, 1H),7.41 (d, *J* = 8 Hz, 3H), 7.33 (d, *J* = 4 Hz, 3H), 7.19 (d, *J* = 8, 16 Hz, 1H), 7.06 (t, *J* = 4, 16 Hz, 1H), 7.00 (d, *J* = 8 Hz, 1H), 4.59-4.50 (m, 2H), 4.41 (t, *J* = 12 Hz, 1H), 3.13 (t, *J* = 4 Hz, 1H), 3.02 (d, *J* = 4 Hz, 1H), 2.55-2.45 (m, 1H), 1.79 (s, 1H). ^{13}C NMR (100 MHz; DMSO-d_6): 171.6, 140.3, 129.5, 128.4, 127.8, 127.7, 126.9, 125.9, 122.3, 122.3, 121.0, 66.9, 60.6, 49.9, 38.1. IR (neat, ν cm^{-1}): 2936, 2864, 1648, 1638, 1628, 1599, 1564, 1465, 1202. HRMS (ESI-TOF) m/z: $[M + H]^+$calculated for ($C_{24}H_{22}N_3$) 352.1808, found 352.1801. M.p.- 133 °C

1-benzyl-5-chloro-2,3,8,13b-tetrahydro-1H-indolo[2,3-b]pyrrolo[3,2-c]quinolone (9b):

Following the general procedure 5-Chlorotryptamine, o-nitrobenzaldehyde and benzyl bromide provided **9b** (81% yield).^{1}H NMR (400 MHz; DMSO-d_6): 7.51 (t, *J* = 4 Hz, 2H), 7.44-7.36 (m, 3H), 7.34-7.25 (m, 2H), 7.22 (d, *J* = 7.22, 2H), 7.18-7.12 (m, *2*H), 6.99-6.88 (m, *2*H), 4.58 (s,1H), 4.53-4.48 (m, 1H), 4.42-4.33 (m, 1H), 2.90-2.88 (m, 2H), 2.25-2.17 (m, 1H), 1.66-1.62 (m, 1H). ^{13}C NMR (100 MHz; DMSO-d_6): 179.6, 171.4, 159.7, 157.4, 140.8, 128.9, 128.9, 128.5, 127.5, 123.1, 121.4, 116.1, 115.9, 115.0, 114.8, 67.4, 61.0, 50.6, 38.4. IR (neat, ν cm^{-1}): 2931, 2827, 1654, 1609, 1573, 1462, 1123, 747. HRMS (ESI-TOF) m/z: $[M + H]^+$calculated for ($C_{24}H_{21}N_3Cl$) 386.1419, 388.1398 found 386.1420, 388.1396 M.p.- 148 °C

5-chloro-1-methyl-2,3,8,13b-tetrahydro-1H-indolo[2,3-b]pyrrolo[3,2-c]quinolone (9c):

Following the general procedure 5-Chlorotryptamine, o-nitrobenzaldehyde and methyl iodide provided **9c** (78% yield).^{1}H NMR (400 MHz; DMSO-d_6): 7.44 (d, *J* = 8 Hz, 1H), 7.29-7.25 (m, 2H), 7.34-7.25 (m, *2*H), 7.18-7.12 (m, 2H), 7.00-6.93 (m, *2*H), 4.27 (s, 1H), 2.99 (s, 3H), 2.98-2.91 (m, 2H), 2.22-2.15 (m, 1H), 1.66-1.61 (m, 1H). ^{13}C NMR (100 MHz; DMSO-d_6): 171.1,

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159.3, 157.0, 137.0, 136.0, 128.0, 122.6, 120.6, 120.8, 115.4, 115.2, 114.5, 114.3, 113.4, 67.5, 54.9, 53.27, 51.5, 43.2, 38.6. IR (neat, ν cm^{-1}): 2251, 2127, 1660, 1650, 1578, 1485, 831. HRMS (ESI-TOF) m/z: $[M + H]^+$calculated for ($C_{18}H_{17}N_3Cl$) 310.1106, 312.1081 found 310.1109, 312.1084. M.p.- 123 °C

1-benzyl-12-fluoro-2,3,8,13b-tetrahydro-1H-indolo[2,3-b]pyrrolo[3,2-c]quinolone (9d):

Following the general procedure tryptamine, 5-fluoro-2-nitrobenzaldehyde and benzyl bromide provided **9d** (85% yield).^{1}H NMR (400 MHz; $CDCl_3$): 7.55 (s,1H), 7.53 (s, 1H), 7.41 (dd, *J* = 4, 12 Hz, 3H), 7.31 (dd, *J* = 8, 20 Hz, 3H), 7.20 (d, *J* = 8 Hz, 1H), 7.12 (dd, *J* = 4, 12 Hz, 1H), 7.05 (t, *J* = 8 Hz, 2H), 6.90 (td, *J* = 4, 8 Hz, 1H), 4.51 (t, *J* = 16 Hz, 2H), 4.41 (d, *J* = 12 Hz, 1H), 3.14 (t, *J* = 8 Hz, 1H), 3.05-2.98 (m, 1H), 2.49-2.41 (m, 1H), 1.79 (dd, *J* = 8, 12 Hz, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): 179.1, 172.4, 160.7, 158.3, 148.7, 139.8, 136.6, 135.2, 128.8, 128.8, 128.6, 127.5, 123.0, 121.8, 121.0, 116.2, 115.9, 115.3, 114.0, 67.5, 61.4, 54.1, 50.4, 38.3. IR (neat, ν cm^{-1}): 2920, 2850, 1640, 1577, 1505, 1433. HRMS (ESI-TOF) m/z: $[M + H]^+$calculated for ($C_{24}H_{21}N_3F$) 370.1714, found 370.1710. M.p.- 158 °C

1-benzyl-5-bromo-2,3,8,13b-tetrahydro-1H-indolo[2,3-b]pyrrolo[3,2-c]quinolone (9e):

Following the general procedure 5-bromotryptamine, *o*-nitrobenzaldehyde and benzyl bromide provided **9e** (77% yield).^{1}H NMR (400 MHz; $CDCl_3$): 7.55 (s, 1H), 7.53 (s, 1H), 7.41 (dd, *J* = 8, 12 Hz, 3H), 7.31 (dd, *J* = 4, 16 Hz, 3H), 7.20 (d, *J* = 8 Hz, 1H), 7.11 (dd, *J* = 4, 8 Hz, 1H), 7.05 (t, *J* = 4 Hz, 2H), 6.90 (td, *J* = 4, 8 Hz, 1H), 4.51 (t, *J* = 16 Hz, 2H), 4.41 (d, *J* = 12 Hz, 1H), 3.14 (t, *J* = 8 Hz, 1H), 3.05-2.99 (m, 1H), 2.49-2.41 (m, 1H), 1.79 (dd, *J* = 8, 12 Hz, 1H).^{13}C NMR (100 MHz; $CDCl_3$): 179.3, 172.6, 160.9, 158.5, 148.9, 140.0, 136.8, 135.4, 129.0, 128.8, 127.7, 123.2, 122.0, 121.2, 116.4, 116.1, 115.5, 115.3, 114.2, 67.7, 61.6, 54.3, 50.6, 38.5. HRMS (ESI-TOF) m/z: $[M + H]^+$calculated for ($C_{24}H_{21}N_3Br$) 432.0896, 430.0913 found 432.0864, 430.0887. M.p.- 168 °C

12-fluoro-1-methyl-2,3,8,13b-tetrahydro-1H-indolo[2,3-b]pyrrolo[3,2-c]quinolone (9f):

Following the general procedure tryptamine, 5-fluoro-2-nitrobenzaldehyde and methyl iodide provided **9f** (80% yield).^{1}H NMR (400 MHz; $CDCl_3$): 7.33 (d, *J* = 8 Hz, 1H), 7.29 (d, *J* = 4 Hz, 1H), 7.23 (s, 1H), 7.21 (d, *J* = 4 Hz, 1H), 7.17 (d, *J* = 8 Hz, 1H), 7.02 (t, *J* = 8 Hz, 1H), 6.98 (t, *J*

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= 4 Hz, 1H), 6.90 (td, *J* = 4, 8 Hz, 1H), 4.18 (s, 1H), 3.21 (t, *J* = 8 Hz, 1H), 3.09 (s, 1H), 3.05-3.00 (m, 1H), 2.49-2.41 (m, 1H), 1.78 (dd, *J* = 8, 12 Hz, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): 173.0, 160.7, 158.3, 149.5, 137.3, 135.2, 129.2, 129.2, 128.5, 123.0, 121.6, 120.8, 115.6, 114.9, 114.0, 68.5, 54.5, 52.0, 43.7, 39.0. HRMS (ESI-TOF) m/z: $[M + H]^+$calculated for ($C_{18}H_{17}N_3F$) 294.2184, found 294.2180. M.p.- 133 °C

1-benzyl-5-methoxy-2,3,8,13b-tetrahydro-1H-indolo[2,3-b]pyrrolo[3,2-c]quinolone (9g):

Following the general procedure 5-methoxytryptamine, o-nitrobenzaldehyde and benzyl bromide provided **9g** (86% yield). ^{1}H NMR (400 MHz; $CDCl_3$): 7.53 (t, *J* = 8 Hz, 2H), 7.45 (d, *J* = 8 Hz, 1H), 7.39 (t, *J* = 8 Hz, 2H), 7.32 (d, *J* = 4 Hz, 1H), 7.19 (dd, *J* = 8, 16 Hz, 3H), 7.10 (d, *J* = 12 Hz, 1H), 7.02 (t, *J* = 8 Hz, 1H), 6.96 (d, *J* = 8 Hz, 1H), 6.85 (d, *J* = 8 Hz, 1H), 4.52 (s, 1H), 4.45 (dd, *J* = 4, 16 Hz, 2H), 3.86 (s, 1H), 3.08-3.01 (m, 2H), 2.51-2.40 (m, 1H), 1.81 (t, *J* = 8 Hz, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): 173.2, 140.6, 139.6, 139.3, 137.0, 132.4, 131.3, 131.1, 129.5, 127.5, 127.3, 125.9, 125.4, 123.5, 117.8, 117.2, 114.6, 67.2, 66.8, 60.6, 56.0, 49.6, 38.1. HRMS (ESI-TOF) m/z: $[M + H]^+$calculated for ($C_{25}H_{24}N_3O$) 382.1914, found 382.1915. M.p.- 149 °C

1-benzyl-11-(trifluoromethyl)-2,3,8,13b-tetrahydro-1H-indolo[2,3-b]pyrrolo[3,2-c]quinolone (9h):

Following the general procedure tryptamine, 2-nitro-5-(trifluoromethyl) benzaldehyde and benzyl bromide provided **9h** (75% yield).^{1}H NMR (400 MHz; $CDCl_3$): 7.69 (s, 1H), 7.67 (s, 2H), 7.56 (s, 4H), 7.48 (s, 3H), 7.41 (d, *J* = 4 Hz, 2H), 7.24 (s, 1H), 4.70 (t, *J* = 16 Hz, 2H), 4.58 (d, *J* = 16 Hz, 1H), 3.30 (d, *J* = 4 Hz, 1H), 2.64 (d, *J* = 8 Hz, 1H), 1.99 (d, *J* = 4 Hz, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): 173.4, 149.5, 139.9, 139.7, 137.4, 136.4, 130.6, 130.6, 129.0, 128.9, 128.7, 127.6, 123.0, 122.2, 120.1, 116.1, 114.6, 67.4, 61.6, 54.8, 50.6, 38.6. IR (neat, ν cm^{-1}): 2920, 2850, 1640, 1577, 1505, 1433. HRMS (ESI-TOF) m/z: $[M + H]^+$calculated for ($C_{25}H_{21}N_3F_3$) 420.1682, found 420.1680. M.p.- 165 °C

1-benzyl-12-chloro-2,3,8,13b-tetrahydro-1H-indolo[2,3-b]pyrrolo[3,2-c]quinolone (9i):

Following the general procedure tryptamine, 5-chloro-2-nitrobenzaldehyde and benzyl bromide provided **9i** (85% yield).^{1}H NMR (400 MHz; $CDCl_3$): 7.55 (s, 1H), 7.54 (s, 1H), 7.42 (t, *J* = 8 Hz, 3H), 7.32 (dd, *J* = 8, 16 Hz, 3H); 7.18 (d, *J* = 8 Hz, 1H), 7.13 (dd, *J* = 4, 12 Hz, 1H), 7.06 (t,

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J = 8 Hz, 1H), 6.98 (dd, *J* = 4, 8 Hz, 1H), 6.89 (t, *J* = 8 Hz, 1H), 4.52 (t, *J* = 8 Hz, 2H), 4.41 (d, *J* = 16 Hz, 1H), 3.15 (t, *J* = 8 Hz, 1H), 3.04-2.91 (m, 1H), 2.51-2.43 (m, 1H), 1.82-1.78 (m, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): 172.4, 160.7, 158.3, 148.7, 139.8, 136.6, 135.2, 128.8, 128.8, 128.6, 127.5, 123.0, 121.8, 121.0, 120.9, 116.2, 115.9, 115.3, 115.1, 114.0, 67.5, 61.4, 54.1, 50.4, 38.3. IR (neat, ν cm^{-1}): 2921, 2853, 1659, 1636, 1576, 1493, 795. HRMS (ESI-TOF) m/z: $[M + H]^+$ calculated for ($C_{24}H_{21}N_3Cl$) 386.1419, 388.1398 found 386.1411, 388.1389. M.p.- 141 °C

12-(trifluoromethyl)-2,3,8,13b-tetrahydro-1H-indolo[2,3-b]pyrrolo[3,2-c]quinolone (9j):

Following the general procedure tryptamine and 2-nitro-5-(trifluoromethyl) benzaldehyde provided **9j** (84% yield).^{1}H NMR (400 MHz; DMSO-d_6): 10.53 (s, 1H), 7.75 (d, *J* = 8 Hz, 1H), 7.51 (d, *J* = 8 Hz, 1H), 7.30 (d, *J* = 8 Hz, 1H), 7.21 (t, *J* = 8 Hz, 1H), 7.05 (d, *J* = 8 Hz, 1H), 6.94 (t, *J* = 8 Hz, 1H), 4.49 (s, 1H), 3.29-3.18 (m, 1H), 2.79 (t, *J* = 12 Hz, 1H), 2.07-1.99 (m, 1H), 1.64 (t, *J* = 8 Hz, 1H). ^{13}C NMR (100 MHz; CD_3OD): 173.7, 170.2, 140.6, 134.2, 133.8, 131.9, 129.1, 126.1, 125.3, 124.7, 122.8, 117.9, 112.8, 62.2, 57.7, 51.4, 40.4. HRMS (ESI-TOF) m/z: $[M + H]^+$calculated for ($C_{18}H_{15}N_3F_3$) 330.1213, found 330.1214. M.p.- 145 °C

Biological assays

Cell culture. Human ovarian carcinoma (A2780), colorectal carcinoma (HCT116) and lung adenocarcinoma (A549) cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen Corp., Grand Island, NY, USA) supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic solution (Invitrogen Corp.) and maintained at 37 ºC in a humidified atmosphere of 5 % (v/v) CO_2.[17 and 21] MCF7 cell line derived from pleural effusion of breast adenocarcinoma from a female patient was grown in similar conditions, supplemented with 1 % MEM non-essential amino acids (Invitrogen Corp.).[22] Normal Humanfibroblastsweregrown in thesameconditions as MCF7 cell line.[17, 21-22] All cell lines were purchase from ATCC (www.atcc.org).

Compound exposure for dose-response curves. Cells were plated at 5000 cells/well in 96-well plates. Media was removed 24 h after platting and replaced with fresh media containing: 0.1 - 100 μM of compounds **9a, 9d-j** or 0.1 % (v/v) DMSO (vehicle control). All the previous solutions were prepared from concentrated stock solutions (in DMSO) of the compounds.

Viability assays. After 48 h of cell incubation in the presence or absence of each compound, cell viability was evaluated with CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay

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(Promega, Madison, WI, USA), using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) as previously described.[17-24] In brief, this is a homogeneous, colorimetric method for determining the number of viable cells in proliferation, cytotoxicity or chemosensitivity assays. The CellTiter 96® AQueous Assay is composed of solutions of MTS and an electron coupling reagent (phenazinemethosulfate, PMS). MTS is bioreduced by cells into a formazan product that is soluble in tissue culture medium. The absorbance of the formazan product at 490 nm can be measured directly from 96-well assay plates without additional processing. The conversion of MTS into the aqueous soluble formazan product is accomplished by dehydrogenase enzymes found in metabolically active cells. The quantity of formazan product was measured in a Bio-Rad microplate reader Model 680 (Bio-Rad, Hercules, CA, USA) at 490 nm, as absorbance is directly proportional to the number of viable cells in culture.

Assessment of apoptosis through Hoechst 33258 staining. HCT116 cells grown as described above were plated at 7500 cells/mL and incubated for 48 h in culture medium containing the compound **9a** or 0.1 % (v/v) DMSO (vehicle control). Hoechst staining (excitation and fluorescence emission 352 and 461nm, respectively) was used to detect apoptotic nuclei as previously described.[16] Briefly, medium was removed, cells were washed with phosphate-buffered saline 1X (PBS) (Invitrogen), fixed with 4 % (v/v) paraformaldehyde in PBS 1X (10 min in the dark) and incubated with Hoechst dye 33258 (Sigma, Missouri, USA; 5 µg/mL in PBS 1X) for another 10 min. After washed with PBS 1X, cells were mounted using 20 µL of PBS: glycerol (3:1; v/v) solution. Fluorescent nuclei were sort out per the chromatin condensation degree and characteristics. Normal nuclei showed non-condensed chromatin uniformly distributed over the entire nucleus. Apoptotic nuclei showed condensate or fragmented chromatin. Some cells formed apoptotic bodies. Plates were photographed in an AXIO Scope (Carl Zeiss, Oberkochen Germany), and three random microscopic fields per sample with ca. 50 nuclei were counted. Mean values were expressed as the percentage of apoptotic nuclei.[21]

Autophagy. For autophagy analysis, $0.5x10^5$ of HCT116 cells were seeded on top of a sterilized cover slide and let to adhere for 24 h. The supernatant was substituted with fresh medium containing compound **9a** (at its IC_{50}). For control purposes, one cover slide with cell monolayer was treated with 0.1% (v/v) DMSO in fresh medium, and another cover slide was treated with fresh medium for 24 h, when rapamycin was added to a final concentration of 50 µg/mL. After

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48 h incubation of cells with the compound **9a** and the control compound, medium was removed and cells were stained according to the instructions of CYTO-ID Autophagy detection kit (ENZO, NY, USA).[19 and 25] Stained cells were imaged using a fluorescence microscope (Carl Zeiss) and autophagy measured using the CYTO-ID® Green dye (excitation and fluorescence emission 463 and 534 nm, respectively; DAPI was used to counterstain the nucleus (excitation and fluorescence emission 358 and 461 nm, respectively) and respective software (ZEN Blue edition, 2011). The total number of cells and the number of cells with autophagolysosomes were counted in at least 5 different images of each sample to calculate the % of cells in autophagocytosis.

Evaluation of mitochondria membrane potential. Mitochondrial transmembrane potential was analyzed using the dye 5,5',6,6'-tetrachloro-1,1',3,3'- tetraethylbenzimi-dazolyl-carbocyanine iodide (JC-1; Abnova Corporation, Walnut, CA, USA). A2780 cell line was seeded into 24-well plates cells at 0.75×10^5 cells/well density and incubated during 24 h. After the incubation, cells were treated with compound **9a** (at the IC_{50}) or 0.01% (v/v) DMSO (vehicle control) and incubated for 48 h. For the measurement of the fluorescence intensity, cells were stained with JC-1 staining solution for 20 min at 37°C in the dark, followed by visualization with a fluorescence Microscope Olympus Bx51 microscope equipped with Olympus DP50 camera using the same exposition time for all samples. Green to red fluorescence ratio of each sample was determined using ImageJ software (National Institutes of Health (NIH), Bethesda, MD, USA).

Detection of reactive oxygen species (ROS). The detection of ROS was performed using the ROS assay (Life Technologies, InvitrogenTM, USA). A2780 cell line was seeded and incubated at 37ºC, 99% (v/v) humidity and 5% (v/v) CO_2 with a cell density of 1 x 105 cells/mL. After 24 h, culture medium was removed and replaced by fresh medium containing compound **9a** (at its IC_{50}) or and 0.01 % (v/v) DMSO (vehicle control). Hydrogen peroxide (H_2O_2) at a concentration of 50 µM was used as a positive control. Cells were harvested after 48 h of exposure to compounds, washed two times with PBS 1X, re-suspended in pre-warmed PBS 1X containing 10 µM H2DCF-DA and incubated for 20 min at 37°C, in absence of light. The levels of DCF positive cells was measured in an Attune cytometer (Applied Biosystems, Foster City, CA, USA) and quantified with the Attune Cytometric Software (Applied Biosystems).

Statistical analysis

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All data were expressed as mean ± SEM from at least three independent experiments. Statistical significance was evaluated using the Student's t-test; $p < 0.05$ was considered statistically significant.

ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra of all products. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Acknowledgements

This work was supported by Shiv Nadar University and The Unidade de Ciências Biomoleculares Aplicadas - UCIBIO which is financed by national funds from FCT/MEC (UID/Multi/04378/2013) and co-financed by the ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007728).

References

1. C. Marti, E. M. Carreira, *Eu. J. Org. Chem*. **2003**, *24*, 2209-2219.
2. (a) S. Sakai, N. Aimi, K. Yamaguchi, H. Ohhira, K. Hori, J. Haginiwa, *Tetrahedron Lett*. **1975**, *16*, 715-718. (b) O. Dideberg, J. Lamotte-Brasseur, L. Dupont, H. Campsteyn, M. Vermeire and L. Angenot, *ActaCrystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.,***1977**, *33*, 1796–1801. (c) C. B. Cui, H. Kakeya, H. Osada, *J. Antibiot.* **1996**, *49*, 832-835. (d) S. Shangary, S. Wang, *Ann. Rev. Pharmaco. Toxcol.***2009**, *49*, 223-241.

View Article Online
DOI: 10.1039/C7NJ04616B

3. (a) S. M. Verbitski, C. L. Mayne, R. A. Davis, G. P. Concepcion, C. M. Ireland, *J. Org. Chem.* **2002**, *67*, 7124-7126. (2) (b) A. Numata, C. Takahashi, Y. Ito, T. Takada, K. Kawai, Y. Usami, E. Matsumura, M. Imachi, T. Ito, T. Hasegawa, T. *Tetrahedron Lett.***1993**, *34*, 2355-2358. (c) A. S. Ratnayake, W. Y. Yoshida, S. L. Mooberry, T. K. Hemscheidt, *J. Org. Chem.* **2001**, *66*, 8717-8717. (d) Erratum: A. S. Ratnayake, W. Y. Yoshida, S. L. Mooberry, T. K. Hemscheidt, *J. Org. Chem.* **2003**, *68*, 1640-1640. (d) R. Jadulco, R. A. Edrada, R. Ebel, A. Berg, K. Schaumann, V. Wray, K. Steube, P. Proksch, P. *J. Nat. Prod*. **2004**, *67*, 78-81.
4. (a) R. T. Brown, in: *Heterocyclic Compounds* (Ed.: J. E. Saxon), Wiley Interscience, New York, **1983**, vol. 25, part 4, pp. 85-97. (b) A. Bischler, B. Napiralsky, *Ber. Dtsch. Chem. Ges.* **1893**, *26*, 1903-1908. (c) R. Grigg, M. F. Aly, V. Sridharan, S. Thianpatanagul, *J. Chem. Soc. Chem. Commun.* **1984**, 182-183. (d) K. Jones, J. Wilkinson, *J. Chem. Soc., Chem. Commun.* **1992**, 1767-1769. (e) W. G. Earley, T. Oh, L. E. Overman, *Tetrahedron Lett.* **1988**, *29*, 3785_3788. (f) A. Madin, C. J. O'Donnell, T. Oh, D. W. Old, L. E. Overman, M. J. Sharp, *Angew. Chem. Int. Ed.* **1999**, *38*, 2934-2936. (g) W. G. B. Beyersbergen van Henegouwen, R. M. Fieseler, F. Rutjes, H. Hiemstra, *Angew. Chem. Int. Ed.* **1999**, *38*, 2214-2217. (h) W. G. B. Beyersbergen van Henegouwen, R. M. Fieseler, F. Rutjes, H. Hiemstra, *J. Org. Chem.* **2000**, *65*, 8317-8325.
5. (a) Z. Zuo, D. Ma, *Angew. Chem. Int. Ed*. **2011**, *50*, 12008–12011. (b) *Angew. Chem*. **2011**, *123*, 12214–12217. (c) J. A. May, R. K. Zeidan, B. M. Stoltz, *Tetrahedron Lett*. **2003**, *44*, 1203 –1205. (d) S. L. Crawley, R. L. Funk, *Org. Lett*. **2003**, *5*, 3169–3171. (e) B. W. Boal, A. W. Schammel, N. K. Garg, *Org. Lett*. **2009**, *11*, 3458–3461; (f) A. W. Schammel, B. W. Boal, L. Zu, T. Mesganaw, N. K. Garg, *Tetrahedron*, **2010**, *66*, 4687–4695.
6. S. Hati, S. Tripathy, P. K. Dutta, R. Agarwal, R. Srinivasan, A. Singh, S. Singh, S. Sen, *Sci. Rep*. **2016**, *6*, 32213.
7. P. Yu, J. M. Cook, *Tetrahedron Lett*. **1997**, *38*, 8799-8802.
8. (a) H. Shimojo, K. Moriyama, H. Togo, *Synthesis*, **2013**, *45*, 2155-2156. (b) Z. Wang, L. Zhu, F. Yin, Z. Su, Z. Li, C. Li, *J. Am. Chem. Soc.*, **2012**, *134*, 4258-4263. (c) F.-Q. Huang, J. Xie, J.-Guo Sun, Y.-W. Wang, X. Dong, L-W. Qi, B. Zhang, *Org.*

View Article Online
DOI: 10.1039/C7NJ04616B

Lett. **2016**, *18*, 684-687. (d) Y. Takeda, S. Okumura, S. Minakata, *Synthesis*, **2013**, *45*, 1029-1033.

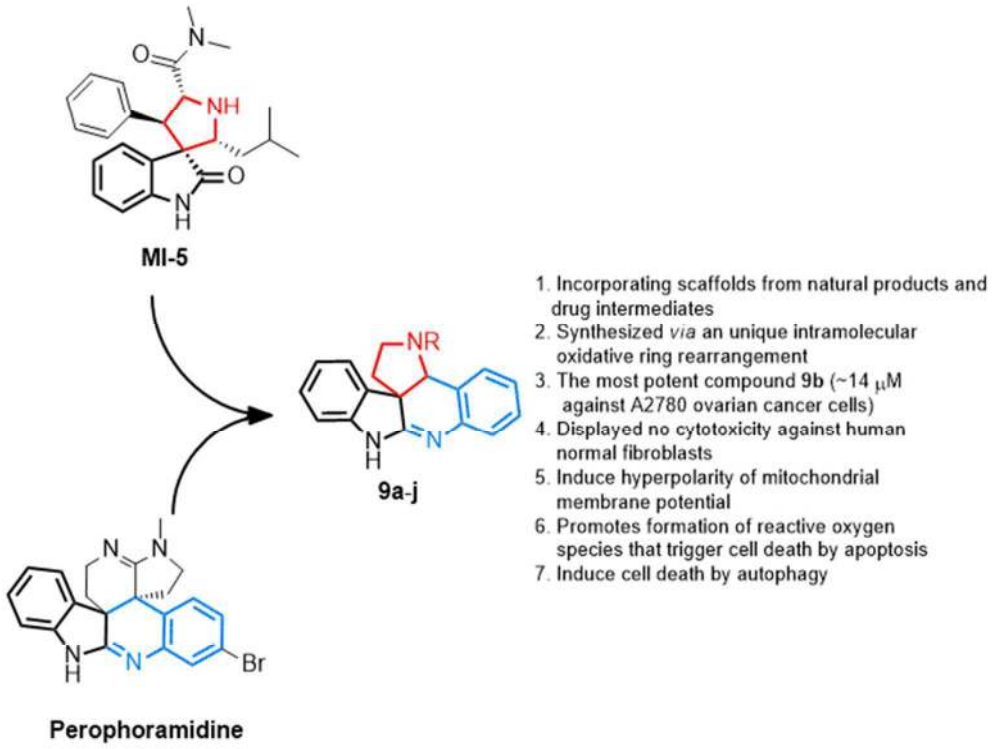
9. (a) L. Jiang, X. Xie, L. Zu, *RSC Adv*. **2015**, *5*, 9204-9207. (b) M. Ito, C. W. Clark, M. Mortimore, J. B. Goh, S. F. Martin, J. Am. Chem. Soc. 2001, 123, 8003-8010.
10. X. Zhang, J. Chen, B. Davis, F. Kiechle. *Arch. Pathol. Lab. Med.*, **1999**, *123*, 921-927.
11. S. Shimizu, T. Yoshida, M. Tsujioka, S. Arakawa, *Int. J. Mol. Sci.,* **2014**, *15*, 3145-3153.
12. J. D. Ly, D. R. Grubb, A. Lawen, *Apoptosis* **2003**, *8*, 115-128.
13. S. W. Perry, J. P. Norman, J. Barbieri, E. B. Brown, H. A. Gelbar, *Biotechniques*, **2011**, *50*, 98-115.
14. A. Per, P. Gergely, Jr, G. Nagy, A. Koncz, K. Banki, *Trends Immunol.***2004**, *25*, 360-367.
15. J. L. Scarlett, P. W. Sheard, G. Hughes, E. C. Ledgerwood, H. H. Ku, M. P. Murphy, *FEBS Lett*. **2000**, *475*, 267-72.
16. D. Wu, P. Yotnda, *J. Vis. Exp*. **2011**, *57*, 3357.
17. A. Silva, D. Luís, S. Santos, J. Silva, A. S. Mendo, L. Coito, T. F. S. Silva, M. Fátima, C. Guedes da Silva, L. M. D. R. S. Martins, A. J. L. Pombeiro, P. M. Borralho, C. M. P. Rodrigues, M. G. Cabral, P. A. Videira, C. Monteiro, A. R. Fernandes. *Drug Metabol. Drug Interact*. **2013**, *28*, 167-176.
18. J. M. Suski, L. M, Bonora, P. Pinton, D. J, M. R. Wieckowski, *Methods Mol. Biol*. **2012**, *810*, 183-205.
19. L. Magdalena, L. Circu, T. Y. Aw. *Free Radic. Biol Med*. **2010**, *48*, 749-762.
20. O. A. Lenis-Rojas, C. Roma-Rodrigues, A. R. Fernandes, F. Marques, D. Pérez-Fernández, J. Guerra-Varela, L. Sánchez, D. Vázquez-García, M. L. Torres, A. Fernández, J. J. Fernández. *Inorg. Chem*. **2017**,*56*, 7127–7144.
21. T. F. S. Silva, L. M. D. R. S. Martins, M. F. C. Guedes da Silva, A. R. Fernandes, A. Silva, P. M. Borralho, S. Santos, C. M. P. Rodrigues, A. J. L. Pombeiro, *Dalton Trans*. **2012**,*41*, 12888-12897.
22. T. F. S. Silva, P. Smoleński, L. M. D. R. S. Martins, M. F. C. Guedes da Silva, A. R. Fernandes, D. Luis, A. Silva, S. Santos, P. M. Borralho, C. M. P. Rodrigues, A. J. L. Pombeiro, *Eur. J. Inorg. Chem*. **2013**, *21*,3651-3658.
23. K. T. Mahmudov, M. F. C. Guedes da Silva, A. Mizar, M. N. Kopylovich, A. R. Fernandes, A. Silva, A. J. L. Pombeiro, *J. Organomet. Chem*. **2014**,*760*, 67-73.

View Article Online
DOI: 10.1039/C7NJ04616B

24. T. F. S. Silva, L. M. D. R. S. Martins, M. F. C. Guedes da Silva, M. L. Kuznetsov, A. R. Fernandes, A. Silva, C. -J. Pan, J.-F. Lee, B.-J. Hwang, A.J.L. Pombeiro, *Chemistry - an Asian Journal*.**2014**,*9*, 1132-1143.

25. O. A. Lenis-Rojas, A. R. Fernandes, C. Rodrigues, P. V. Baptista, F. M. Marques, D. Pérez-Fernández, J. Guerra, L. E. Sanchez, D. Vazquez-Garcia, M. Lopez-Torres, A. Fernández, J. J. Fernández Sánchez. *Dalton Trans*., **2016**, *45*, 19127-19140.

View Article Online
DOI: 10.1039/C7NJ04616B



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