Short Communication

Mild Synthetic Approach to Novel Indole-1-Carbinols and Preliminary Evaluation of Their Cytotoxicity in Hepatocarcinoma Cells

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A mild and versatile method for the synthesis of some novel indole-1-carbinols has been developed *via* one-pot reaction of indoles and paraformaldehyde in the presence of an excess of CaO, MgO, ZnO or TiO_2. The solvent-free reaction provided all the indole derivatives in moderate to good yields and short reaction times. Moreover, the effect of some selected indole-1-carbinols on cell proliferation of the hepatoma cell line FaO was evaluated.

Keywords: Apoptosis / Cytotoxicity / Hepatocarcinoma / Indole-1-carbinols / Solvent-free reaction

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Introduction

Indole is probably the most widespread heterocyclic framework, present in a plethora of natural biologically active molecules [1]. In particular, indole-3-carbinol (I3C), a dietary component found exclusively in *Brassica* vegetables as a metabolite of glucobrassicin, has emerged as an efficient carcinogenesis modulator with a pleiotropic mode of action. (Figure 1) [2].

However, the bioactivity of I3C may be plausibly related to its conversion, in the acidic gastric milieu, to more stable and active oligomers such as 3,3′-diindolylmethanes (DIMs), indolo[3,2*b*]-carbazole (ICZ) and linear as well as cyclic trimers (LTr_1, CTr). Consequently, indole-3-carbinol and DIMs are now widely used as scaffolds in the development of new anticancer agents [2, 3]. Recently, some remarkable attempts to improve the stability and the metabolic profile of indole-3-carbinol have been reported in the literature [4]. The best results were obtained with compound OSU-A9, a novel I3C analogue, which is very stable in acidic conditions and has a higher apoptosis-inducing potency, due to the presence of a 3-chloro-2-nitrobenzene sulfonyl group linked to the indole nitrogen [4] (Figure 2).

From a structural point of view, the characteristic acid instability of I3C is associated to the high propensity of the vinylogous hemiaminal moiety of the indole ring to dehydrate. Preventing this mechanism through a substitution could solve this drawback and may lead to an optimization of the anticancer activity. Thus, in the present research we report an extremely simple, mild and solvent-free method for the synthesis of some novel indole-1-carbinols, which are I3C structural isomers in which the *N*1-position is functionalized with a hydroxymethyl group.

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Abbreviations: I3C, indole-3-carbinol; **DIMs,** 3,3'-diindolylmethanes; **ICZ,** indolo[3,2*b*]-carbazole; **LTr_1**; **CTr,** linear and cyclic trimers; **PARP,** poly(ADP-ribose) polymerase; **DMSO,** dimethylsulfoxide; **DMF,** dimethylformamide; **I1C,** indole-1-carbinol.

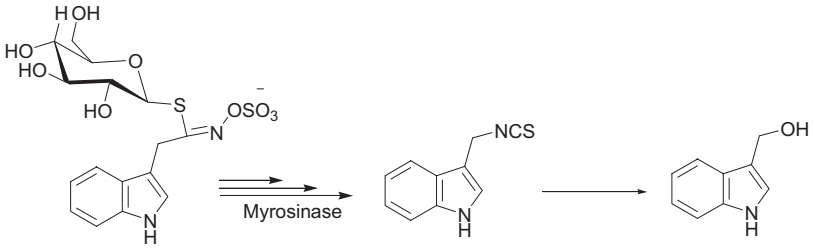


Figure 1. Glucobrassicin as precursor of indole-3-carbinol (I3C).

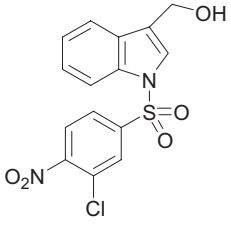
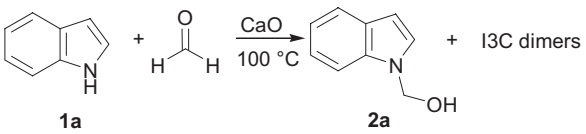


Figure 2. OSU-A9.

Results and discussion

In contrast to benzotriazole [5], the N-alkylation and in particular the N-hydroxyalkylation of indoles have not often been investigated, probably due to the reduced nucleophilicity of their N–H function [6]. To date, apart from a few mild procedures [7], many of the methods reported in the literature provide indole-1-carbinols in very low yields and long reaction times [7b, 8]. Mostly, the reduction of 1-indolyl carbamates [9] or strong basic conditions [10a–c] also involving organomethallic reagents are used [5c, 10d]. Consequently, the development of a simple and efficient method to achieve the N-hydroxymethylation of indoles under mild conditions, in very short reaction times and in solvent-free conditions, represents an important challenge.

In the beginning of our study, with the aim of searching for a new protocol to I3C dimers, indole **1a** was allowed to react with formaldehyde, derived from paraformaldehyde, under solvent free conditions, at 100°C, in the presence of an excess of CaO. Surprisingly, apart from the expected 3,3′-diindolyl methane, we observed the formation of indole-1-carbinol **2a**.



Scheme 1. Reaction between indole **1a** and formaldehyde at 100°C.

It was also evidenced a new dimeric derivative (Scheme 1). Mechanistically, we presume that a different reaction pathway can be conceived for the formation of the last compound (paper in preparation).

This observation diverted our attention toward the N-hydroxyalkylation of indole. We began our study by investigating all the parameters involved in the reaction with the aim of improving the yields, the regiochemistry and the scope of the reaction with respect to indole-1-carbinol **2a**.

We first explored the role of the CaO observing that the reaction did not occur in its absence [7a]. Besides, the experiment did not proceed at all at room temperature, both with or without the alkaline oxide. Fixing the reaction temperature at 65°C showed to be the best manner to obtain indole-1-carbinol **2a** with a high regioselectivity. In fact, higher temperatures directed the reaction towards dimer formation. The determining effect of the amount of CaO on the reaction proceeding was also immediately apparent.

Indeed, as shown in Table 1, we observed that when calcium oxide was used in large excess, the reaction seemed to proceed smoothly, while the use of CaO in catalytic or stoichiometric amounts did not seem to produce any effect. Therefore, the alkaline earth metal oxide may act as a multifunctional agent, showing a synergic effect together with the temperature in the activation of the reaction, provoking a rapid conversion of paraformaldehyde to free formaldehyde

Table 1. Base effect on reaction between indole **1a** and formaldehyde at 65°C.

Substrate	Oxide[a]/1a	Time (min)	Product	Yield (%)[c]
1a	–	120	–	–
1a	0.1/1	120	**2a**	traces
1a	1/1	120	**2a**	5–7
1a	5/1	120	**2a**	25–55
1a	17/1	30[b]	**2a**	25–65

[a] Best results with CaO and MgO.
[b] No traces of dimers also for longer times.
[c] Lit. Yield (%) 34 [7a], not reported [10a], 67 [20]

and neutralizing the traces of formic acid produced during prolonged heating.

We assumed that CaO might induce a partial N–H proton abstraction and, despite the modest ionic character of N–Ca bond, formaldehyde could be so reactive to undergo a nucleophilic attack, generating indole-1-carbinol **2a.**

To test our hypothesis, we ran our experiments with MgO, ZnO and TiO_2. As expected, the oxides possessing strongly electropositive cations, such as MgO and CaO, afforded the best yields (Table 1).

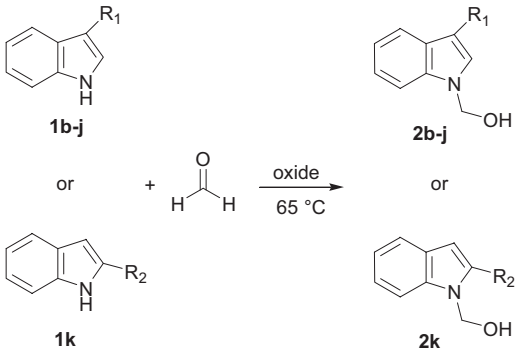
We also investigated the use of potassium hydroxide, but, unfortunately, no traces of compound **2a** could be detected. A possible explanation is related to the thermal decomposition under strong basic conditions, of indole-1-carbinol to regenerate indole **1a** and HCHO [10a].

Different solvents were studied in order to optimize the reaction conditions further. Obviously, as the mechanism is a typical nucleophilic addition, we focused our interest on dimethylsulfoxide (DMSO), dimethylformamide (DMF), acetonitrile and acetone, strongly dissociating polar aprotic solvents which tend to favour attack at the indole nitrogen. As a matter of fact, no reaction improvement was evidenced by using tetrahydrofuran (THF) or apolar solvents such as toluene or hexane. Besides, polar protic solvents have been banned, in order to avoid a nucleophile competition or a reduction of the strength of our indole nitrogen.

Overall, we noticed a sizeable improvement of the yields when running the reaction in the presence of DMSO, but after removal of the solvent, only a small amount of carbinol **2a** could be isolated, due to its instability at high temperature (Table 2).

The hypothesis that the reaction, if successful with **1a**, would allow similar results when different indole derivatives are used, led us to carry out some other exploratory reactions with compounds **1b–n** (Scheme 2).

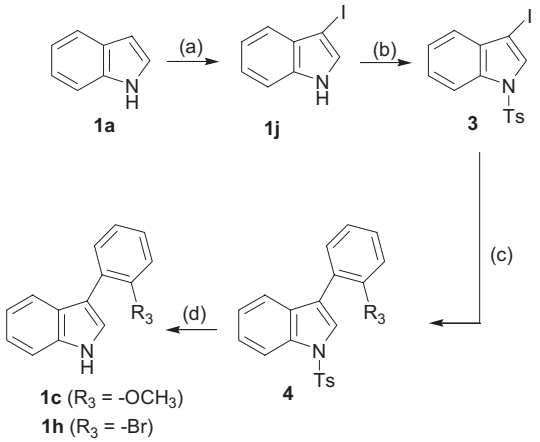
Almost all indole substrates were purchased from commercial sources, apart from compounds **1i** and **1j**, which were prepared according to literature [11], and indoles **1c** and **1h**, obtained via the Suzuki coupling synthetic approach [12] (Scheme 3).



1b R_1= -CH_3; **1c** R_1= -2-OCH_3Ph; **1d** R_1= -CHO; **1e** R_1= -$COCH_3$; **1f** R_1= -$COOCH_3$; **1g** R_1= -CN; **1h** R_1= -2-BrPh; **1i** R_1= -Br; **1j** R_1= -I; **1k** R_2= -CH_3

No reaction with: **1l** R_2= -$COOC_2H_5$; **1m** R_2= -Ph; **1n** R_2= -4-ClPh;

Scheme 2. Synthesis of 2- and 3-substituted *N*-carbinols.



Reagents and conditions: (a) I_2, KOH, DMF; (b) TsCl, NaOH, TBABr, CH_2Cl_2; (c) 2-methoxy or 2- bromo benzeneboronic acid, $Pd(PPh_3)_4$,Na_2CO_3, EtOH, toluene, H_2O; (d) NaOH 3 M, MeOH.

Scheme 3. Synthesis of indole substrates **1c** and **1h**.

The developed protocol showed to be valid for the substrates **1b–k** (Table 3). Unfortunately, no reaction was observed by using substrate **1l**. We presume a nucleophilicity decrease of the correspondent indolyl anion due to its bidentate ligand behaviour. Similarly, the lack of reactivity of indoles **1m** and **1n** might be related both to electronic and steric substituent effects (Scheme 2).

In particular, as shown in Table 2, the reactions carried out with compounds **1d–g**, showed to be extremely regioselective for the related carbinols; in fact, no traces of dimers were noticed also for longer reaction times. Interestingly, products

Table 2. Solvent effect on reaction between indole **1a** and formaldehyde at 65°C.

Entry	Solvent	Time (min)	Yield (%)
1	DMSO	30	90[a]
2	DMF	60	34[a]
3	CH_3CN	120	22[a]
4	Acetone	120	5[b]
5	THF	120	–
6	Toluene	120	–
7	Hexane	120	–

[a] GC yields.
[b] Isolated yields

Table 3. Reaction of indoles **1b–k** with formaldehyde at 65°C.

Substrate	R_1, R_2	Oxide[a]/1	Time (min)	Product	Yield (%)	Lit. Yield (%)
1b	$R_1 = -CH_3$	–	120	–	–	
1b		17/1	30[b]	**2b**	43–55	10 [8], 80 [20]
1c	R_1 = -2-OCH_3Ph	–	120	–	–	
1c		17/1	30[b]	**2c**	50–70	–
1d	$R_1 = -CHO$	–	120[c]	**2d**	15	33 [21]
1d		17/1	120[c]	**2d**	65	
1e	$R_1 = -COCH_3$	–	120[c]	**2e**	10	
1e		17/1	120[c]	**2e**	60	–
1f	$R_1 = -COOCH_3$	–	120[c]	**2f**	15	–
1f		17/1	120[c]	**2f**	70	
1g	$R_1 = -CN$	–	120[c]	**2g**	8	
1g		17/1	120[c]	**2g**	67	49 [21]
1h	R_1 = -2-BrPh	17/1	60[b]	**2h**	65	–
1i	R_1 = -Br	17/1	30	**2i**	20[d]	–
1j	R_1 = -I	17/1	30	**2j**	18[d]	–
1k	$R_2 = -CH_3$	–	120	**2k**	–	–
1k		17/1	30[b]	**2k**	45–57	–

[a] Lower yields with TiO_2.
[b] Longer times facilitate the formation of dimers.
[c] No traces of dimers also for longer times.
[d] Highly unstable products.

2d–g were also detected in very low yields without the use of any alkaline oxide. This observed behavior is presumably due to the presence of an electron-withdrawing substituent which helps the removal of the N–H proton by reducing its pK_a.

We applied the optimized procedure also to a selection of aliphatic (propanal, butanal, hexanal, heptanal and dodecanal) and aromatic aldehydes (benzaldehyde, 2-methoxybenzaldehyde, 3-methoxybenzaldehyde, 4-methoxybenzaldehyde, 2-nitrobenzaldehyde, 3-nitrobenzaldehyde, 4-nitrobenzaldehyde, piperonal, 2-thiophenaldehyde, 2-furaldehyde and 3-formyl indole) but the results were unsatisfactory. In particular, with the less reactive aromatic substrates, no *N*-carbinol was never observed. As a consequence, no reaction also with ketones (2- octanone, 2-hexanone, 4′-methylacetophenone and 1-(*p*-methoxyphenyl)-2-propanone).

By employing aliphatic aldehydes with α-hydrogens, we observed only the formation of 1-alkenyl-1-*H*-indoles, certainly derived from *in situ* dehydration of the correspondent *N*-carbinols.

The effect of indole-3-carbinol and of some selected new compounds on cell proliferation of the hepatoma cell line FaO was also evaluated. We first investigated the possible role of electron donor and electron-withdrawing substituents in 3-position of the indole ring, on the cytotoxic activity. With this respect, compounds **2a**, **2b**, **2d** and **2e** have been the first compounds of choice.

FaO cells were treated with an increasing concentration of compounds **2a**, **2d**, **2e** and **2b** as well as indole-3-carbinol, for 24 hours. The results indicated a dose-dependent decrease of cell viability as measured by NRU, compared to control (Figure 3).

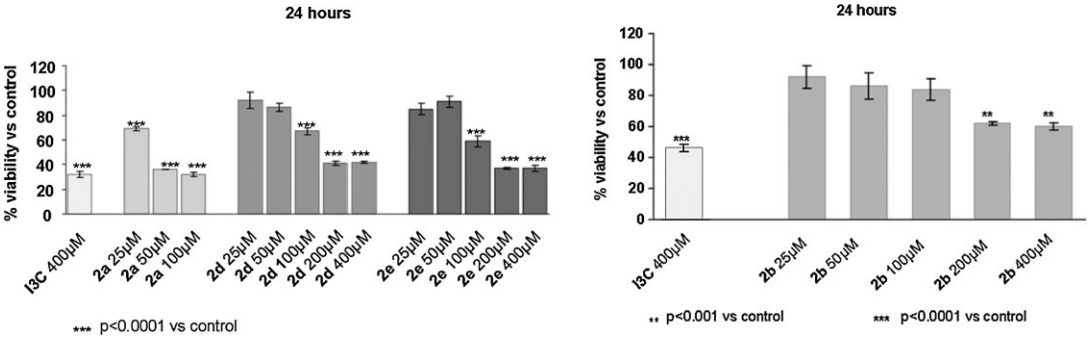


Figure 3. Effect of increasing concentrations of I3C and derivatives **2a**, **2d**, **2e** and **2b** on FaO cell viability determined by NRU assay after 24 hours of treatment. Data are expressed as percentage of viable cells as assessed by Neutral Red uptake. Results are the means ± SD from three separate experiments.

In particular, the derivatives **2a**, **2d** and **2e** were two to four fold more potent than I3C in suppressing the viability of FaO cells showing IC_{50} values ranging from 100 μM to 200 μM (Figure 3).

Since preliminary evaluations of metabolic transformations [13] did not evidence intracellular formation of formaldehyde or dimer derivatives, we suppose that the observed cytotoxic activity is related to other interactions. Further studies aimed at the synthesis and the evaluation of the cytotoxicity of the most important I1Cs' metabolites are now in progress.

In order to evaluate the possible apoptotic effect of I3C and of our new derivatives, we determined the expression of the poly(ADP-ribose) polymerase (PARP) protein by Western blot analysis. The nuclear enzyme PARP transfers ADP-ribose moieties from NAD^+ to various nuclear proteins, and poly(ADP)-ribosylation has been reported to be responsible for changes in nuclear structure and function. A pro-apoptotic function of PARP has been proposed based on the finding that its overexpression stimulates apoptosis [14] and that treatment with a specific chemical inhibitor of PARP, 3-aminobenzamide, suppressed apoptosis [15]. Following caspase-3 activation, PARP is cleaved at Asp214 early in apoptosis; the cleaved PARP loses enzymatic activity [16] and the resulting N-terminal and C-terminal PARP fragments accelerate apoptosis [17].

The results shown in Figure 4 clearly indicate that, at the highest tested concentration, I3C as well as derivatives **2a**, **2d** and **2e** caused an increase of the cleaved PARP protein in FaO cells (Figure 4).

Further studies are needed in order to better characterize the I3C and **2a**, **2d**, **2e** proapoptotic effect in FaO cells. Moreover, similar investigations are now in progress with the other indole-1-carbinol derivatives **2c**, **2f**, **2g**, **2h** and **2k**.

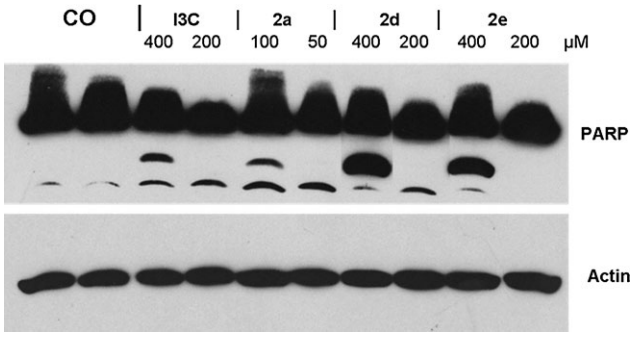


Figure 4. Western blot analysis of PARP in FaO cells treated with increasing concentrations of indole-3-carbinol (I3C) and derivatives **2a**, **2d** and **2e** for 24 hours. Control cells (CO) were treated with an equivalent amount of the solvent alone. Actin was used as loading control.

In view of their *in vivo* application via oral administration, we also tested the stability in acidic conditions of compounds **2a**, **2b**, **2d**, **2e**, **2f** and **2g**, monitoring the experiments *via* NMR analysis [4a]. We observed that after treatment with labeled HCl, the most stable compounds were derivatives **2e** and **2f**, while the others produced an acidic reaction mixture consisting of dimers and other side products. All the other derivatives are now under investigation in similar experimental conditions.

In conclusion, we have developed a simple and versatile synthetic procedure to prepare several *N*-carbinols. This process might represent a valuable alternative to the drastic reaction conditions usually employed for the N-hydroxymethylation of indoles. This makes our method a convenient tool for the preparation of a wide variety of indole-1-carbinols. Furthermore. all the tested derivatives showed an apoptopic effect on FaO cells. Work continues on yield improvement and on the determination of the antitumoral effect of all the other novel derivatives, in order to determine an accurate structure/activity relationship, which will be discussed elsewhere.

Experimental

Chemistry

Unless otherwise stated, all reagents were purchased from commercial sources and used without further purification. Reaction progress was monitored by TLC using Aldrich silica gel 60 F254 (0.25 mm) with detection by UV. Chromatography was performed using Aldrich silica gel (60–120) mesh size with freshly distilled solvents. ^1H and ^{13}C NMR were recorded by Varian 300 and 400 MHz using tetramethylsilane as internal standard. Microanalysis for CHN were performed by a Carlo Erba 1106 Elemental Analyzer. GC-MS: Low resolution mass spectrometric experiments were carried out on a Saturn 2000 ion-trap coupled with a Varian 3800 gas chromatograph (Varian, Walnut Creek, CA) operating under EI conditions (electron energy 70 eV, emission current 20 μA, ion-trap temperature 200°C, manifold temperature 80°C, automatic gain control (AGC) target 21.000) with the ion trap operating in scan mode (scan range from *m/z* 40–400 at a scan rate of 1 scan/s). Aliquots of 1 μL of solutions 1.0×10^{-5} M in chloroform have been introduced into the gas chromatographer inlet. A CIP Sil-8 CB Lowbleed/MS capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) was used. The oven temperature was programmed from 150°C (held for 2 min) to 310°C at 30°C/min (held for 2 min). The temperature was then ramped to 350 at 20°C/min. The transfer line was maintained at 250°C and the injector port (30:1 split) at 230°C. IR spectra were recorded in KBr or neat on a Perkin-Elmer 1310 spectrophotometer.

Nuclear magnetic resonance analysis of acid stability.

Indole-1-carbinols **2a**, **2b**, **2d**, **2e** and **2f** were dissolved in 0.75 mL of CD_3OD. To each solution, 100 μL of deuterium-labeled HCl (35 wt% in D_2O, 99 atom% D) was added and nuclear magnetic resonance (NMR) spectra were recorded in a 300 MHz NMR spectrometer at room temperature at different time intervals.

General synthetic procedure for the synthesis of indole-1-carbinols ***2a–k***

A mixture of indole **1** (8.54 mmol), paraformaldehyde (10.25 mmol) and oxide (145.18 mmol) was stirred for the period indicated (TLC) at 65°C. After reaction, the crude mixture was washed with acetone and filtered through celite. The concentrated filtrate was flash chromatographed (hexane/ethyl acetate 3:1, 5:1 or 1:3) on silica gel, obtaining the desired product.

(1H-Indol-1-yl)methanol ***2a***

Sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): 7.62 (d, J = 7.8 Hz, 1H), 7.45 (d, J = 7.8 Hz, 1 H), 7.25 (t, 1H), 7.16 (m, 2H), 6.53 (d, J = 3.3, 1 H), 5.61 (d, J = 4.8 Hz, 2 H) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): δ 135.93, 128.80, 125.89, 121.24, 119.00, 118.42, 110.12, 109.06, 68.70 ppm. EI-MS *m/z* (%): 147 (55), 130 (4), 117 (100), 103 (1), 90 (40), 89 (45). IR (neat): 3250, 2980, 1625, 1470, 1260 cm^{-1}. Anal. Calcd for C_9H_9NO: C 73.47, H 6.12 N 9.52. Found: C 73.40, H 6.03, N 9.49.

(3-Methyl-1H-indol-1-yl)methanol ***2b***

Sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.59 (d, J = 7.5 Hz, 1H), 7.39 (d, J = 7.5 Hz, 1H), 7.27 (t, 1 H), 7.19 (t, 1 H), 6.91 (s, 1H), 5.50 (s, 2H), 2.34 (s, 3H) ppm. ^{13}C NMR (300 MHz, DMSO): δ 135.83, 128.79, 125.96, 121.21, 118.84, 118.53, 110.12, 109.56, 68.35, 9.67 ppm. EI-MS *m/z* (%): 161 (77), 144 (8), 139 (100), 103 (10), 77 (12). IR (neat): 3300, 2860, 1600, 1480, 1260 cm^{-1}. Anal. Calcd for $C_{10}H_{11}NO$: C 74.53, H 6.83, N 8.69. Found: C 74.49, H 6.82, N 8.70.

(3-(2-Methoxyphenyl)-1H-indol-1-yl)methanol ***2c***

Gummy. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78 (d, J = 7.8 Hz, 1H), 7.62 (d, J = 7.8 Hz, 1H), 7.53 (d, J = 8.1 Hz, 1H), 7.28 (m, 2H), 7.19 (t, 1H), 7.04 (q, 2H), 5.67 (s, 2H), 5.30 (s, 1H), 3.85 (s, 3H) ppm. ^{13}C NMR (300 MHz, DMSO): δ 156.33, 144.01, 135.66, 129.71, 128.13, 127.21, 123.70, 121.35, 120.60, 119.97, 119.76, 111.70, 110.60, 68.78, 54.36 ppm. EI-MS *m/z* (%): 253 (25), 223 (100), 208 (42), 180 (26), 152 (20), 130 (8). IR (KBr): 3410, 1650, 1420, 1260, 1130 cm^{-1}. Anal. Calcd for C_{16} $H_{15}NO_2$: C 75.89, H 6.38, N 5.53. Found: C 75.90, H 6.33, N.5.52.

1-(Hydroxymethyl)-1H-indole-3-carbaldehyde ***2d***

White solid (m.p. 101–103°C). ^{1}H NMR (300 MHz, DMSO): δ 10.06 (s, 1H), 8.47 (s, 1H), 8.21 (d, J = 8.1 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.47–7.36 (m, 2H), 6.92 (t, 1H), 5.73 (d, 2H) ppm. ^{13}C NMR (300 MHz, DMSO): δ 190.22, 145.64, 141.71, 130.09, 128.77, 12786, 126.20, 122.65, 116.77, 74.90 ppm. EI-MS *m/z* (%): 175 (23), 147 (20), 146 (55), 116 (100). IR (KBr): 3350, 2980, 2850, 1725, 1660, 1280 cm^{-1}. Anal. Calcd for $C_{10}H_9NO_2$: C 68.57, H 5.14, N 8.00. Found: C 68.55, H 5.16, N 7.87.

1-(1-(Hydroxymethyl)-1H-indol-3-yl)ethanone ***2e***

White solid (m.p. 128–130°C). ^{1}H NMR (300 MHz, DMSO): δ 8.51 (s, 1H), 8.32 (d, J = 8.7 Hz, 1H), 7.75 (d, J = 8.7 Hz, 1H), 7.37 (m, 2H), 6.84 (t, J = 7.5 Hz, 1H), 5.70 (d, J = 7.5 Hz, 2H), 2.57 (s, 3H) ppm. ^{13}C NMR (300 MHz, DMSO): δ 218.51, 136.42, 127.65, 126.58, 122.93, 122.27, 121.56, 116.20, 109.99, 69.61, 28.14 ppm. EI-MS *m/z* (%): 189 (27), 174 (12), 146 (45), 116 (100). IR (KBr): 3400, 2850, 1715, 1660, 1420, 1300 cm^{-1}. Anal. Calcd for $C_{11}H_{11}NO_2$: C 69.84, H 5.82, N 7.41. Found: C 69.82, H 5.79, N 7.40.

Methyl 1-(hydroxymethyl)-1H-indole-3-carboxylate ***2f***

White solid (m.p. 111–113°C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (d, J = 7.5 Hz, 1H), 7.62 (s, 1H), 7.38 (d, J = 7.5 Hz, 1H), 7.20–7.14 (m, 2H), 5.43 (s, 2H), 3.73 (s, 3H) ppm. ^{13}C NMR (300 MHz, DMSO): δ 169.81, 137.02, 126.65, 126.41, 123.00, 122.47, 121.43, 116.12, 109.67, 69.99, 52.84 ppm. EI-MS *m/z* (%): 205 (23), 175 (74), 161 (5), 144 (100), 116 (27), 89 (22). IR (KBr): 3270, 1750, 1650, 1260, 1160 cm^{-1}. Anal. Calcd for $C_{11}H_{11}NO_3$: C 64.39, H 5.36, N 6.83. Found: C 64.41, H 5.34, N 6.82.

1-(Hydroxymethyl)-1H-indole-3-carbonitrile ***2g***

White solid (m. p. 105–107°C). ^{1}H NMR (300 MHz, DMSO): δ 8.45 (s, 1H), 7.84 (d, J = 8.5 Hz, 1H), 7.75 (d, J = 8.5 Hz, 1H), 7.50–7.38 (m, 2H), 6.93 (t, J = 7.2 Hz, 1H), 5.72 (d, J = 7.2 Hz, 2H) ppm. ^{13}C NMR (300 MHz, DMSO): δ 141.87, 139.93, 132.55, 128.72, 127.44, 123.88, 121.07, 117.29, 89.26, 74.81 ppm. EI-MS *m/z* (%): 172 (45), 142 (68), 116 (100). IR (KBr): 3300, 2860, 2250, 1600, 1480, 1260 cm^{-1}. Anal. Calcd for $C_{10}H_8N_2O$: C 69.77, H 4.65, N 16.28. Found: C 69.76, H 4.65, N 16.29.

(3-(2-Bromophenyl)-1H-indol-1-yl)methanol ***2h***

Gummy. ^{1}H NMR (300 MHz, DMSO): δ 7.93 (d, J = 8.2 Hz, 1H), 7.86 (s, 1H), 7.77 (d, J = 8.2 Hz, 1H), 7.73 (d, J = 7.8 Hz, 1H), 7.56 (t, J = 7.8 Hz, 2H), 7.39–7.24 (m, 3H), 6.61 (t, J = 7.2 Hz, 1H), 5.71 (d, J = 7.2 Hz, 2H) ppm. ^{13}C NMR (300 MHz, DMSO): δ 136.30, 134.95, 128.69, 127.43, 127.07, 126.11, 124.69, 122.61, 120.78, 120.13, 118.51, 109.68, 69.93 ppm. EI-MS *m/z* (%): 303 [M+2] (24), 301 (22), 271 (45), 194 (100), 116 (67), 77 (40). IR (KBr): 3310, 1640, 1420, 1260, 1130 cm^{-1}. Anal. Calcd for $C_{15}H_{12}$ BrNO: C 59.80, H 3.99, N 4.65. Found: C 59.77, H 4.00, N 4.62.

(3-Bromo-1H-indol-1-yl)methanol ***2i***

White solid (decomposes). EI-MS *m/z* (%): 227 [M+2] (100), 225 (98), 197 (25), 195 (23), 116 (50), 89 (20).

(3-Iodo-1H-indol-1-yl)methanol ***2j***

White solid (decomposes). EI-MS *m/z* (%): 273 (100), 243 (88), 116 (65), 89 (23).

(2-Methyl-1H-indol-1-yl)methanol ***2k***

Sticky oil . ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.54 (d, J = 8.4 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H), 7.21 (t, J = 8.4 Hz, 1H), 7.13 (t, J = 8.4 Hz, 1H), 6.81 (s, 1H), 5.38 (s, 2H), 2.28 (s, 3H) ppm. ^{13}C NMR (300 MHz, DMSO): δ 141.59, 133.02, 130.96, 125.41, 124.48, 114.83, 105.56, 105.43, 64.95, 19.04 ppm. EI-MS *m/z* (%): 161 (50), 144 (16), 130 (100), 117 (5), 103 (20). IR (neat): 3310, 2840, 1610, 1480, 1260 cm^{-1}. Anal. Calcd for C_{10} $H_{11}NO$: C 74.53, H 6.83, N 8.69. Found: C 74.51, H 6.79, N 8.66.

Biological general methods

Cell line and culture

Rat FaO cell line was supplied by Interlab Cell Line Collection (Servizio Biotecnologie, IST, Genova, Italy). FaO cells were maintained in Dulbecco's medium (DMEM plus Glutamax I, Invitrogen S.r.l., Milano, Italy) supplemented with penicillin, streptomycin and 10% heat-inactivated fetal calf-serum (FCS) (Invitrogen) in a humidified atmosphere of 5% CO_2/95% air, at 37°C. Indole-3-carbinol (I3C) and compounds **2a**, **2b**, **2d** and **2e**

were dissolved in DMSO and were added to the culture media to the final concentrations specified in the text. Control cells were treated with an equivalent amount of the solvent alone.

Cell viability

Cell viability was determined by the uptake of Neutral Red (NRU) by the lysosomes of viable cells.

Determination of viability of the adherent cells by NRU assay was performed according to Borefreund and Puerner [18]. The value obtained for treated cells was expressed as percentage of the value obtained in control cells. All experiments were performed in triplicate and were repeated at least three times.

Western blotting analysis

Cell lysates, obtained by homogenization with lysis buffer (1× PBS, 1% IGEPAL, 0.5% sodium deoxycholate, 0.1% SDS with 100 μg/mL phenylmethylsulfonylfluoride (PMSF), 300 μg/mL aprotinin and 100 μg/mL sodium orthovanadate, all purchased from Sigma), were incubated 30 min on ice and centrifuged at 12.000 rpm at 4°C, and the supernatants were recovered. Protein concentration was determined according to the method of Bradford [19] using BSA as standard (DC Protein Assay, Bio Rad Laboratories, Hercules, CA). For immunoblotting analysis, equal amounts of proteins were electrophoresed on SDS 8% polyacylamide gels. After gel electrotransfer onto nitrocellulose membranes, at 800 mA for 4 h at 4°C, to ensure equivalent protein loading and transfer in all lanes, the membranes and gels were stained with 0.5% (w/v) Ponceau S red (ICN Biomedicals) in 1% acetic acid, and with Coomassie blue (ICN Biomedicals) in 10% acetic acid, respectively. Before staining, gels were fixed in 25% (v/v) isopropanol and 10% acetic acid (Sigma). After blocking in TBS containing 0.05% Tween 20 (Sigma) (TBS-T) and 5% non-fat dry milk, overnight at 4°C, membranes were washed in TBS-T and incubated with the appropriate primary antibodies diluted in blocking buffer. The same membrane was used to detect the expression of PARP and actin. Depending on the origin of primary antibody, filters were incubated with anti-mouse, or anti-rabbit horseradish peroxidase-conjugated IgGs (Santa Cruz Biotechnology). Immunoreactive bands were identified with chemiluminescence detection system, as described by the manufacturer (Supersignal Substrate, Pierce, Rockford, IL). For immunoblotting experiments, the rabbit polyclonal anti-poly(ADP-ribose) polymerase (PARP) (Cell Signaling Technologies, Beverly, MA) and mouse monoclonal anti-actin (clone AC-40) (Sigma-Aldrich, Milano, Italy) were used.

Statistical analysis

Instat software (GraphPad Prism5, San Diego, CA) was used to analyze the data. One-way analysis of variance (ANOVA) with *post hoc* analysis using Tukey's multiple comparison test was used for parametric data. The results of multiple observations were presented as the means ± S.D of at least 3 separate experiments. A p value of <0.05 was considered statistically significant.

Supporting information

^{1}H and ^{13}C NMR spectra.

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