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PII: S0045-2068(18)31395-6  
DOI: <https://doi.org/10.1016/j.bioorg.2019.02.007>  
Reference: YBIOO 2782

To appear in: *Bioorganic Chemistry*

Received Date: 3 December 2018  
Revised Date: 28 January 2019  
Accepted Date: 3 February 2019

Please cite this article as: M. Demurtas, A. Baldisserotto, I. Lampronti, D. Moi, G. Balboni, S. Pacifico, S. Vertuani, S. Manfredini, V. Onnis, Indole derivatives as multifunctional drugs: Synthesis and evaluation of antioxidant, photoprotective and antiproliferative activity of indole hydrazones, *Bioorganic Chemistry* (2019), doi: <https://doi.org/10.1016/j.bioorg.2019.02.007>

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## Indole derivatives as multifunctional drugs: synthesis and evaluation of antioxidant, photoprotective and antiproliferative activity of indole hydrazones.

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### Abstract

Two series of indole derivatives **4-17**, **20-22** were easily prepared and assayed for their radical-scavenging ability. Arylidene-1H-indole-2-carbohydrazones showed different extent antioxidant activity in DPPH, FRAP and ORAC assays. Good antioxidant activity is related to the number and position of hydroxyl groups on the arylidene moiety as well as to the presence of methoxy or 4-(diethylamino) group. On the contrary low antioxidant activity is showed by the isomeric 1H-indol-2-yl(methylene)-benzohydrazides. Furthermore, hydrazones **4-17** showed photoprotective capacities with satisfactory *in vitro* SPF as compared to the commercial PBSA sunscreen filter. The indole **16** and **17**, showing the best antioxidant and photoprotective profile, were included in different formulation and their topical release was evaluated. Varying the formulation composition, it was possible to optimize skin adsorption and solubility of the active indole in the formulation. The antiproliferative effect of the hydrazones **4-17** was tested on human erythroleukemia K562 and melanoma Colo-38 cells. Hydrazones **11**, **16** and **17** showed growth inhibition at sub micromolar concentrations on both cell lines. These results indicate indole hydrazones as potential multifunctional molecules especially in the treatment of neoplastic diseases being the good antioxidant properties of **16** and **17** correlated to their high antiproliferative activity.

### Keywords

Indole hydrazones, Polyhydroxylated compounds, Antioxidant activity, Photoprotective agents, Antiproliferative activity.

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## 1. Introduction

In recent years, a new concept of drug design has emerged that has revolutionized pharmacology: the multi-target approach. A multi-target active ingredient can be designed to act simultaneously on multiple targets. There are numerous advantages that can offer: not only from a pharmacological point of view, characterized by a better efficacy and minimal adverse effects [1], but there is also a reduction in costs and development times before reaching the market [2]. In this context, attention has been focused on the research of compounds with multifunctional activities, that are equipped with both antioxidant and UV-filtering capabilities, to counteract the oxidative damage caused by free radicals and, at the same time, protect during exposure to UV radiation. The latter are the main cause of reactive oxygen species (ROS) and reactive nitrogen species (RNS) formation and are closely related to the skin photo-aging process [3], to skin inflammation and skin diseases, such as erythema and hyperpigmentation, and from the process of carcinogenesis, including the onset of melanoma [4]. Indole ring is one of the most widespread naturally occurring compounds as component of biologically active products derived from plants, animals and marine organisms. Due to its broad range of biological activities, it is a privileged structure in medicinal chemistry. In recent years, indole has emerged as a versatile molecular skeleton for the development of novel anticancer agents [5-7], in particular tubulin inhibitors [8]. Noteworthy are also the central pharmacological activities of indole derivatives that act as anticonvulsants, antidepressants and hypnotic-sedatives [9]. Some indole alkaloids exert considerable pharmacological activity:  $\delta$ -yohimbine and reserpine are examples of drugs active on the circulatory system, while biological studies conducted on indole-3-carbinol, present in relevant concentrations in vegetables such as broccoli, cabbage and cauliflowers, have revealed interesting data on the anti-carcinogenic, antioxidant and anti-atherogenic effects [10]. The indole ring is present in some clinically used drugs, such as triptans, NSAIDs and  $\beta$ -antagonist [11]. Several indole derivatives have been reported for their antioxidant activity [12-17]. Among naturally occurring indoles tryptophan, an essential amino acid involved in numerous biological processes, is the biological parent of serotonin which is central neurotransmitter, and cardiovascular and gastrointestinal signaling molecule. Together with melatonin, serotonin is a powerful scavenger of free radicals and hormone regulator of circadian rhythms in organisms [18]. The indole ring in melatonin has been indicated as the center responsible for antioxidant properties, due to its high resonance stability and low activation energy barrier in direct reactions against free radicals [19]. The high level of free radicals can cause damage to biomolecules and oxidative stress DNA damages have been recognized a leading cause of various types of cancers [20]. For some polyphenols, an anti-melanoma activity is known [21], a malignant and aggressive tumor form associated with intense exposure to UV radiation.

A number of indole-based compounds have been reported for their potential anticancer activities [22]. Hydrazone-indole derivatives have been reported to induce apoptosis [23], and to interact with tubulin [24-27].

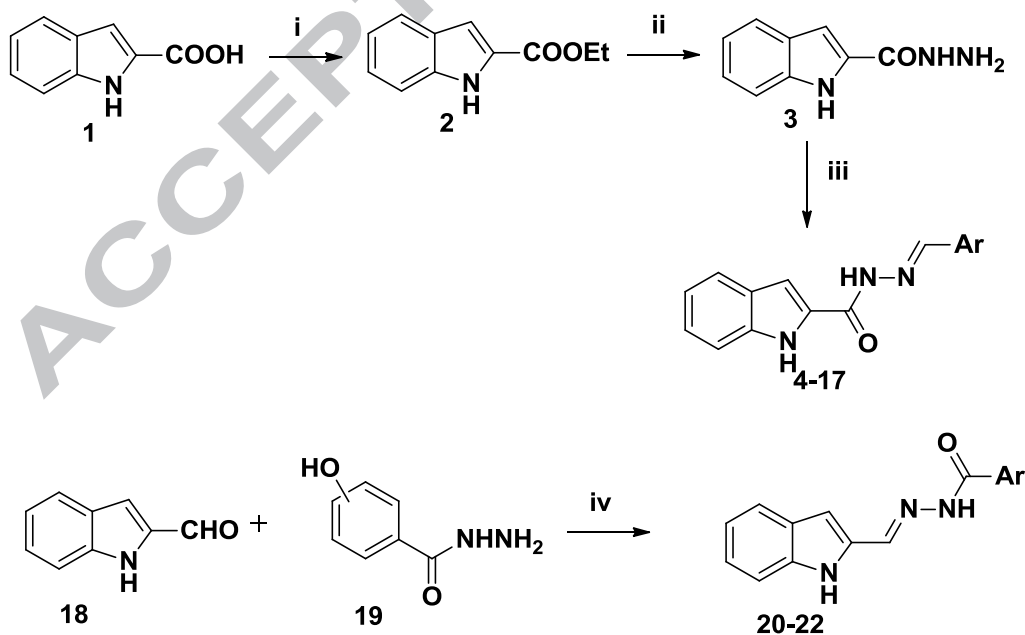
We have previously reported on radical-scavenging activity and antiproliferative activity of benzofuranhydrazones [28]. So, prompted from the above described findings we here report a small library of indole-hydrazone compounds, designed, synthesized and tested, as well as their release from a series of topical formulations.

The aim was to identify new molecules with dualistic activity, in which the strong antioxidant properties were possibly associated with other pharmacological abilities, in particular photoprotection and antiproliferative activity. In this context, the functional dualism sought would allow both to neutralize the oxidative damage promoted by free radicals, generated by UVA radiation, and to protect against UVB, responsible for direct damage to DNA and the onset of cancer in the skin [29].

## 2. Results and Discussion

### 2.1. Chemistry

The target hydrazones **4-17** (Table 1) were synthesized as shown in Scheme 1. 1*H*-Indole-2-carboxylic acid (**1**) was converted into ethyl ester (**2**) [30]. The ethanolic solution of intermediate **2** and hydrazine hydrate was refluxed to achieve 1*H*-Indole-2-carbohydrazide (**3**) [31]. Hydrazones **4-17** were obtained in good to excellent yield by coupling the hydrazide **3** with the appropriate hydroxyarylaldehydes in ethanol. The isomeric indole benzohydrazones **20-22** were obtained by coupling indole-2-carboxaldehyde **18** with the appropriate hydroxybenzohydrazide **19**. All the newly synthesized compounds were in agreement with expected analytical data.



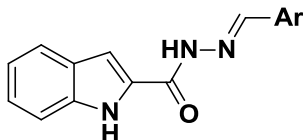
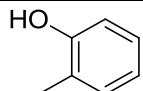
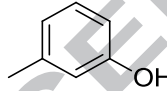
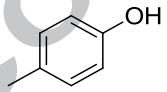
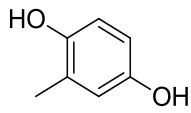
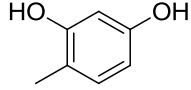
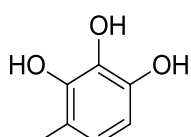
**Scheme 1.** Reagents and conditions: (i) Ethanol, H<sub>2</sub>SO<sub>4</sub>, reflux, 6 h; (ii) NH<sub>2</sub>NH<sub>2</sub>·H<sub>2</sub>O, EtOH, reflux 3 h, yields 64-90%; (iii) ArCHO, EtOH, reflux, 18 h; iv) EtOH, reflux, 18 h, yields 70-75%.

The IR and NMR spectral data are consistent with the assigned structure. According to the literature, the presence of a singlet downfield resonating (8.30-9.49 ppm)  $\text{CH}=\text{N}$  signal, exclusively accounts for formation of *E*-isomers [32].

## 2.2 Antioxidant activity

The evaluation of the antioxidant properties of the hydrazones **4-17** and **20-22** was performed by 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity (DPPH), Ferric Reducing Antioxidant Power (FRAP), and Oxygen radical absorbance capacity (ORAC) methods. Results are shown in Tables 1, 2 and are expressed as  $\mu\text{molTE/g}$  for DPPH, FRAP, and ORAC tests. For the best interpretation of the results of the DPPH, for each compound the concentration was sought to inhibit 50% of the radical. However, in some cases it was not possible to obtain data reflecting 50% inhibition, even if the tested compounds are all in the standard calibration line.

**Table 1.** Antioxidant activity of the indole derivatives **4-17**.

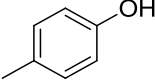
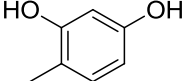
		DPPH <sup>a</sup>	FRAP <sup>a</sup>	ORAC <sup>a</sup>
Compd.	Ar	( $\mu\text{molTE/g}$ )	( $\mu\text{molTE/g}$ )	( $\mu\text{molTE/g}$ )
4		8.71 ± 1.85	34.56 ± 1.2	-
5		59.21 ± 1.0	960.94 ± 7.1	21031.02 ± 31.03
6		233.16 ± 5.7	1559.13 ± 11.5	35124.02 ± 64.55
7		9958.30 ± 13.6	4378.91 ± 14.7	21700.40 ± 28.73
8		2004.80 ± 6.0	873.06 ± 1.9	13014.11 ± 25.10
9		12846.01 ± 16.8	10655.78 ± 25.1	16621.77 ± 34.41

10		4187.20 ± 7.4	4137.42 ± 8.8	4505.29 ± 49.27
11		206.65 ± 3.7	2667.35 ± 6.5	16838.33 ± 41.07
12		221.9 ± 2.3	43.04 ± 2.1	-
13		38.66 ± 0.1	115.78 ± 2.2	-
14		<< 19.1 <sup>b</sup>	97.04 ± 1.9	-
15		14.15 ± 0.12	25.89 ± 1.0	-
16		2436.60 ± 15.29	3760.80 ± 8.07	22761.19 ± 18.48
17		1783.54 ± 13.1	97.4 ± 1.1	9398.69 ± 14.92

<sup>a</sup>Each value was obtained from three experiments (mean ± SE); <sup>b</sup>LOQ limit of quantification; - not tested.

**Table 2.** Antioxidant activity of the indole derivatives 20-22.

Compd.	Ar			
		DPPH <sup>a</sup> (μmolTE/g)	FRAP <sup>a</sup> (μmolTE/g)	ORAC <sup>a</sup> (μmolTE/g)
19		37.95 ± 9.23	243.38 ± 3.85	-

20		$28.58 \pm 0.7$	$257.61 \pm 4.3$	-
21		$125.0 \pm 3.26$	$209.37 \pm 8.47$	-

<sup>a</sup>Each value was obtained from three experiments (mean  $\pm$  SE); <sup>b</sup>LOQ limit of quantification; - not tested.

Hydrazones **4-17** DPPH result analysis indicates that high antioxidant capacity is correlated to high number of hydroxyl groups present on the arylidene ring. Indole derivatives **4-6** bearing a single hydroxyl group on the arylidene ring showed weak activity. The introduction of a second (compounds **7** and **8**) or a third hydroxy group (compounds **9** and **10**) leads to the best compounds of the series. Nevertheless, the hydroxyl group position also affects antioxidant activity, in fact indole **8** is about 5 times more active than analog **7** and the 2,3,4-trihydroxyarylidene derivative **9** is about 3 times more active than the 2,4,6-trihydroxyarylidene analog **10**. The replacement of one hydroxyl group with an alkoxy group (compounds **11-13**) or halogen atoms (compounds **14-15**) led reduction in activity. On the contrary the introduction of a 4-diethylamino group (indole **16**) or naphthyl ring (indole **17**) produced increase in activity as compared to compounds **4**, **8** and **12**. All hydrazones **20-22** showed weak activity demonstrating the importance of atoms double bond position on the linker between indole and hydroxylaryl rings.

On FRAP analysis, the most powerful derivatives were **5**, **6**, **7**, **8**, **9**, **10**, **11** and **16**. According to the results obtained from the DPPH test, in general activity increases with an increase in the number of phenolic hydroxyl groups on the aromatic ring and the reducing capacity is again conditioned by the position of hydroxyl groups. The moving of 2-hydroxy group of indole **4** into 3- (indole **5**) or 4-position (indole **6**) led to a progressive activity enhancement. The introduction on compound **5** of a 4-methoxy group (indole **11**) produced a further activity increase, while the analogs **12** and **13** showed weak activity. Confirming DPPH results, the replacement of 4-hydroxy group of compound **8** with a 4-diethylamino group (indole **16**) produced increase in activity.

The compounds that from DPPH to FRAP tests showed the most interesting profile, were further investigated in terms of antioxidant capacity by performing the ORAC test, in order outline a complete activity profile. The 4-hydroxyarylidene derivative **6** demonstrated the best antioxidant capacity towards the peroxy radicals (ROO $\cdot$ ). Shifting the 4-hydroxy group into 3-position (indole **5**) led reduction in activity. The introduction of second hydroxy group in 2-position afforded the poor active indole **8**, while the replacement of the 4-hydroxy with a 4-diethylamino group (indole **16**) restore the antioxidant properties. Similarly, the introduction on compound **5** of 4-methoxy group (indole **11**) caused reduction in activity. The presence of a trihydroxyarylidene moiety (compounds **9** and **10**) drop the activity as compared to a dihydroxyarylidene (compounds **7** and **8**), however the 2,3,4-trihydroxy derivative **9** showed better activity than the 2,4,6-trihydroxy analog **10**.

### 2.3 Evaluation of filtering parameters

On the indolehydrazones **5-11**, **16** and **17** showing antioxidant properties, *in vitro* tests were conducted to determine the parameters fundamental for the relative evaluation of the filtering power: critical wavelength ( $\lambda_c$ ) and SPF (Sun Protection Factor). The Solar Protection Effectiveness Evaluation System specifies a SPF primarily representing a measure of UVB protection [33] is related to the UV absorption of substances. The trend of the UV spectra of the compounds **5-11**, **16** and **17** between 290 and 400 nm was then recorded (data not shown) and compared with that of the commercial Phenyl Benzimidazole Sulfonic Acid filter (PBSA) used as a reference UVB filter ( $\lambda_{max}$  302 nm). All indole **5-11**, **16** and **17** have an absorption spectrum in which the  $\lambda_{max}$  is shifted towards longer wavelengths, between 330 and 350 nm, well above the maximum absorption of PBSA, and the absorption curves of the compounds analyzed are more extensive than the reference one. Hydrazones **17** and **16** showed the more evident bathochromic displacement. Indole **16** bears a 2-hydroxy-4-(diethylamino)arylidene moiety that has a positive effect on absorption. While indole **17** presents a naphthylarylidene moiety that produces increase in the degree of conjugation accounting for the shift of the maximum absorbance peak towards the visible region.

The preliminary study on the filtering properties to determine the UV-protective potential of hydrazones **5-11**, **16** and **17** was performed *in vitro*. Whereas a new method in ISO 24443: 2012 was established and standardized for the *in vitro* evaluation of UVA protection, there is no universally approved *in vitro* method for the assessment of protection in the UVB range. The *in vitro* evaluation of the SPF parameter was initially proposed by Diffey-Robson in 1989 [34] and the method is still widely applied.

After recording the UV absorption spectra of the hydrazones **5-11**, **16** and **17** and the reference PBSA, the values relating to the parameters were extrapolated to determine their filtering power (Table 3).

**Table 3.** UV-filtering activity of hydrazones **5-11**, **16**, **17** in solution.

Compound	SPF	UVA/UVB	UVAPF0	$\lambda_c$ (nm)
<b>5</b>	10,42	0,43	1,77	346
<b>6</b>	10,05	0,81	2,56	352
<b>7</b>	5,13	1,96	4,60	363
<b>8</b>	5,78	1,93	4,97	363
<b>9</b>	5,01	2,09	4,32	362
<b>10</b>	4,34	2,66	6,19	367
<b>11</b>	5,75	1,34	2,87	356
<b>16</b>	2,21	1,39	9,55	392



<b>17</b>	4,81	1,71	8,55	385
<b>PBSA</b>	3,4	0,29	1,03	322

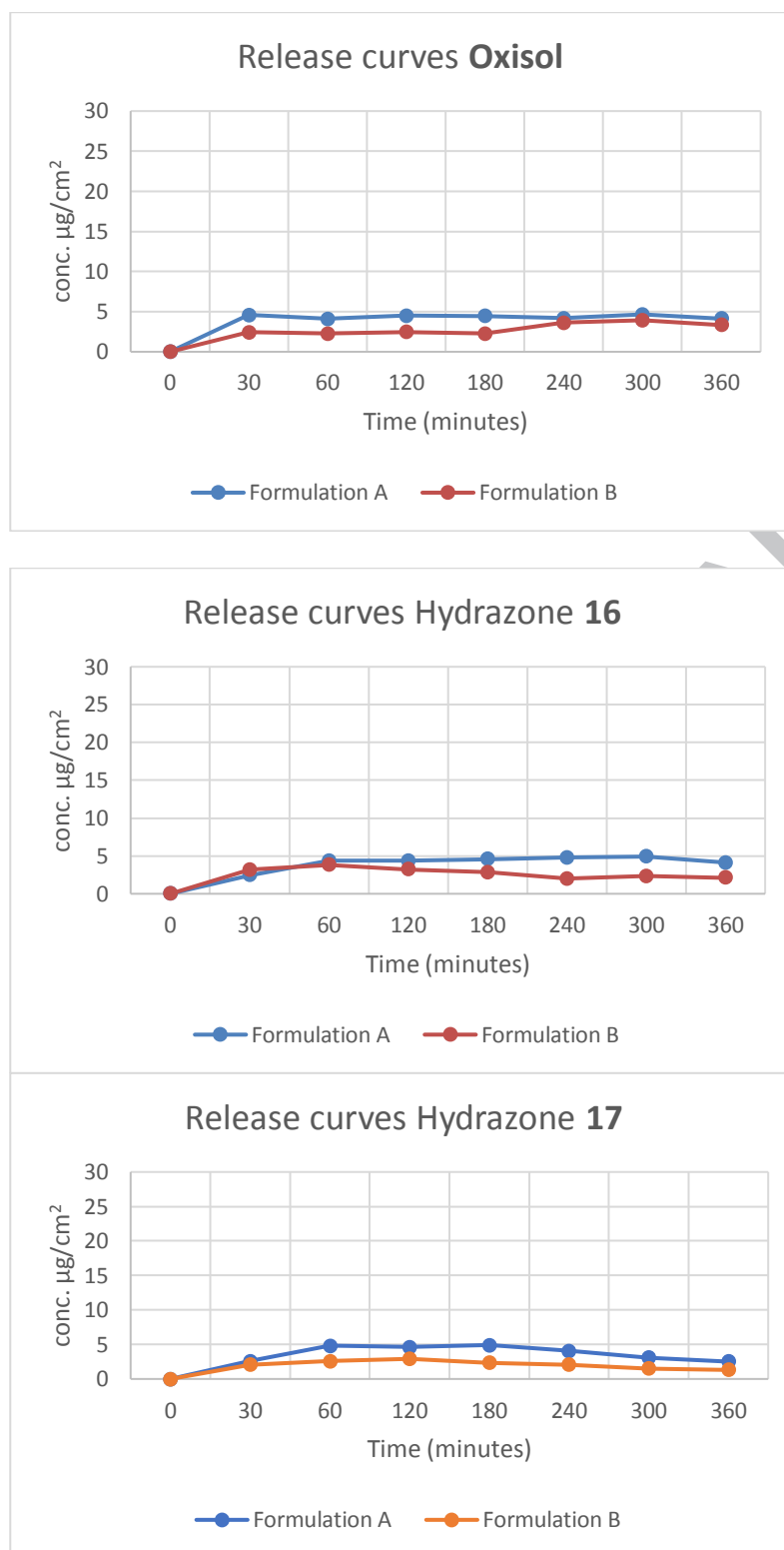
All indole derivatives **5-11**, **16** and **17** showed better filtering characteristics than the reference compound PBSA. A wide-spectrum solar product, able to protect the skin at the same time from UVA and UVB rays, should have a value of  $\lambda_c$  greater than 370 nm. Based on this criterion, the values of the indoles **16** and **17** emerge. Only the hydrazones **5** and **6** showed an appreciable SPF value. The UVA/UVB absorbance ratio is another parameter that gives a good idea of which UV region is best blocked by each substance [35]. As defined by the EU recommendation on the effectiveness of sunscreen products (2006/247 / EC), this ratio should be worth at least 1/3. This requirement is satisfied by almost all the indolehydrazones **5-11**, **16** and **17**, most of which characterized by values higher than 1. As close to 1 is the ratio, the more similar it will be the absorption of the molecule in the UVA region to that in the region UVB. This means that indoles **7-10**, **11**, **16**, and **17** mainly absorb in the UVA region. Finally, the value of UVAPF0, determined in relation to the guidelines provided by ISO-24443, showed that all the tested indoles possess a better UVA Protection Factor than the reference commercial filter. In particular, the UVAPF0 values of the **17** and **16** samples emerge.

#### 2.4 *In vitro* release studies

The indolehydrazones **16** and **17** due to their best dualistic activity profile (antioxidant and at the same time UV-filtering properties) were selected in to *in vitro* evaluate their behavior as potential active ingredients in topical formulations. Depending on the function of the active ingredient, it is possible to discriminate two types of formulations: to act as a sunscreen, the active ingredient must remain in the most superficial part of the skin, while to act as an antioxidant, it is desirable that this also penetrates into the underlying layers of the dermis.

For this purpose, the formulation devised for Oxisol, a molecule with dualistic activity [36], was taken into consideration and optimized for indoles **16** and **17**. Based on the structure of both the compounds, two topical formulations with different degrees of polarity were prepared: Formulation A) to obtain high release of the active substance performing activity after absorption into the skin and Formulation B) to obtain limited release, as needed in the case of solar filters, through a better solubilization of the active ingredient in the formulation. The effective release of the active ingredients from each of the two topical formulations optimized for the compounds **16** and **17** was determined through the Franz Cells system. This last represent one of the most common system for the study of *in vitro* release and of the permeation of active ingredients from semi-solid forms [34]. This system consists of two vertical chambers, defined as donors and recipients, separated by a synthetic or biological membrane which acts as a support and a means of separation between the formulation and the receiving medium [37]. The receptor phase was sampled within 6 h to measure

indoles **16**, **17** and Oxisol amount in the receiving chamber, the resulting release curves were reported in Figure 1.



**Fig. 1.** Permeation profiles of Oxisol, and hydrazones **16**, **17**. Line blue correspond to formulation A (optimized for skin adsorption), line orange correspond to Formulation B (optimized to best solubilize the active in formula).

The tested indoles and the reference Oxisol are characterized by a difference in the 0.8% and 2% range between the release curves of the two different formulations. The Oxisol release curves from formulation A and B maintain the same trend: the delta tends to decrease after 240 minutes. Differently, indole **16** and **17** did not show an appreciable difference between the two release curves in the initial 60 and 30 minutes respectively; in any case, after the first hour, the release increases for the formulation A and the difference between the two curves settles around 2%. In conclusion, the obtained data indicate that it is possible to modify the release of the indoles **16** and **17** from the formulation designed ad hoc by simply varying the percentages of its components, thus influencing the polarity of the formulation, depending on the activity of the active ingredient and end-use of the preparation.

### 2.5 Antiproliferative activity on Human melanoma Colo38 and erythroleukemia K562 cell lines

The hydrazones **4-17** were tested on human melanoma Colo38 cell line as well as on human erythroleukemic K562 cells to determine their antiproliferative activity. All the compounds have been tested on both cell lines to evaluate the relative IC<sub>50</sub> values expressed in  $\mu\text{M}$  concentration (Table 4). Hydrazone **11** exhibited greatest activity of the series, displaying an antiproliferative effect on both the Colo38 and K562 cells at nanomolar concentrations (IC<sub>50</sub> values  $0.59\pm 0.03$  and  $0.067\pm 0.001$   $\mu\text{M}$  respectively). The removal of the 4-methoxy group to give the hydrazone **5** produced extensive reduction in activity. The shift of the 3-hydroxy group into 4-position (hydrazone **6**) restore the antiproliferative activity against Colo38 cell line, while the activity against K562 cells is not affected. Good activity is showed by the di-hydroxy substituted hydrazones **7** and **8**. On the contrary the introduction of third hydroxyl group led to reduction in activity especially for the 2,4,6-trihydroxy analog **10** and against K562 cells. The replacement of the 4-hydroxy group of hydrazone **8** with 4-(dimethylamino) group (hydrazone **17**) led to increase in activity against K562 cells and at same time the submicromolar antiproliferative activity against Colo38 cells is maintained. The hydrazone **16** showed an inhibitory activity profile very similar to compound **17** on both the cell lines (IC<sub>50</sub> values between 0.54 and 0.83  $\mu\text{M}$ ).

**Table 4.** Effects of the indole derivatives **4-17** on the proliferation of Colo38 and K562 cells.

Compd.	IC <sub>50</sub> ( $\mu\text{M}$ )	
	Colo38	K562
<b>4</b>	>100	>100
<b>5</b>	$10.02\pm 0.91$	$47.80\pm 7.80$
<b>6</b>	$0.73\pm 0.05$	$46.11\pm 4.30$
<b>7</b>	$2.96\pm 0.13$	$8.60\pm 0.60$

<b>8</b>	0.57±0.05	4.31±0.40
<b>9</b>	6.13±0.26	5.39±0.01
<b>10</b>	8.94±0.13	54.52 ±0.11
<b>11</b>	0.59±0.03	0.067±0.001
<b>12</b>	>100	>100
<b>13</b>	>100	>100
<b>14</b>	>100	>100
<b>15</b>	>100	>100
<b>16</b>	0.54±0.15	0.63±0.05
<b>17</b>	0.83±0.09	0.63±0.04

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### 3. Conclusion

Continuing our research aiming to develop possible multifunctional drugs, we selected the indole scaffold in view of its interesting known properties. Indolehydrazones **4-17** showed different extent of radical-scavenging ability towards the nitrogen radical by the DPPH test, to reduce ferric ion by the FRAP test and to inhibit the oxidative degradation caused by peroxy radicals using the ORAC test. The SAR data obtained by the antioxidant screening of the benzofuran derivatives have shown good antioxidant activity and is related to the number and position of hydroxyl groups on the arylidene moiety as well as to the presence of methoxy or 4-(diethylamino) group. The indole derivatives **4-17** showed photoprotective capacities with satisfactory *in vitro* SPF as compared to the commercial PBSA sunscreen filter. Compounds **16** and **17** confirmed their relevant filtering activity when included in topical formulations. Skin adsorption and solubility of the active indoles were optimized by varying the formulation composition indicating a possible use of such compounds as sunscreen filters. Additionally, hydrazones **11**, **16** and **17** showed growth inhibition at sub micromolar concentrations on human erythroleukemia K562 and melanoma Colo-38 cells. These results indicate indole hydrazones as potential multifunctional molecules mainly in the treatment of neoplastic diseases being the good antioxidant properties of **16** and **17** correlated to their high antiproliferative activity.

### 4. Experimental section

#### 4.1 General methods.

All commercially available solvents and reagents were used without further purification. Standard samples were purchased from Sigma-Aldrich, Milan, Italy. NMR spectra were recorded on an Inova 500 spectrometer (Varian, Palo Alto, CA, USA). The chemical shifts ( $\delta$ ) are reported in part per million downfield from tetramethylsilane (TMS), which was used as internal standard, and the spectra were recorded in

hexadeuteriodimethylsulphoxide (DMSO-d<sub>6</sub>). Infrared spectra were recorded on a Vector 22 spectrometer (Bruker, Bremen, Germany) in Nujol mulls. The main bands are given in cm<sup>-1</sup>. Positive-ion electrospray ionization (ESI) mass spectra were recorded on a double-focusing MAT 95 instrument (Finnigan, Waltham, MA, USA) with BE geometry. Melting points (mp) were determined on a SMP1 Melting Point apparatus (Stuart Scientific, Stone, UK) and are uncorrected. All products reported showed NMR spectra in agreement with the assigned structures. The purity of the tested compounds was determined by combustion elemental analyses conducted by the Microanalytical Laboratory of the Chemistry Department of the University of Ferrara with a MT-5 CHN recorder elemental analyzer (Yanagimoto, Kyoto, Japan) and the values found were within 0.4% of theoretical values. The spectrophotometer used for antioxidant analysis is a Beckman Coulter™, DU®530, Life Science UV / VIS spectrophotometer, Single Cell Module. The instrument used to conduct ORAC analyzes is the Thermo Fluoroskan Ascent FL® Microplate Fluorometer and Luminometer, linked to Ascent Software® software for data control and processing. In the sample loading phase, 96-well plates with a black background were used. Spectrophotometric analysis for the detection of filter parameters were conducted with a UV-VIS spectrophotometer SHIMADZU UV-2600 240 V.

Ethyl 1*H*-Indole-2-carboxylate (**2**) was prepared as previously described [30] starting from 1*H*-Indole-2-carboxylic acid. 1*H*-Indole-2-carbohydrazide (**3**) was prepared as previously described [31].

## 4.2. Chemistry

4.2.1. *General procedure for the synthesis of hydrazones (4-17)*. A mixture of hydrazide **3** (1 mmol) and the appropriate aldehyde (1 mmol) in EtOH (10 mL) was refluxed for 18h. After cooling the formed precipitate was filtered off and purified by crystallization from the adequate solvent to give the hydrazone derivatives.

4.2.1.1. *(E)-N'-(2-hydroxybenzylidene)-1*H*-indole-2-carbohydrazide (4)*. Yield 64%. Mp >250 °C (EtOH). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 6.92 (m, 2H, Ar), 7.05 (m, 1H, Ar), 7.21 (m, 1H, Ar), 7.28 (m, 2H, Ar), 7.45 (d, *J* = 8.0 Hz, 1H, Ar), 7.56 (m, 1H, Ar), 7.66 (d, *J* = 8.0 Hz, 1H, Ar), 8.63 (s, 1H, CH), 11.16 (s, 1H, OH), 11.79 (s, 1H, NH), 12.09 (s, 1H, NH). IR (Nujol) 3294, 1654, 1617 cm<sup>-1</sup>. *m/z* 280 (M + H)<sup>+</sup>. Anal. Calcd for C<sub>16</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>: C, 68.81; H, 4.69; N, 15.05. Found: C, 68.89; H, 4.70; N, 15.02. [39]

4.2.1.2. *(E)-N'-(3-hydroxybenzylidene)-1*H*-indole-2-carbohydrazide (5)*. Yield 84%. Mp >250 °C (EtOH) Lit. 278-281 °C [39]. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 6.81 (m, 1H, Ar), 7.04 (m, 1H, Ar), 7.10 (d, *J* = 7.5 Hz, 1H, Ar), 7.18-7.27 (m, 4H, Ar), 7.43 (d, *J* = 8.5 Hz, 1H, Ar), 7.65 (d, *J* = 8.0 Hz, 1H, Ar), 8.34 (s, 1H, CH), 9.58 (s, 1H, OH), 11.75 (s, 1H, NH), 11.79 (s, 1H, NH). IR (Nujol) 3255, 1638, 1580 cm<sup>-1</sup>. *m/z* 280 (M + H)<sup>+</sup>. Anal. Calcd for C<sub>16</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>: C, 68.81; H, 4.69; N, 15.05. Found: C, 68.73; H, 4.68; N, 15.08.

4.2.1.3. *(E)-N'-(4-hydroxybenzylidene)-1*H*-indole-2-carbohydrazide (6)*. Yield 80%. Mp >250 °C (EtOH) Lit. 278-279 °C [40]. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 6.82 (d, *J* = 8.0 Hz, 2H, Ar), 7.03 (m, 1H, Ar), 7.19 (m, 1H, Ar), 7.43 (d, *J* = 8.0 Hz, 2H, Ar), 7.56 (d, *J* = 8.0 Hz, 2H, Ar), 7.65 (d, *J* = 7.5 Hz, 1H, Ar), 8.33 (s, 1H, CH), 9.88 (s, 1H, OH), 11.64 (s, 1H, NH), 11.71 (s, 1H, NH). IR (Nujol) 3223, 1608 cm<sup>-1</sup>. *m/z* 280 (M + H)<sup>+</sup>. Anal. Calcd for C<sub>16</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>: C, 68.81; H, 4.69; N, 15.05. Found: C, 68.74; H, 4.70; N, 15.07.

4.2.1.4. (*E*)-*N'*-(2,5-dihydroxybenzylidene)-1*H*-indole-2-carbohydrazide (**7**). Yield 80%. Mp >250 °C (EtOH) Lit. 278-279 °C [40]. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 6.74 (m, 2H, Ar), 7.05 (m, 2H, Ar), 7.22 (m, 1H, Ar), 7.31 (s, 1H, Ar), 7.46 (d, *J* = 8.0 Hz, 1H, Ar), 7.67 (d, *J* = 8.0 Hz, 1H, Ar), 8.57 (s, 1H, CH), 8.95 (s, 1H, OH), 10.26 (s, 1H, OH), 11.78 (s, 1H, NH), 11.99 (s, 1H, NH). IR (Nujol) 3507, 3345, 3266, 1662 cm<sup>-1</sup>. *m/z* 296 (M + H)<sup>+</sup>. Anal. Calcd for C<sub>16</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>: C, 65.08; H, 4.44; N, 14.23. Found: C, 65.15; H, 4.43; N, 14.20.

4.2.1.5. (*E*)-*N'*-(2,4-dihydroxybenzylidene)-1*H*-indole-2-carbohydrazide (**8**). Yield 86%. Mp >250 °C (EtOH) Lit. 280-282 °C [40]. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 6.35 (m, 2H, Ar), 7.06 (m, 1H, Ar), 7.21 (m, 1H, Ar), 7.33 (m, 2H, Ar), 7.46 (d, *J* = 8.0 Hz, 1H, Ar), 7.66 (d, *J* = 8.0 Hz, 1H, Ar), 8.50 (s, 1H, CH), 9.92 (s, 1H, OH), 11.35 (s, 1H, OH), 11.76 (s, 1H, NH), 11.92 (s, 1H, NH). IR (Nujol) 3417, 1607, 1554 cm<sup>-1</sup>. *m/z* 296 (M + H)<sup>+</sup>. Anal. Calcd for C<sub>16</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>: C, 65.08; H, 4.44; N, 14.23. Found: C, 65.00; H, 4.43; N, 14.28.

4.2.1.6. (*E*)-*N'*-(2,3,4-trihydroxybenzylidene)-1*H*-indole-2-carbohydrazide (**9**). Yield 71%. Mp >250 °C (EtOH). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 6.40 (d, *J* = 8.0 Hz, 1H, Ar), 6.81 (d, *J* = 8.0 Hz, 1H, Ar), 7.06 (m, 1H, Ar), 7.23 (m, 2H, Ar), 7.45 (d, *J* = 8.5 Hz, 1H, Ar), 7.67 (d, *J* = 8.0 Hz, 1H, Ar), 8.46 (s, 1H, OH), 8.50 (s, 1H, CH), 9.44 (s, 1H, OH), 11.43 (s, 1H, OH), 11.80 (s, 1H, NH), 11.98 (s, 1H, NH). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 106.7, 110.8, 114.1, 115.5, 123.1, 124.1, 124.9, 126.9, 130.1, 132.9, 135.9, 140.0, 150.5, 151.8, 152.5, 160.3. IR (Nujol) 3412, 3324, 3250, 1641, 1616 cm<sup>-1</sup>. *m/z* 312 (M + H)<sup>+</sup>. Anal. Calcd for C<sub>16</sub>H<sub>13</sub>N<sub>3</sub>O<sub>4</sub>: C, 61.73; H, 4.21; N, 13.50. Found: C, 61.79; H, 4.23; N, 13.47.

4.2.1.7. (*E*)-*N'*-(2,4,6-trihydroxybenzylidene)-1*H*-indole-2-carbohydrazide (**10**). Yield 77%. Mp >250 °C (EtOH). Lit. 256-258 °C [40]. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 5.85 (s, 2H, Ar), 7.05 (m, 1H, Ar), 7.22 (m, 2H, Ar), 7.45 (d, *J* = 8.5 Hz, 1H, Ar), 7.66 (d, *J* = 8.0 Hz, 1H, Ar), 8.79 (s, 1H, CH), 9.79 (s, 2H, OH), 11.06 (s, 1H, OH), 11.74 (s, 1H, NH), 11.92 (s, 1H, NH). IR (Nujol) 3347, 1642, 1611 cm<sup>-1</sup>. *m/z* 312 (M + H)<sup>+</sup>. Anal. Calcd for C<sub>16</sub>H<sub>13</sub>N<sub>3</sub>O<sub>4</sub>: C, 61.73; H, 4.21; N, 13.50. Found: C, 61.68; H, 4.22; N, 13.54.

4.2.1.8. (*E*)-*N'*-(3-hydroxy-4-methoxybenzylidene)-1*H*-indole-2-carbohydrazide (**11**). Yield 90%. Mp 158-160 °C (EtOH). Lit. 150-152 °C [40]. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 3.81 (s, 3H, CH<sub>3</sub>), 6.98 (d, *J* = 8.0 Hz, 1H, Ar), 7.10 (m, 2H, Ar), 7.24 (m, 3H, Ar), 7.45 (d, *J* = 8.0 Hz, 1H, Ar), 7.66 (d, *J* = 7.5 Hz, 1H, Ar), 8.30 (s, 1H, CH), 9.31 (s, 1H, OH), 11.71 (s, 1H, NH), 11.77 (s, 1H, NH). IR (Nujol) 3300, 1621, 1563 cm<sup>-1</sup>. *m/z* 310 (M + H)<sup>+</sup>. Anal. Calcd for C<sub>17</sub>H<sub>15</sub>N<sub>3</sub>O<sub>3</sub>: C, 66.01; H, 4.89; N, 13.58. Found: C, 66.07; H, 4.90; N, 13.54.

4.2.1.9. (*E*)-*N'*-(2-hydroxy-4-methoxybenzylidene)-1*H*-indole-2-carbohydrazide (**12**). Yield 74%. Mp > 250 °C (EtOH). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 3.77 (s, 3H, OCH<sub>3</sub>), 6.49-6.53 (m, 2H, Ar), 7.34-7.68 (m, 6H, Ar), 8.54 (s, 1H, CH), 11.49 (s, 1H, OH), 11.76 (s, 1H, NH), 12.0 (s, 1H, NH). IR (Nujol) 3315, 3241, 1651, 1630, 1606 cm<sup>-1</sup>. *m/z* 310 (M + H)<sup>+</sup>. Anal. Calcd for C<sub>17</sub>H<sub>15</sub>N<sub>3</sub>O<sub>3</sub>: C, 66.01; H, 4.89; N, 13.58. Found: C, 65.95; H, 4.91; N, 13.62.

4.2.1.10. (*E*)-*N'*-(2-hydroxy-3-ethoxybenzylidene)-1*H*-indole-2-carbohydrazide (**13**). Yield 87%. Mp 214-216 °C (EtOH). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 1.35 (t, *J* = 7.0 Hz, 3H, CH<sub>3</sub>), 4.06 (q, *J* = 7.0 Hz, 2H, CH<sub>2</sub>), 6.85 (t, *J* = 8.0 Hz, 1H, Ar), 7.04 (d, *J* = 8.0 Hz, 1H, Ar), 7.16 (d, *J* = 8.0 Hz, 1H, Ar), 7.20-7.68 (m, 5H, Ar), 8.64 (s, 1H, CH), 10.85 (s, 1H,

OH), 11.81 (s, 1H, NH), 12.1 (s, 1H, NH). IR (Nujol) 3320, 1655, 1621, 1605  $\text{cm}^{-1}$ .  $m/z$  324 (M + H)<sup>+</sup>. Anal. Calcd for  $\text{C}_{18}\text{H}_{17}\text{N}_3\text{O}_3$ : C, 66.86; H, 5.30; N, 13.00. Found: C, 66.80; H, 5.32; N, 13.03.

4.2.1.11. (*E*)-*N'*-(5-chloro-2-hydroxybenzylidene)-1*H*-indole-2-carbohydrazide (**14**). Yield 81%. Mp > 250 °C (EtOH). <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta$  6.95 (d,  $J$  = 8.0 Hz, 1H, Ar), 7.07 (m, 1H, Ar), 7.23 (m, 1H, Ar), 7.33 (s, 1H, Ar), 7.44 (m, 2H, Ar), 7.46 (d,  $J$  = 8.5 Hz, 1H, Ar), 7.68 (d,  $J$  = 8.0 Hz, 1H, Ar), 8.62 (s, 1H, CH), 11.18 (s, 1H, OH), 11.80 (s, 1H, NH), 12.18 (s, 1H, NH). IR (Nujol) 3342, 3325, 1666  $\text{cm}^{-1}$ .  $m/z$  314 (M + H)<sup>+</sup>. Anal. Calcd for  $\text{C}_{16}\text{H}_{12}\text{ClN}_3\text{O}_3$ : C, 61.25; H, 3.86; N, 13.39. Found: C, 61.19; H, 3.87; N, 13.42.

4.2.1.11. (*E*)-*N'*-(5-bromo-2-hydroxybenzylidene)-1*H*-indole-2-carbohydrazide (**15**). Yield 81%. Mp > 250 °C (EtOH) Lit. 278-280 °C [41]. <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta$  6.98 (d,  $J$  = 7.0 Hz, 1H, Ar), 7.07 (m, 1H, Ar), 7.23 (m, 1H, Ar), 7.33 (s, 1H, Ar), 7.44 (m, 2H, Ar), 7.68 (d,  $J$  = 8.5 Hz, 1H, Ar), 7.81 (m, 1H, Ar), 8.61 (s, 1H, CH), 11.19 (s, 1H, OH), 11.81 (s, 1H, NH), 12.18 (s, 1H, NH). IR (Nujol) 3312, 1668, 1605  $\text{cm}^{-1}$ .  $m/z$  358 (M + H)<sup>+</sup>, 360 (M + 2 + H)<sup>+</sup>. Anal. Calcd for  $\text{C}_{16}\text{H}_{12}\text{BrN}_3\text{O}_3$ : C, 53.65; H, 3.38; N, 11.73. Found: C, 53.70; H, 3.37; N, 11.70.

4.2.1.12. (*E*)-*N'*-(4-(diethylamino)-2-hydroxybenzylidene)-1*H*-indole-2-carbohydrazide (**16**). Yield 80%. Mp 200-202 °C (EtOH). <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta$  1.11 (t,  $J$  = 7.5 Hz, 6H, CH<sub>3</sub>), 3.35 (q,  $J$  = 7.5 Hz, 4H, CH<sub>2</sub>), 6.13 (s, 1H, Ar), 6.28 (d,  $J$  = 8.0 Hz, 1H, Ar), 7.06 (m, 1H, Ar), 7.21 (m, 3H, Ar), 7.45 (d,  $J$  = 8.0 Hz, 1H, Ar), 7.66 (s, 1H, Ar), 8.42 (s, 1H, CH), 11.35 (s, 1H, OH), 11.74 (s, 1H, NH), 11.83 (s, 1H, NH). <sup>13</sup>C NMR (DMSO- $d_6$ ):  $\delta$  15.6, 46.9, 100.6, 106.3, 106.8, 109.7, 110.0, 115.5, 123.1, 124.8, 126.8, 130.2, 133.2, 134.5, 139.9, 152.4, 153.3, 160.1, 162.7. IR (Nujol) 3295, 1635, 1592  $\text{cm}^{-1}$ .  $m/z$  351 (M + H)<sup>+</sup>. Anal. Calcd for  $\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}_2$ : C, 68.55; H, 6.33; N, 15.99. Found: C, 68.49; H, 3.32; N, 16.03.

4.2.1.13. (*E*)-*N'*-((2-hydroxynaphthalen-1-yl)methylene)-1*H*-indole-2-carbohydrazide (**17**). Yield 85%. Mp >250 °C (EtOH). <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta$  7.08 (d,  $J$  = 7.5 Hz, 1H, Ar), 7.24 (d,  $J$  = 7.5 Hz, 1H, Ar), 7.42 (d,  $J$  = 7.5 Hz, 1H, Ar), 7.48-7.60 (m, 3H, Ar), 7.64 (d,  $J$  = 8.0 Hz, 1H, Ar), 7.71 (d,  $J$  = 8.0 Hz, 1H, Ar), 7.89-8.27 (m, 3H, Ar), 9.46 (s, 1H, CH), 11.86 (s, 1H, OH), 12.22 (s, 1H, NH), 12.69 (s, 1H, NH). IR (Nujol) 3322, 1673, 1620, 1571  $\text{cm}^{-1}$ .  $m/z$  330 (M + H)<sup>+</sup>. Anal. Calcd for  $\text{C}_{20}\text{H}_{15}\text{N}_3\text{O}_2$ : C, 72.94; H, 4.59; N, 12.76. Found: C, 72.99; H, 4.61; N, 12.71.

4.2.2. General procedure for the synthesis of hydrazones (**19-21**). A mixture of indole-2-carboxaldehyde **18** (0.14g, 1 mmol) and the appropriate hydroxybenzohydrazide (1 mmol) in EtOH (10 mL) was refluxed for 18h. After cooling the formed precipitate was filtered off and purified by crystallization from the adequate solvent to give the hydrazone derivatives.

4.2.2.1. (*E*)-*N'*-((1*H*-indol-2-yl)methylene)-3-hydroxybenzohydrazide (**19**). Yield 70%. Mp >250 °C (EtOH). <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta$  6.82 (s, 1H, Ar), 7.00 (d,  $J$  = 8.0 Hz, 2H, Ar), 7.15 (m, 2H, Ar), 7.32 (m, 2H, Ar), 7.44 (d,  $J$  = 7.5 Hz, 1H, Ar), 7.55 (d,  $J$  = 8.0 Hz, 1H, Ar), 8.47 (s, 1H, CH), 9.75 (s, 1H, OH), 11.56 (s, 1H, NH), 11.74 (s, 1H, NH). IR (Nujol) 3331, 1655, 1596  $\text{cm}^{-1}$ .  $m/z$  280 (M + H)<sup>+</sup>. Anal. Calcd for  $\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}_2$ : C, 68.81; H, 4.69; N, 15.05. Found: C, 68.89; H, 4.68; N, 15.01.

4.2.2.2. (*E*)-*N'*-((1*H*-indol-2-yl)methylene)-4-hydroxybenzohydrazide (**20**). Yield 75%. Mp >250 °C (EtOH). <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta$  6.77 (s, 1H, Ar), 6.84 (d,  $J$  = 8.0 Hz, 2H, Ar), 6.96-7.53 (m, 4H, Ar), 7.80 (d,  $J$  = 8.0 Hz, 2H,

Ar), 8.43 (s, 1H, CH), 10.06 (s, 1H, OH), 11.49 (s, 1H, NH), 11.56 (s, 1H, NH). IR (Nujol) 3282, 1645, 1607, 1581  $\text{cm}^{-1}$ .  $m/z$  280 ( $M + H$ )<sup>+</sup>. Anal. Calcd for  $\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}_2$ : C, 68.81; H, 4.69; N, 15.05. Found: C, 68.76; H, 4.70; N, 15.09.

4.2.2.3. (*E*)-*N'*-((1*H*-indol-2-yl)methylene)-2,4-dihydroxybenzohydrazide (**21**). Yield 72%. Mp >235-237 °C (EtOH). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  7.04 (m, 2H, Ar), 7.22 (m, 2H, Ar), 7.32 (m, 2H, Ar), 7.44 (d,  $J = 8.5$  Hz, 1H, Ar), 7.62 (d,  $J = 8.0$  Hz, 1H, Ar), 8.68 (s, 1H, CH), 10.12 (s, 2H, OH), 11.69 (s, 2H, NH). IR (Nujol) 3420, 1606  $\text{cm}^{-1}$ .  $m/z$  296 ( $M + H$ )<sup>+</sup>. Anal. Calcd for  $\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}_3$ : C, 65.08; H, 4.44; N, 14.23. Found: C, 65.02; H, 4.45; N, 14.28.

### 4.3. Biological assays

#### 4.3.1. DPPH assay

The DPPH<sup>•</sup> radical scavenging activity of the synthesized derivatives was measured according to the method of Wang et al [42] modified as previously reported [28]. The results are expressed as  $\mu\text{mol TE/g}$  corresponding to an inhibition of the radical equal to 50%, except for some compounds.

#### 4.3.2. FRAP test

The FRAP antioxidant capacity of indoles **4-17** and **20-22** was estimated according to the method described previously [43]. The absorbance of the reaction mixture was read at 593 nm. The values are expressed as  $\mu\text{mol TE/g}$  compound.

#### 4.3.3. Oxygen Radical Absorbance Capacity (ORAC)

Based on a protocol previously reported and modified in our previous work [44], the peroxy radical scavenging efficacy was measured. Sample solutions were prepared using phosphate buffer solution (pH 7.4). A series of Trolox solutions (40-240  $\mu\text{M}$ ) was prepared by diluting with phosphate buffer solution (pH 7.4) a solution of Trolox (2 mM). Sample solution, Trolox dilution (25  $\mu\text{L}$ ), or phosphate buffer solution (pH 7.4) used as blank was placed in wells of a 96-well black microplate (VWR). The fluorescence measurements were carried out at 37 °C and recorded at 5 min intervals up to 30 min after the addition of AAPH. The ORAC values were calculated according to the method of Cao et al. [45] and expressed as Trolox equivalents ( $\mu\text{mol TE}/\mu\text{mol}$ ).

#### 4.3.4. Evaluation of filtering parameters

The method followed is an adaptation of the official method for determining the value of SPF *in vitro* [46]. 0.000034 ( $\pm 0.0000033$ ) M solutions of the indole hydrazones **5-11**, **16** and **17** were prepared in MeOH and each of the absorption spectra was recorded.

To calculate the SPF value *in vitro*, the absorbance values obtained were transformed into transmittance values, using the equation below:

$$A(\lambda) = -\text{Log}[T(\lambda)]$$

The transmittance spectrum was elaborated with the SPF calculator software (version 2.1, Shimadzu, Milan, Italy) to obtain the values of SPF, UVA / UVB, UVAPF and  $\lambda$  critical.

#### 4.3.5. Formulations



For each molecule studied, two standard oil / water emulsions (Formulation A and Formulation B) were prepared. Once the ingredients of the aqueous phase and of the oil phase have been added, each phase has been heated up to a temperature between 60 and 70 °C. At this point, the oil phase was added to the aqueous phase, under mechanical stirring. As a third step the test compound was added during the subsequent cooling of the emulsion, maintained in continuous agitation, to preserve it from possible thermal degradation. Finally, the emulsion was cooled up to 25° C and kept in the fridge until the next day of analysis. The formulation compositions are the following: Formulation A: Aqua, Ethylhexyl Stearate, Tribehenin PEG-20 Esters, Butyrospermum Parkii, Olea Europaea Oil Unsaponifiables, Oxisol or indole **16** or indole **17**, Xanthan Gum, Caprylic/Capric Triglyceride, Cetearyl Alcohol, Dicaprylyl Carbonate.

Formulation B: Aqua, Cetearyl Alcohol, Tribehenin PEG-20 Esters, Butyrospermum Parkii, Olea Europaea Oil Unsaponifiables, Indole **16** or indole **17**, Xanthan Gum, Caprylic/Capric Triglyceride.

#### 4.3.6. Franz cells apparatus

The synthetic membrane employed in Franz diffusion cells experiments in this study was a Cuprophan Regenerated cellulose from Medicell (London, UK), membrane thickness (11.5µm +/- 0,5µm), molecular-weight cut off range (10,000 Da). The effective diffusion area of the Franz cells was 0.6 cm<sup>2</sup> and receptor volume was 4.3 mL.

The membranes were conditioned with the receiving solution (DMSO), before being mounted on the diffusion cells. The system was set up by placing the membrane between the compartment containing the receiving medium and the donor compartment. The formulation (0.5 grams) was placed in the donor, in contact with the membrane; the donor compartment was sealed with Parafilm® to avoid any loss of the emulsion components. Franz cells are immersed in a thermostat bath which guarantees a membrane surface temperature of 37 ± 1 ° C, keeping the receiving solvent continuously magnetic stirred. Using a glass syringe, 500 µl of sample were taken and replaced by 500 µl of fresh receiving medium, after 30 minutes, 1 hour and then every hour thereafter up to 6 hours and the absorbance of samples at λ<sub>max</sub> was measured using a UV-VIS spectrophotometer. For each compound a calibration line and a release curve have been constructed.

#### 4.3.7. Growth inhibition assays

Cell growth inhibition assays were carried out using two human cancer cell lines, melanoma Colo38 and erythroleukemia K562 [47, 48, 28]. Cell lines were maintained in RPMI 1640, supplemented with 10% fetal bovine serum (FBS), penicillin (100 Units mL<sup>-1</sup>), streptomycin (100 µg mL<sup>-1</sup>) and glutamine (2 mM) (complete medium); the pH of the medium was 7.2 and the incubation was performed at 37°C in a 5% CO<sub>2</sub> atmosphere. Indole derivatives were dissolved in MeOH/DMSO 10% to obtain 20 mM stock solutions and diluted before cell treatment in MeOH 100%. All the hydrazones **4-17** were added at serial dilutions to the cell cultures and incubated for 3 days. Cells were then harvested, suspended in physiological solution and counted with a Z2 Coulter Counter (Coulter Electronics, Hialeah, FL, USA). The cell number/ml was determined as IC<sub>50</sub> after 3

days of culture, when untreated cells are in log phase of cell growth. Untreated cells were placed in every plate as negative control.

### Acknowledgment

This work was supported by the Fondazione di Sardegna under the project "Innovative antioxidant molecules for the food and health industry" (CUP F71I17000180002 to GB and VO), by University of Ferrara (Grant FAR 2017 to SM, SV and IL) and Ambrosialab srl (Ferrara, Italy)

### Conflict of interest

The authors declare no conflict of interest.

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UV-filtering activity

	SPF	UVA/UVB	UVA/PA
$\lambda_c$ (nm)	392	2.21	1.39
		9.55	

Antioxidant activity

	DPFH	FRAP	ORAC
$\mu\text{molTE/g}$	12844.01 $\pm$ 14.8	10668.78 $\pm$ 26.1	14421.77 $\pm$ 244.4

Antiproliferative activity

	Colo38	K562
$\text{IC}_{50}$ ( $\mu\text{M}$ )	0.844018	0.638008

ACCEPTED MANUSCRIPT

**Highlights**

\*Indole hydrazones were designed and easy synthesized.

\*Synthesized compounds were assayed *in vitro* as antioxidants and photoprotective agents.

\* Synthesized compounds were assayed *in vitro* for their antiproliferative activity on human melanoma and leukemia cells.

\* The hydrazones **16** and **17** showed antioxidant activity in DPPH, FRAP and ORAC test as well as photoprotective effect.

\* The hydrazones **11**, **16** and **17** inhibited the growth of Colo-38 and K-562 cells with sub-micromolar IC<sub>50</sub> values.