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R. Amara, H. Awad, D. Chaker, G. Bentabed-Ababsa, F. Lassagne, et al.. Conversion of Isatins to Tryptanthrins, Heterocycles Endowed with a Myriad of Bioactivities. European Journal of Organic Chemistry, 2019, 2019 (31-32), pp.5302-5312. 10.1002/ejoc.201900352 . hal-02161845

HAL Id: hal-02161845

https://univ-rennes.hal.science/hal-02161845

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Conversion of isatins to tryptanthrins, heterocycles endowed with a myriad of bioactivities

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Abstract: The numerous bioactivities exhibited by tryptanthrins led chemists to develop synthetic approaches towards this important scaffold. In particular, conversion of isatins into tryptanthrins has been the subject of several studies. In this context, by using iodine and potassium hydroxide at room temperature, we have discovered a simple way to convert isatin and seven of its 5-substituted derivatives (Bu, F, Cl, Br, I, OMe and OCF_3) into the corresponding tryptanthrins. Furthermore, a mechanism was proposed to explain this original reactivity. Finally, we evaluated the antibacterial, antifungal, antioxidant and cytotoxic properties of the synthesized tryptanthrins. The unprecedented inhibition of human 20S constitutive proteasome was finally evaluated.

Introduction

The alkaloid tryptanthrin (unsubstituted indolo[2,1-*b*]quinazoline-6,12-dione, **1-H**) is present in a variety of natural sources such as dried roots of indigo[1] and leaves of *Couroupita guianensis* (cannonball tree).[2] It can also be found in cultures including fungus *Candida lipolytica*[3] and yeast.[4] As traditional component of dyes and constituent of traditional medicinal herbal treatments,[5] tryptanthrin has experienced a very rich history since the elucidation of its structure 50 years ago.[6] Indeed, the long list of biological properties associated with this tetracycle has justified the study of many of its derivatives.[3]

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First, tryptanthrins show biological properties such as antibacterial, antifungal, antiprotozoal and other antiparasitic activities. Towards *Mycobacterium tuberculosis*, tryptanthrin (**1-H**) is as effective as isoniazid, streptomycin and ethambutol which are powerful antitubercular agents.[7] It is also effective against bacillus (antibiotic) and dermatophytes (fungistatic activity).[8] Tryptanthrins are promising drugs against toxoplasmosis,[9] while they display antitrypanosomal activities, in particular against *Trypanosoma brucei* responsible for the African sleeping sickness,[10] as well as antimalarial properties.[11]

Second, the anti-inflammatory activities attributed to **1-H**[12] can result from its ability to inhibit cyclooxygenase-2 and 5-lipooxygenase mediated syntheses of prostaglandins and leukotrienes which are pro-inflammatory mediators.[13] Tryptanthrin (**1-H**) can alternately inhibit the biocatalysed nitric oxide over-production and mast cell degranulation,[14] at the origin of several inflammatory diseases. It has potential for the treatment of diseases such as allergic atopic dermatitis[15] and inflammatory bowel colitis.[16]

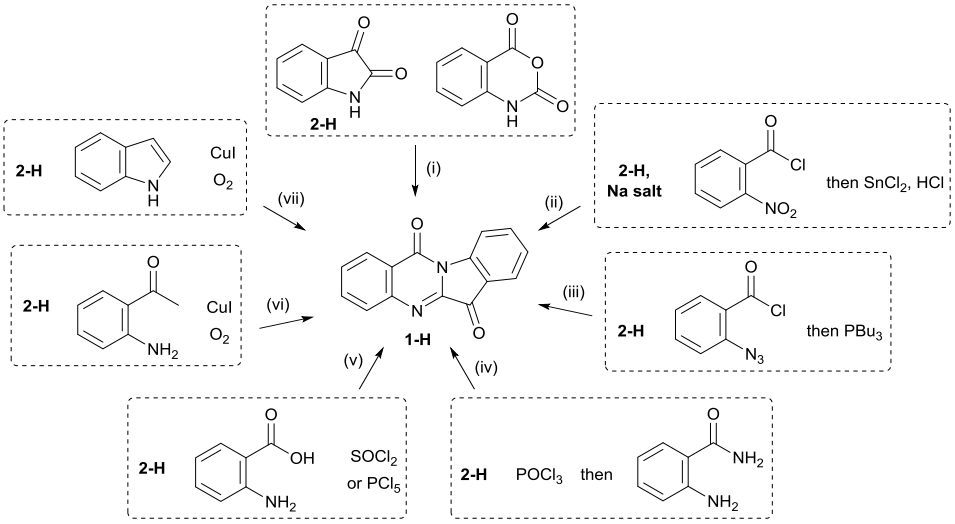
Some tryptanthrins are inhibitors of the indoleamine 2,3-dioxygenase 1[17] and 2,[18] and of the tryptophan 2,3-dioxygenase,[19] which are important therapeutic targets due to their involvement in cancers, neurological disorders and other diseases linked to pathological tryptophan metabolism. The chemopreventive activity of tryptanthrin (**1-H**) is also reported, for example towards tumor promotion[20] or adverse effects of environmental pollutants such as chlorinated dioxins.[21] A cell protected effect against oxidative stress has also been proposed.[22]

The antineoplastic properties of tryptanthrin (**1-H**) have been associated with cell cycle arrest[23] and cell differentiation promotion,[1] leading to apoptosis[24] and reduced proliferation.[25] Furthermore, tryptanthrin (**1-H**) has shown some activity towards the inhibition of angiogenesis, which plays an important role in tumor growth, invasion and metastasis.[26] It prevents the action of topoisomerase I and II, both essential for solving DNA topological problems, and also causes cytotoxicity in several human cancer cell lines such as neuroblastoma and leukemia cells.[27] Finally, it is capable of reversing multidrug resistance, one of the main causes of failure for cancer chemotherapy.[28]

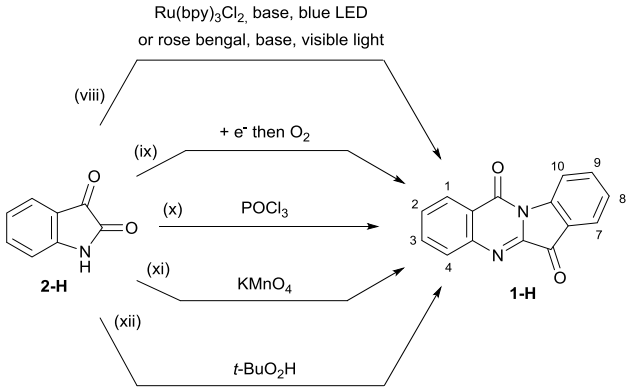
Because of its numerous bioactivities, different synthetic approaches to access tryptanthrin (**1-H**) and its substituted derivatives have been reported.[4-5,29] As unsubstituted isatin (**2-H**) can be easily obtained by oxidation of indole, oxindole or indigo,[30] it constitutes the most general starting material towards **1-H** (Schemes 1 and 2). Other approaches are less straightforward and will not be detailed here. Isatoic anhydride is the most commonly used partner of **2-H** (reaction (i) in Scheme 1);[31] one can also cite 2-nitrobenzoyl chloride (reaction (ii) for which a reduction step is needed),[32] 2-azidobenzoyl chloride (intramolecular aza-Wittig reaction (iii)),[33] anthranilamide

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(reaction (iv) using $POCl_3$),[31h] anthranilic acid (reaction (v) in the presence of $SOCl_2$[34] or PCl_5),[11a] and more recently 2-aminoacetophenone and indole (reactions (vi)[29g] and (vii),[35] both using CuI and oxygen). These ways are in general suitable for synthesis of tryptanthrins with different substituents on each side. Self-condensation of isatin can be performed as an alternative method to access tryptanthrin (**1-H**), as well as its 2,8-disubstituted (and in principle 1,7-, 3,9-, 4,10-, etc.) analogues bearing the same substituents (Scheme 2). In addition to the photoredox-catalyzed reactions recently published (reaction (viii))[31a,36] and cathodic reduction (reaction ix),[37] methods using $POCl_3$ (reaction (x))[38] or oxidizing agents such as $KMnO_4$ (xi)[8b,39] and *t*-BuO_2H (xii)[29e] were reported to achieve such a dimerization.



Scheme 1. Methods for converting isatin (**2-H**) into tryptanthrin (**1-H**) that can be extended to tryptanthrins with different substituents on each benzo ring.

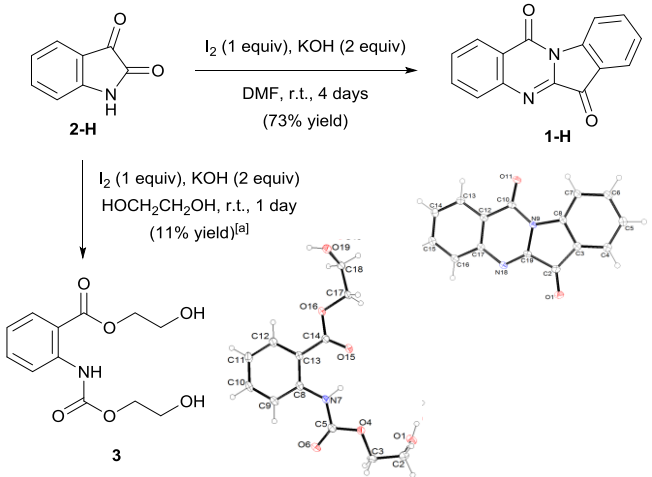


Scheme 2. Methods for converting isatin (**2-H**) into tryptanthrin (**1-H**) that can be extended to its 2,8-disubstituted (and in principle 1,7-, 3,9-, 4,10-, etc.) analogues bearing the same substituents.

Intrigued by the conditions reported in the literature to convert isatin (**2-H**) into its 5-iodo derivative **2-I**,[40] we simply treated **2-H** by iodine and potassium hydroxide in DMF.[41] By this way, we obtained a bright yellow compound for which the structure of tryptanthrin (**1-H**) was unambiguously attributed by X-ray diffraction (Scheme 3, top). This result prompted us to extend the reaction to substituted isatins **1-R** (Table 1; R = substituent), and to assess some of their biological properties. Particularly, in the course of our search for new non-peptide proteasome inhibitors,[42] we evaluated their ability to inhibit human 20S proteasome.

Results and Discussion

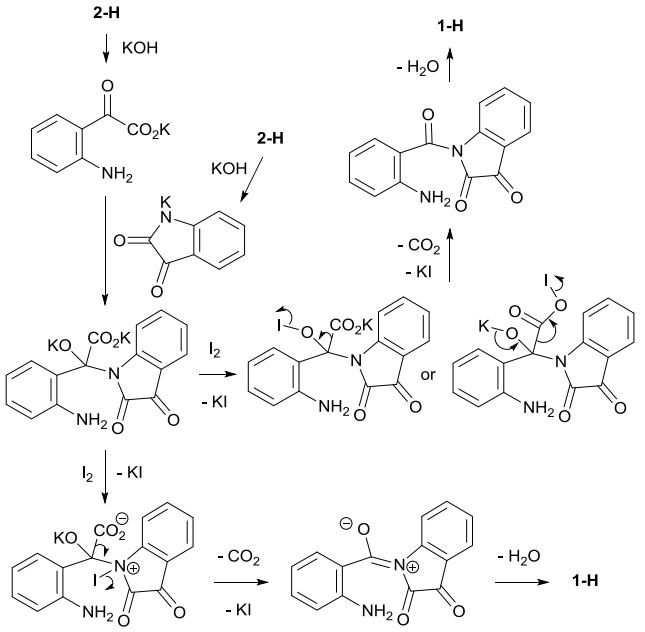
Synthesis



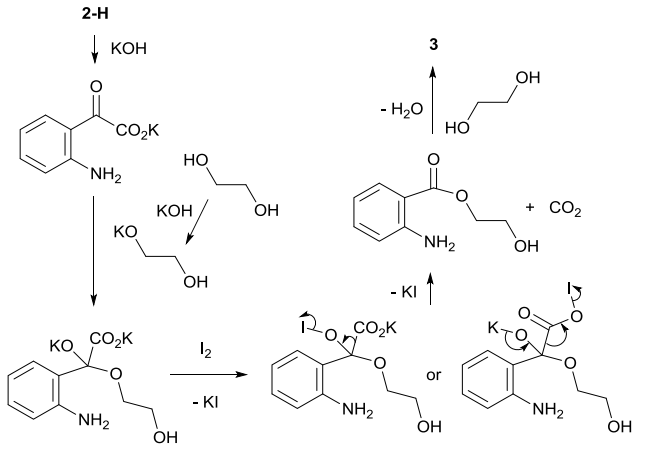
Scheme 3. Conversion of isatin (**2-H**) into tryptanthrin (**1-H**) or **3** by using iodine and potassium hydroxide in DMF or ethylene glycol, respectively. ORTEP diagrams (30% probability) of **1-H** and **3**. [a] **1-H** also formed in <20% yield.

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First, we tried to get clues on the conversion of **2-H** to **1-H**. Without iodine or potassium hydroxide, the reaction did not occur, indicating that both are playing a role in the reaction mechanism. When DMF was replaced by ethylene glycol to favour a polar mechanism, the competitive formation of compound **3** was observed; it was isolated in 11% yield while tryptanthrin was also formed in a <20% yield under these conditions (Scheme 3, bottom). These results led us to propose the pathways shown in Schemes 4 and 5 in which potassium hydroxide and iodine are involved.



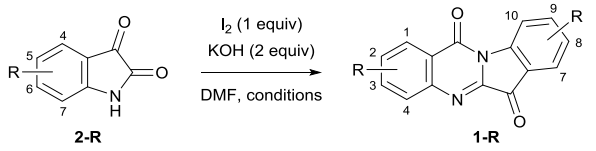
Scheme 4. Mechanism proposed for the conversion of isatin (**2-H**) into **1-H** by using iodine as oxidant and potassium hydroxide.



Scheme 5. Mechanism proposed for the conversion of isatin (**2-H**) into **3** by using iodine as oxidant and potassium hydroxide in ethylene glycol.

Given the ease with which N-C2 (lactam) cleavage of isatin can occur through nucleophilic attack of hydroxides (e.g. aqueous KOH, r.t., 0.5 h),[43] it seemed to us that it could be the first step of both reaction pathways. Next, nucleophilic attacks at C3 are well-known, and could here take place with the potassium salt of isatin (Scheme 4) or ethylene glycol (Scheme 5). An oxidation step is then needed, step probably performed by iodine. Even if N-I bonds are sometimes put forward,[44] and can be here advanced as key step in the pathway giving **1-H** (Scheme 4, bottom), O-I bonds would allow a common way to **1-H** and **3**. In addition, the latter were proposed to rationalize oxidative cleavage of C2-C3 isatin bonds using (diacetoxyiodo)benzene.[45] It is not obvious to decide which of the oxygens (that of the alkoxide or carboxylate group) interacts with iodine in this decarboxylative oxidation.[46] As no cyclization can occur in the case of **3**, the amino group is finally engaged in a carbamoylation step with carbon dioxide in ethylene glycol (Scheme 5).

Table 1. Synthesis of substituted tryptanthrins.



Entry	**2-R**[a]	Conditions	**1-R**,[a] Yields[b] (%)
1	**2-H**	r.t., 4 days	**1-H**, 73
2	**2-Bu** (5-Bu)	r.t., 4 days	**1-Bu** (2,8-diBu), 70%
3	**2-F** (5-Cl)	r.t., 8 days then 100 °C, 3 h	**1-F** (2,8-diF), 58
4	**2-Cl** (5-Cl)	r.t., 4 days	**1-Cl** (2,8-diCl), 53
5	**2'-Cl** (7-Cl)	r.t., 4 days	**1'-Cl**, 0
6	**2-Br** (5-Br)	r.t., 3 days	**1-Br** (2,8-diBr), 15[b]
7	**2'-Br** (4-Br)	r.t., 4 days then 100 °C, 19 h	**1'-Br**, 0[b]
8	**2-I** (5-I)	r.t., 4 days	**1-I** (2,8-diI), 40
9	**2-OMe** (5-OMe)	r.t., 4 days	**1-OMe** (2,8-diOMe), 80
10	**2-OCF3** (5-OCF_3)	r.t., 4 days	**1-OCF3** (2,8-diOCF_3), 34

[a] The substituent location is given into brackets. [b] After purification (see experimental part). [c] Degradation noticed.

We next studied the impact of isatin substituents on the efficiency of the reaction (Table 1). Unlike 5-substituted isatins, the 7-chloroisatin (**2'-Cl**) and 4-bromoisatin (**2'-Br**) did not furnish the expected tryptanthrins **1'-Cl** and **1'-Br** (entries 5 and 7). As previously observed in the reaction of 5,7-dichloroisatin with phosphorus(V) oxychloride,[38a] the presence of an electron-withdrawing group next to the isatin nitrogen could reduce its ability to act as a nucleophile. Probably for a similar reason, the presence of electron-withdrawing groups such as halogens (entries 3, 4, 6 and 8) and trifluoromethoxy (entry 10; Figure 1) at the 5-position of the starting isatin led to the expected tryptanthrins in yields ranging from 15 to 58%, lower than with bare isatin (73%; entry 1). In contrast, while a similar yield was

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recorded from 5-butylisatin (70%, entry 2; Figure 1), an increased reactivity was noticed in the presence of a methoxy group at C5 (entry 9; Figure 1). Thus, our method is perfectly suitable to synthesize tryptanthrins 2,8-disubstituted by electron-donating groups under very mild conditions. However, as noticed when using $POCl_3$,[38a] it is not appropriate to prepare monosubstituted derivatives of tryptanthrin; indeed, treating a 1:1 mixture of isatin and 5-chloroisatin under the same reaction conditions furnished a mixture of the tryptanthrins **1-H** and **1-Cl** in addition to monochlorinated tryptanthrins (~1:1:1 ratio).

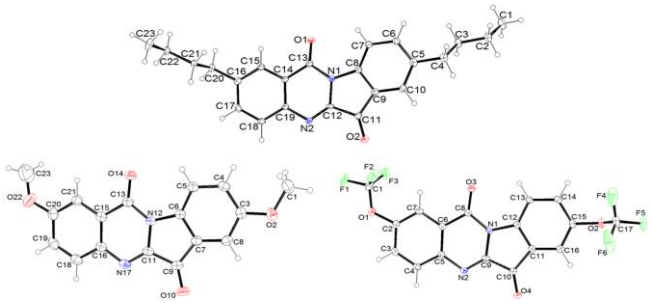


Figure 1. ORTEP diagrams (30% probability) of **1-Bu** (top), **1-OMe** (bottom left) and **1-OCF3** (bottom right).

Table 3. Antioxidant activity of the tryptanthrins **1-R** (R = substituent).

Compound	RSA (%)[a] at t = 0 min	RSA (%)[a] at t = 30 min
1-H	40	40
1-Bu	30	39
1-F	74	81
1-Cl	82	82
1-Br	48	49
1-I	55	58
1-OMe	49	51
1-OCF3	60	65

[a] At a concentration of 5 mg.mL^{-1} in DMSO at room temperature.

Bioactivity

As discussed in the introduction, if the biological activities of bare tryptanthrin (**1-H**) has been extensively studied, 2,8-disubstituted derivatives similar to those prepared here were scarcely studied. 2,8-Difluorotryptanthrin (**1-F**) was evaluated for its ability to inhibit indoleamine 2,3-dioxygenase (IDO) 1[17] and 2,[18] but did not prove better than **1-H**. In order to see if the IDO1 inhibitory activity of **1-H** was related to its redox potential, its radical scavenging properties was also analysed, but no reduction of the diphenylpicrylhydrazyl (DPPH) radical was observed.[17] Besides, 2,8-dibromotryptanthrin (**1-Br**) showed promising antitumor activity against lung and gastric cancer.[47] Its antibacterial and antifungal activities were determined (0.6 µg.mL^{-1} minimum inhibitory concentration (MIC) against the gram-positive methicillin-resistant *Staphylococcus aureus*, and 4 µg.mL^{-1} MIC against *Malassezia furfur*), and although good, proved similar to those exhibited by **1-H**.[48]

In search for improved bioactivities conferred by our substituents, it was of interest to evaluate the synthesized compounds in various biological assays. They were first screened for their antibacterial and antifungal activity (Table 2). None of the tested compounds inhibit the growth of *E. coli*, *P. aeruginosa* and *L. monocytogenes*. When compared with parent **1-H**, the **1-I** and **1-OMe** derivatives have a similar effect on the growth of *S. aureus* whereas **1-F**, **1-Cl** and **1-Br** show a more significant inhibition. The compound **1-F** also inhibits *E. faecium* while **1-Br** is more active on *C. albicans*. The *in vitro* antioxidant activity of the synthesized tryptanthrins is immediate and does not exceed 60% of DPPH radical scavenger activity (RSA), except for the halogenated compounds **1-F** and **1-Cl** that respectively show 74 and 82% scavenging (Table 3).

Recently, human mesenchymal stem cells were confidently used in the area of toxicology.[49] Particularly, adipose human mesenchymal stem cells were considered as a potential new cell substrate for expecting preliminary chemical doses for toxicity assays.[50] We thus evaluated the cytotoxicity of four compounds, **1-H**, **1-Cl**, **1-I** and **1-OMe**, against human mesenchymal stem cells proliferation and viability. To this purpose, their antiproliferative activity was assessed by calculating the index of growth inhibition [1 - (number of cells in treated well/number of cells in control well)] at different concentrations.

The results depicted in Figure 2 show an antiproliferative effect of all the tested compounds. However, the effect of **1-OMe** and **1-I** is already marked at a low 0.1 mg.mL^{-1} concentration while **1-H** and **1-Cl** show less toxicity effects when used at low doses. The viability of the human mesenchymal stem cells in the presence of **1-H**, **1-Cl**, **1-I** and **1-OMe** at different concentrations was also determined (Figure 3). While the viability (assessed using the trypan blue stain) does not exceed 60% when cells are treated over 48 h with 0.01 mg.mL^{-1}, **1-I** and **1-OMe** show a high toxicity effect when used concentrated at 0.1 mg.mL^{-1}. Interestingly, the microscopy examination confirmed these results (Figure 4).

Table 2. Antibacterial and antifungal activity of the tryptanthrins **1-R** (R = substituent).[a]

Compound	Amount (µg dissolved in DMSO)	*Staphylococcus aureus*	*Enterococcus faecium*	*Listeria monocytogenes*	*Candida albicans*
1-H[b]	450	10	0	-[d]	0
1-Bu[c]	150	0	0	0	0
1-F[c]	150	23	10	0	5
1-Cl[b]	450	19	0	-[d]	0
1-Br[c]	150	21	0	0	13
1-I[b]	450	10	0	-[d]	0
1-OMe[b]	450	10	0	-[d]	0
1-OCF3[c]	150	5	0	0	0
DMSO	-	0	0	0	0
Reference compound		18 Vancomycin (30 µg)	24	30 Ampicillin (25 µg)	13 Nystatin (416 UI)

[a] The diameters of zones of inhibition are given in mm. [b] 50 µL/well. [c] 30 µL/well. [d] Not tested.

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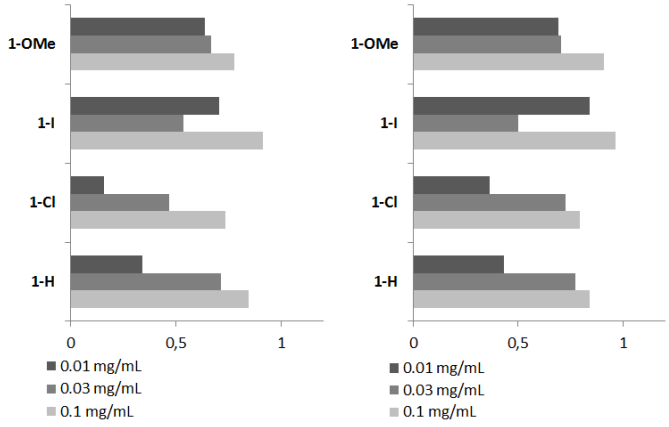


Figure 2. Antiproliferative activity of **1-H**, **1-Cl**, **1-I** and **1-OMe** at 0.01, 0.03 and 0.10 mg.mL^{-1}. 5.103 human mesenchymal stem cells were treated for 24 h (left) and 48 h (right). The proliferation activity was assessed by the calculation of the index of growth inhibition.

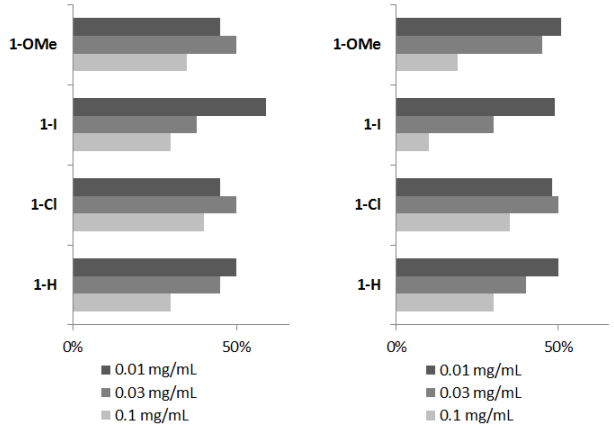


Figure 3. Cell viability of human mesenchymal stem cells treated with **1-H**, **1-Cl**, **1-I** and **1-OMe** at 0.01, 0.03 and 0.10 mg.mL^{-1} for 24 h (left) and 48 h (right).

As proteasome is a target for cancer therapy, very important efforts have been made in recent years in order to discover new inhibitors of this complex, multi-catalytic and proteolytic enzyme.[51] Three peptide proteasome inhibitors bearing an electrophilic warhead are currently marketed for the treatment of multiple myeloma, a blood cancer.[51] In order to circumvent peptide limitations in therapy, the development of non-peptide inhibitors, especially heterocyclic compounds, is growing.[42,51a-d,52] The heterocyclic structure of the prepared tryptanthrins **1-R** and their described cytotoxicity prompted us to test their ability to inhibit purified human constitutive 20S proteasome *in vitro*. At first, their effect on the three proteasome proteolytic activities was evaluated by following the hydrolysis of the corresponding fluorogenic specific substrate in the presence of 50 µM solution of **1-R** or in their absence (see Eq. (1) in the experimental section and Figure 5a.).

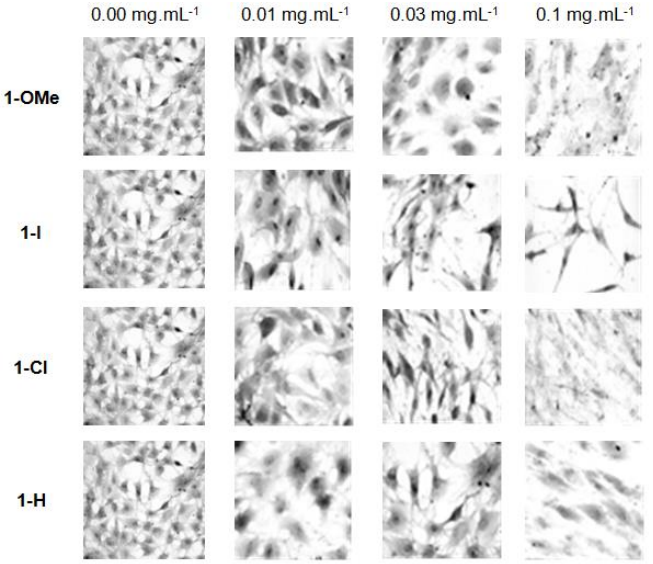


Figure 4. Microscopy examination after 48 h treatment of human mesenchymal stem cells treated with **1-H**, **1-Cl**, **1-I** and **1-OMe** at 0.00, 0.01, 0.03 and 0.1 mg.mL^{-1}.

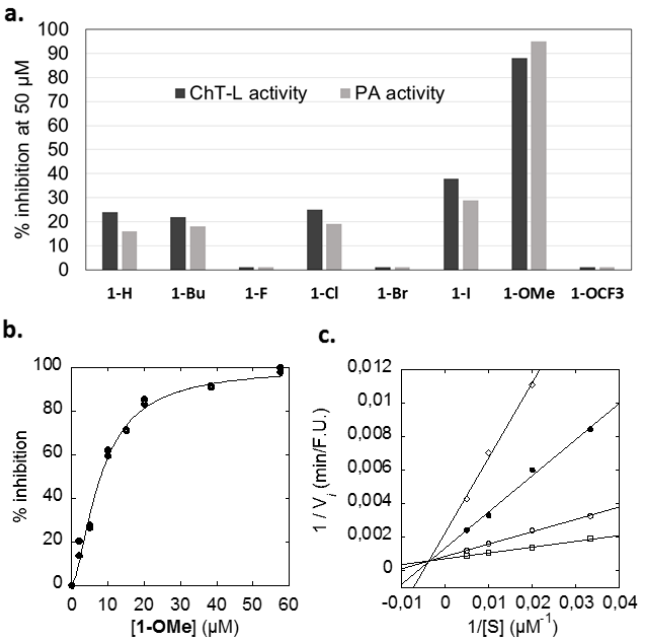


Figure 5. Effects of tryptanthrins on human 20S constitutive proteasome (pH 8, 37 °C). **a.** Inhibition of the ChT-L and PA activities by 50 µM tryptanthrins **1-R**. **b.** Inhibition profile of the PA activity (substrate Z-LLE-βNA, 50 µM) by **1-OMe** showing an IC_{50} of 8.0 ± 0.5 µM. **c.** Double-reciprocal Lineweaver-Burk plot for the inhibition of the ChT-L activity by tryptanthrin **1-OMe**; [20S proteasome] = 0.3 nM; [**1-OMe**] = 0 µM (□); [**1-OMe**] = 10 µM (○); [**1-OMe**] = 20 µM (•); [**1-OMe**] = 38 µM (◊); F.U., fluorescence arbitrary unit; S, substrate Suc-LLVY-AMC.

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No inhibition of the trypsin-like (T-L) activity was detected, whereas the chymotrypsin-like (Ch-TL) and the post-acid (PA) activities were moderately to strongly inhibited by compounds **1-H**, **1-Bu**, **1-Cl**, **1-I**, and **1-OMe** (Figure 5a.). For the most potent tryptanthrin **1-OMe**, the concentrations leading to 50% inhibition (IC_{50} value) were determined, showing that the PA activity (IC_{50} = 7.0 ± 0.5 µM) was more efficiently inhibited than the ChT-L activity (IC_{50} = 15.0 ± 0.8 µM) (Figure 5b.). This is an interesting feature, since co-inhibition of ChT-L and PA activities was shown to enhance cytotoxicity of proteasome inhibitors.[53] As the detection of the ChT-L activity was easier, a kinetic study was realized at a fixed enzyme concentration and various substrate (Suc-LLVY-AMC) and tryptanthrin **1-OMe** concentrations. It was analyzed by Lineweaver-Burk plots that showed a mixed inhibition mechanism (Figure 5c.). Such an allosteric modulation of the proteasome activity, already observed with non-peptide inhibitors[42b,54] and also peptide inhibitors,[55] indicated that binding occurred at a site other than the ChT-L active site, while resulting in inhibition of enzyme activity.

Conclusions

Thus, in view of the numerous potential applications of tryptanthrins, we reported an original route towards 2,8-disubstituted derivatives under mild conditions and proposed a mechanism to explain this unexpected reactivity. The early biological evaluation of our compounds **1-R** was done, and those substituted with halogeno and methoxy groups proved to be more bioactive than the reference **1H**. The antibacterial (*S. aureus*), antifungal (*C. albicans*) and antioxidant activities were higher for **1-F**, **1-Cl** and **1-Br** substituted tryptanthrins. Tryptanthrins **1-OMe** and **1I** were shown to be cytotoxic and antiproliferative agents. This last feature might be due to their ability to inhibit the ChT-L and PA activities of the 20S proteasome, with IC_{50} values in the micromolar range (15 µM and 7 µM, respectively) for **1-OMe**.

If substituted tryptanthrins are of interest by themselves, as disclosed all through this article, they also constitute a promising tetracyclic scaffold to access natural products such as phaitanthrin A and cephalanthrin A,[38b] compounds that can be obtained by nucleophilic attack onto the tryptanthrin ketone.[56] To highlight how such additions can easily happen, we could cite cite the non-optimized formation of compounds **4a** and **4b**.

The dichloro derivative of phaitanthrin A **4a** was obtained in the course of an attempt to dissolve **1-Cl** in acetone and obtain crystals suitable for X-ray diffraction (Figure 6, left). As to **4b** (right), it was formed as undesirable product in the course of an attempt to deprotometallate **1-H** in tetrahydrofuran at room temperature by using a metal amide formed from butyllithium (a slight excess of the latter is enough to rationalize the formation of the butylated alcohol **4b**). Interestingly, both structures obtained by X-ray diffraction show intermolecular hydrogen bonds at the crystal state.

Therefore, combining our mild-formation of tryptanthrins with nucleophilic additions will allow the exploration of the chemical space around this tetracyclic indoloquinazolinedione derivative, in search for original biologically active molecules.

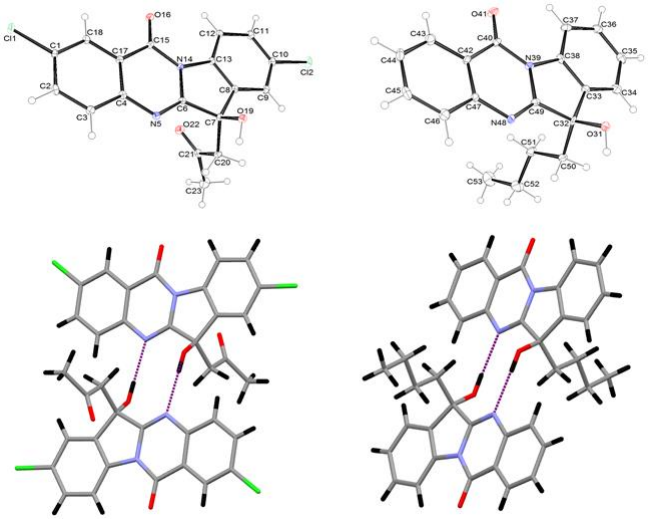


Figure 6. ORTEP diagrams (30% probability) and visualizations of the intermolecular hydrogen bonds of **4a** (1.981 Å; left) and **4b** (1.993 Å; right). Blue: nitrogen; Red: oxygen; Green: chlorine.

Experimental Section

General synthetic and analytical details. Column chromatography separations were achieved on silica gel (40-63 µm). Melting points were measured on a Kofler apparatus. IR spectra were taken on a Perkin-Elmer Spectrum 100 spectrometer. Unless otherwise cited in the product description, ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker Avance III spectrometer at 300 MHz and 75 MHz, respectively. ^{1}H chemical shifts (δ) are always given in ppm relative to the solvent residual peak and ^{13}C chemical shifts are relative to the central peak of the solvent signal.[57] 7-Chloroisatin (**2'-Cl**) and 5-butylisatin (**2-Bu**) were prepared as described previously[58] while the other isatins were purchased.

General crystallographic details. The samples were studied using Mo-Kα radiation (λ = 0.71073 Å) at the temperature given in the crystal data. For compounds **1-H** and **3**, the X-ray diffraction data were collected using APEXII Bruker-AXS diffractometer (graphite monochromatized). The structure was solved by direct methods using the SIR97 program,[59] and then refined with full-matrix least-square methods based on F^2 (SHELX-97)[60] with the aid of the WINGX program.[61] All non-hydrogen atoms were refined with anisotropic atomic displacement parameters. For **1-Bu**, **1-OMe**, **1-OCF3**, **4a** and **4b**, the X-ray diffraction data were collected using D8 VENTURE Bruker AXS diffractometer (multilayer monochromatized). The structure was solved by dual-space algorithm using the *SHELXT* program,[62] and then refined with full-matrix least-square methods based on F^2 (*SHELXL-2014*).[63] All non-hydrogen atoms were refined with anisotropic atomic displacement parameters. Except oxygen linked hydrogen atoms that was introduced in the structural model through Fourier difference maps analysis in the case of **4a** and **4b**, H atoms were finally included in their calculated positions. The molecular diagrams were generated by ORTEP-3 (version 2.02).[64]

Biological evaluation.

The antibacterial, antifungal and antioxidant activity was determined as described previously.[65]

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Antiproliferative activity. Healthy human adult adipose-derived mesenchymal stem cells cell line MSCs (ATCC, PCS-500-011) was used at passage 4. Cells were seeded to a density of 5.103 cells.cm^{-2} in each well of a six-well plate (Corning). Cells were treated with the synthesized compounds **1-H**, **1-Cl**, **1-I** and **1-OMe** at concentrations of 0.01, 0.03 and 0.10 mg.mL^{-1}. All the molecules were reconstituted in DMSO. After 24 and 48 h, cells were counted and the inhibition growth index was calculated. The viability was assessed using the trypan blue stain. The viability of adipose-derived MSCs, solubilized in the same DMSO concentration used for synthetic compounds, is considered as reference control. The toxicity effect of **1-H**, **1-Cl**, **1-I** and **1-OMe** compounds was assessed also by evaluating the apoptotic features of MSCs by microscopy examination (Nikon inverted microscope).

Proteasome inhibition. Purified human 20S constitutive proteasome was purchased from Boston Biochem. Proteasome activities were determined as previously described.[66] Briefly, the enzyme-catalyzed hydrolysis of the appropriate fluorogenic substrate (Suc-LLVY-AMC, concentration 20 µM for the ChT-L activity; Z-LLE-βNA concentration 50 µM for the PA activity; Boc-LRR-AMC, concentration 50 µM for the T-L activity) was followed in microplates for 45 min, at 37 °C and pH 8, by monitoring the fluorescence emission of the released fluorophore (λ_{exc} = 360 nm and λ_{em} = 460 nm for 7-amino-4-methyl-coumarin AMC and λ_{exc} = 340 nm and λ_{em} = 404 nm for β-naphtylamine βNA). Substrates were dissolved in DMSO. Tryptanthrins were also dissolved in DMSO in order to obtain 5 mM stock solutions except for **1-I** (3.5 mM), **1-OMe** (3.8 mM) and **1-OCF3** (4.3 mM). The DMSO percentage was maintained to 2% (v/v) in all experiments, except for those related to **1-I**, **1-OMe** and **1-OCF3** (2.5 %). The mixtures of the enzyme and the tryptanthrins were incubated for 15 min prior treatment by the substrate. The initial rates in the presence of DMSO (control, V_0) or of the tested tryptanthrin at concentration [I] (V_i) were used to determine the inhibition percentage and IC_{50} values according to equations (1) and (2). Data were fitted to equation (2) using the Kaleidagraph software.

$$\%\ inhibition = 100 \times \left(1 - \frac{V_i}{V_0}\right) \qquad (1)$$

$$\%\ inhibition = \frac{100 \times [I]^n}{IC_{50}^n + [I]^n} \qquad (2)$$

Synthesis of the tryptanthrins from the corresponding isatins.

Indolo[2,1-*b*]quinazoline-6,12-dione or tryptanthrin (1-H). To isatin (**2-H**; 2.1 g, 14 mmol) and KOH (1.6 g, 28 mmol) in DMF (30 mL) was added dropwise at room temperature a solution of I_2 (3.6 g, 14 mmol) in DMF (30 mL). After stirring for 4 days, the reaction mixture was poured onto iced water (200 mL) containing NH_4OH (8 mL) and $Na_2S_2O_3$ (14 g). The precipitate formed after 10 h in a fridge was collected by filtration and washed by AcOEt (4 x 30 mL) before drying under vacuum to afford **1-H** in 73% yield (1.3 g) as a yellow powder: mp 260-262 °C (lit.[67] 261-262 °C); IR (ATR): 689, 755, 777, 867, 925, 1039, 1103, 1116, 1165, 1185, 1311, 1353, 1457, 1593, 1680, 1724, 3036, 3070, 3438, 3750 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.41 (t, 1H, *J* = 7.5 Hz), 7.66 (td, 1H, *J* = 7.7 and 0.9 Hz), 7.77 (td, 1H, *J* = 7.8 and 1.2 Hz), 7.84 (td, 1H, *J* = 7.7 and 1.5 Hz), 7.90 (d, 1H, *J* = 7.5 Hz), 8.01 (d, 1H, *J* = 7.8 Hz), 8.41 (dd, 1H, *J* = 8.0 and 1.1 Hz), 8.60 (d, 1H, *J* = 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 118.1 (CH), 122.0 (C), 123.8 (C), 125.5 (CH), 127.3 (CH), 127.7 (CH), 130.4 (CH), 130.8 (CH), 135.3 (CH), 138.4 (CH), 144.4 (C), 146.4 (C), 146.7 (C), 158.2 (C), 182.7 (C). The spectral data are analogous to those described previously.[67] Crystal data for **1-H.** $C_{15}H_8N_2O_2$, *M* = 248.23, *T* = 150(2) K, monoclinic, *P* $2_1/n$, *a* = 7.2839(5), *b* = 7.5706(5), *c* = 19.4214(14) Å, *β* = 91.033(3) °, *V* = 1070.79(13) Å^3, *Z* = 4, *d* = 1.54 g cm^{-3}, *µ* = 0.105 mm^{-1}. A final refinement on F^2 with 2455 unique intensities and 172 parameters converged at $\omega R(F^2)$ = 0.1094 ($R(F)$ = 0.0481) for 1732 observed reflections with $I > 2\sigma(I)$. CCDC 1898458. These data are close to those reported previously.[68]

2,8-Dibutylindolo[2,1-*b*]quinazoline-6,12-dione (1-Bu). To 5-butylisatin (**2-Bu**; 1.3 g, 6.4 mmol) and KOH (0.73 g, 13 mmol) in DMF (14 mL) was added dropwise at room temperature a solution of I_2 (1.3 g, 6.4 mmol) in DMF (14 mL). After stirring for 4 days, the reaction mixture was poured onto iced water (90 mL) containing NH_4OH (4 mL) and $Na_2S_2O_3$ (6 g). The precipitate formed after 10 h in a fridge was collected by filtration and chromatographed over silica gel (eluent: AcOEt-heptane 50:50) to afford **1-Bu** in 70% yield (0.80 g) as a yellow powder: mp 160 °C; IR (ATR): 706, 759, 787, 839, 847, 863, 1044, 1102, 1119, 1135, 1207, 1315, 1350, 1431, 1478, 1588, 1603, 1662, 1725, 2858, 2870, 2930, 2959, 3052 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H, *J* = 7.4 Hz), 0.96 (t, 3H, *J* = 7.4 Hz), 1.31-1.46 (m, 4H), 1.59-1.75 (m, 4H), 2.70 (t, 2H, *J* = 7.7 Hz), 2.80 (t, 2H, *J* = 7.8 Hz), 7.57 (dd, 1H, *J* = 8.3 and 2.0 Hz), 7.65 (dd, 1H, *J* = 8.4 and 2.1 Hz), 7.70 (d, 1H, *J* = 1.5 Hz), 7.92 (d, 1H, *J* = 8.1 Hz), 8.22 (d, 1H, *J* = 1.8 Hz), 8.49 (d, 1H, *J* = 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0 (CH_3), 14.0 (CH_3), 22.3 (CH_2), 22.4 (CH_2), 33.4 (CH_2), 33.4 (CH_2), 35.2 (CH_2), 35.8 (CH_2), 117.8 (CH), 122.3 (C), 123.7 (C), 124.9 (CH), 126.8 (CH), 130.7 (CH), 135.8 (CH), 138.4 (CH), 142.5 (C), 144.2 (C), 144.6 (C), 144.9 (C), 146.2 (C), 158.2 (C), 182.9 (C). Crystal data for **1-Bu.** $C_{23}H_{24}N_2O_2$, *M* = 360.44, *T* = 150(2) K, triclinic, *P* -1, *a* = 5.1503(12), *b* = 13.656(3), *c* = 13.902(3) Å, *α* = 74.392(7), *β* = 89.234(8), *γ* = 85.397(7) °, *V* = 938.7(3) Å^3, *Z* = 2, *d* = 1.275 g cm^{-3}, *µ* = 0.082 mm^{-1}. A final refinement on F^2 with 4316 unique intensities and 246 parameters converged at $\omega R(F^2)$ = 0.2119 ($R(F)$ = 0.0856) for 3008 observed reflections with $I > 2\sigma(I)$. CCDC 1898459.

2,8-Difluoroindolo[2,1-*b*]quinazoline-6,12-dione (1-F). To 5-fluoroisatin (**2-F**; 0.82 g, 5.0 mmol) and KOH (0.57 g, 10 mmol) in DMF (10 mL) was added dropwise at room temperature a solution of I_2 (1.3 g, 5.0 mmol) in DMF (10 mL). After stirring for 8 days at r.t. and 3 h at 100 °C, the reaction mixture was poured onto iced water (70 mL) containing NH_4OH (3 mL) and $Na_2S_2O_3$ (5 g). The precipitate formed after 10 h in a fridge was collected by filtration and chromatographed over silica gel (eluent: AcOEt-heptane 60:40) to afford **1-F** in 58% yield (0.41 g) as a greenish powder: mp > 260 °C (lit.[35] 295-297 °C); IR (ATR): 704, 744, 776, 814, 835, 888, 1037, 1099, 1120, 1162, 1206, 1242, 1270, 1306, 1350, 1470, 1568, 1606, 1678, 1729, 3081, 3130 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.50 (td, 1H, *J* = 8.6 and 2.7 Hz), 7.53-7.60 (m, 2H), 8.01-8.10 (m, 2H), 8.63 (dd, 1H, *J* = 8.9 and 4.1 Hz); ^{13}C NMR ($CDCl_3$) δ 112.3 (CH, d, *J* = 24 Hz), 113.5 (CH, d, *J* = 25 Hz), 119.9 (CH, d, *J* = 7 Hz), 123.7 (CH, d, *J* = 24 Hz), 125.0 (CH, d, *J* = 24 Hz), 125.8 (C, d, *J* = 9 Hz), 133.4 (CH, d, *J* = 9 Hz), 142.4 (C, d, *J* = 3 Hz), 143.3 (C), 143.3 (C), 143.9 (C), 157.1 (C, d, *J* = 4 Hz), 161.4 (C, d, *J* = 249 Hz), 163.4 (C, d, *J* = 253 Hz), 181.5 (C, d, *J* = 3 Hz). The spectral data are analogous to those described previously.[35]

2,8-Dichloroindolo[2,1-*b*]quinazoline-6,12-dione (1-Cl). To 5-chloroisatin (**2-Cl**; 2.5 g, 14 mmol) and KOH (1.6 g, 28 mmol) in DMF (30 mL) was added dropwise at room temperature a solution of I_2 (3.6 g, 14 mmol) in DMF (30 mL). After stirring for 4 days, the reaction mixture was poured onto iced water (200 mL) containing NH_4OH (8 mL) and $Na_2S_2O_3$ (14 g). The precipitate formed after 10 h in a fridge was collected by filtration and chromatographed over silica gel (eluent: AcOEt-heptane 60:40) to afford **1-Cl** in 53% yield (1.2 g) as a yellow powder: mp > 260 °C (lit.[35] 289-291 °C); IR (ATR): 700, 709, 760, 781, 845, 1015, 1024, 1038, 1133, 1170, 1187, 1214, 1255, 1335, 1437, 1462, 1554, 1590, 1603, 1671, 1722, 1734, 2841, 2943, 2971, 3075 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.75 (dd, 1H, *J* = 8.6 and 2.3 Hz), 7.66 (dd, 1H, *J* = 8.4 and 2.4 Hz), 7.88 (d, 1H, *J* = 2.1 Hz), 7.97 (d, 1H, *J* = 8.7 Hz), 8.40 (d, 1H, *J* = 2.4 Hz), 8.58 (d, 1H, *J* = 8.7 Hz); ^{13}C NMR ($CDCl_3$) δ 119.4 (CH), 123.3 (C), 124.9

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(C), 125.5 (CH), 127.4 (CH), 132.4 (CH), 133.8 (C), 135.8 (CH), 137.1 (C), 138.0 (CH), 144.1 (C), 144.3 (C), 145.2 (C), 156.9 (C), 181.2 (C). The spectral data are analogous to those described previously.[35] Crystal data for **4a.** Due to our efforts to solubilize **1-Cl** in acetone in order to get suitable crystals for X-ray diffraction, we observed in the structure the addition of an acetone molecule onto the tryptanthrin ketone (cetolization) to afford **4a**[56a] (the other acetone molecule co-crystallized with the product: $C_{18}H_{12}Cl_2N_2O_3$,C_3H_6O, M = 433.27, T = 150(2) K; triclinic, P -1, a = 8.7184(8), b = 9.5852(7), c = 13.7891(11) Å, α = 72.409(3), β = 88.633(3), γ = 64.794(2) °, V = 986.48(14) Å^3, Z = 2, d = 1.459 g cm^{-3}, μ = 0.360 mm^{-1}. A final refinement on F^2 with 4509 unique intensities and 268 parameters converged at $\omega R(F^2)$ = 0.1826 ($R(F)$ = 0.0702) for 3617 observed reflections with $I > 2\sigma(I)$. CCDC 1898460.

2,8-Dibromoindolo[2,1-*b*]quinazoline-6,12-dione (1-Br). To 5-bromoisatin (**2-Br**; 0.90 g, 4.0 mmol) and KOH (0.46 g, 8.0 mmol) in DMF (9 mL) was added dropwise at room temperature a solution of I_2 (1.0 g, 4 mmol) in DMF (9 mL). After stirring for 3 days, the reaction mixture was poured onto iced water (60 mL) containing NH_4OH (2 mL) and $Na_2S_2O_3$ (4 g). The precipitate formed after 10 h in a fridge was collected by filtration and chromatographed over silica gel (eluent: AcOEt-heptane 60:40) to afford **1-Br** in 15% yield (0.12 g) as a yellow powder: mp > 260 °C (lit.[38a] 319-321 °C); IR (ATR): 741, 782, 803, 845, 1037, 1182, 1262, 1301, 1330, 1459, 1585, 1674, 1730, 2852, 2921 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.89 (d, 1H, J = 8.4 Hz), 7.91 (dd, 1H, J = 8.4 and 2.1 Hz), 7.96 (dd, 1H, J = 8.6 and 2.3 Hz), 8.03 (d, 1H, J = 1.8 Hz), 8.52 (d, 1H, J = 8.7 Hz), 8.56 (d, 1H, J = 2.1 Hz); ^{13}C NMR ($CDCl_3$) δ 118.2 (C), 120.7 (C), 121.9 (CH), 122.8 (C), 125.3 (CH), 130.1 (CH), 130.5 (CH), 136.3 (C), 137.4 (CH), 137.7 (CH), 141.8 (C), 145.8 (C), 151.0 (C), 157.6 (C), 185.0 (C). The spectral data are analogous to those described previously.[38a]

2,8-Diiodoindolo[2,1-*b*]quinazoline-6,12-dione (1-I). To 5-iodoisatin (**2-I**; 1.9 g, 7.0 mmol) and KOH (0.80 g, 14 mmol) in DMF (15 mL) was added dropwise at room temperature a solution of I_2 (1.8 g, 7.0 mmol) in DMF (15 mL). After stirring for 4 days, the reaction mixture was poured onto iced water (100 mL) containing NH_4OH (4 mL) and $Na_2S_2O_3$ (7 g). The precipitate formed after 10 h in a fridge was collected by filtration and washed by AcOEt (4 x 15 mL) before drying under vacuum to afford **1-I** in 40% yield (0.70 g) as a yellow powder: mp > 270 °C (lit.[38a] 338-340 °C); IR (ATR): 733, 845, 856, 1036, 1051, 1117, 1178, 1188, 1303, 1341, 1422, 1458, 1544, 1583, 1678, 1725, 2921, 3024, 3058, 3081 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.73 (d, 1H, J = 8.7 Hz), 8.10 (dd, 1H, J = 8.7 and 1.2 Hz), 8.14 (dd, 1H, J = 8.4 and 1.8 Hz), 8.22 (d, 1H, J = 1.8 Hz), 8.38 (d, 1H, J = 8.4 Hz), 8.77 (d, 1H, J = 1.8 Hz); ^{13}C NMR ($CDCl_3$) δ 91.3 (C), 96.5 (C), 119.8 (CH), 119.8 (C), 123.4 (C), 124.9 (C), 132.2 (CH), 134.1 (CH), 136.6 (CH), 143.7 (C), 144.3 (CH), 145.9 (C), 146.6 (CH), 156.4 (C), 180.8 (C). The spectral data are analogous to those described previously.[38a]

2,8-Dimethoxyindolo[2,1-*b*]quinazoline-6,12-dione (1-OMe). To 5-methoxyisatin (**2-OMe**; 2.5 g, 14 mmol) and KOH (1.6 g, 28 mmol) in DMF (30 mL) was added dropwise at room temperature a solution of I_2 (3.6 g, 14 mmol) in DMF (30 mL). After stirring for 4 days, the reaction mixture was poured onto iced water (200 mL) containing NH_4OH (8 mL) and $Na_2S_2O_3$ (14 g). The precipitate formed after 10 h in a fridge was collected by filtration and chromatographed over silica gel (eluent: AcOEt-heptane 60:40) to afford **1-OMe** in 80% yield (1.7 g) as a yellow powder: mp > 270 °C (lit.[35] 285-288 °C); IR (ATR): 703, 732, 780, 807, 843, 1014, 1023, 1073, 1108, 1137, 1168, 1219, 1256, 1285, 1319, 1345, 1436, 1464, 1479, 1556, 1603, 1665, 1721, 2841, 2943, 2973, 3027, 3053 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.89 (s, 3H), 3.98 (s, 3H), 7.29 (dd, 1H, J = 9.0 and 2.7 Hz), 7.36-7.40 (m, 2H), 7.81 (d, 1H, J = 2.7 Hz), 7.93 (d, 1H, J = 8.7 Hz), 8.50 (d, 1H, J = 8.7 Hz); ^{13}C NMR ($CDCl_3$) δ 56.1 (CH_3), 56.2 (CH_3), 108.3 (CH), 108.4 (CH), 119.3 (CH), 123.5 (C), 124.3 (CH), 124.9 (CH), 125.5 (C), 132.5 (CH), 140.4 (C), 141.0 (C), 142.9 (C), 157.6 (C), 158.9 (C), 161.5 (C), 182.7 (C). The spectral data are analogous to those described previously.[35] Crystal data for **1-OMe.** $C_{17}H_{12}N_2O_4$, M = 308.29, T = 295 K, monoclinic, P 2$_1$/n, a = 18.012(7), b = 3.8582(15), c = 20.150(7) Å, β = 100.88(2) °, V = 1375.1(9) Å^3, Z = 4, d = 1.489 g cm^{-3}, μ = 0.108 mm^{-1}. A final refinement on F^2 with 3109 unique intensities and 210 parameters converged at $\omega R(F^2)$ = 0.2482 ($R(F)$ = 0.0904) for 1838 observed reflections with $I > 2\sigma(I)$. CCDC 1898461.

2,8-Bis(trifluoromethoxy)indolo[2,1-*b*]quinazoline-6,12-dione (1-OCF3).[36] To 5-(trifluoromethoxy)isatin (0.46 g, 2.0 mmol) and KOH (0.23 g, 4.0 mmol) in DMF (5 mL) was added dropwise at room temperature a solution of I_2 (0.51 g, 2.0 mmol) in DMF (5 mL). After stirring for 4 days, the reaction mixture was poured onto iced water (30 mL) containing NH_4OH (1 mL) and $Na_2S_2O_3$ (2 g). The precipitate formed after 10 h in a fridge was collected by filtration and chromatographed over silica gel (eluent: AcOEt-heptane 60:40) to afford **1-OCF3** in 34% yield (0.14 g) as a yellow powder: mp 246-247 °C; IR (ATR): 677, 781, 801, 837, 852, 863, 1040, 1104, 1125, 1160, 1210, 1256, 1292, 1474, 1605, 1683, 1741, 2852, 2924, 2958, 3081 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.65 (ddd, 1H, J = 8.9, 2.6 and 0.8 Hz), 7.69 (ddd, 1H, J = 8.9, 2.7 and 0.7 Hz), 7.77-7.78 (m, 1H), 8.09 (d, 1H, J = 9.0 Hz), 8.26 (dd, 1H, J = 2.4 and 0.9 Hz), 8.69 (d, 1H, J = 8.7 Hz); ^{1}H NMR ((CD_3)$_2$SO) δ 7.92 (dd, 1H, J = 9.0 and 1.5 Hz), 7.96 (d, 1H, J = 1.3 Hz), 7.98 (dd, 1H, J = 9.0 and 2.5 Hz), 8.13 (d, 1H, J = 8.5 Hz), 8.18 (d, 1H, J = 1.5 Hz), 8.56 (d, 1H, J = 8.5 Hz); ^{13}C NMR (125 MHz, (CD_3)$_2$SO, 378 K) δ 116.6 (CH), 117.3 (CH), 118.4 (CH), 119.5 (CF_3, q, J = 257 Hz), 119.6 (CF_3, q, J = 256 Hz), 123.3 (C), 124.3 (C), 127.3 (CH), 129.3 (C), 132.1 (CH), 143.7 (C), 144.7 (C), 144.9 (C), 146.3 (C), 148.3 (C), 156.2 (C), 180.2 (C). Crystal data for **1-OCF3.** $C_{17}H_6F_6N_2O_4$, M = 416.24, T = 150(2) K, triclinic, P -1, a = 6.9024(9), b = 7.5517(10), c = 14.893(2) Å, α = 85.430(5), β = 78.965(5), γ = 89.996(5) °, V = 759.42(18) Å^3, Z = 2, d = 1.820 g cm^{-3}, μ = 0.178 mm^{-1}. A final refinement on F^2 with 3466 unique intensities and 262 parameters converged at $\omega R(F^2)$ = 0.1114 ($R(F)$ = 0.0464) for 2593 observed reflections with $I > 2\sigma(I)$. CCDC 1898462.

2-Hydroxyethyl [2-((2-hydroxyethoxy)carbonyl)amino]benzoate (3).

To a stirred solution of isatin (0.15 g, 1.0 mmol) in ethylene glycol (5 mL) at room temperature was added KOH (0.11 g, 2.0 mmol), followed by I_2 (0.25 g, 1.0 mmol). After stirring for 1 day, the reaction mixture was poured onto a saturated aqueous solution of $Na_2S_2O_3$ (50 mL) and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated. Purification was achieved by column chromatography over silica gel using AcOEt-Heptane 70:30 as eluent. Compound **3** was obtained (Rf = 0.24) in 11% yield (30 mg) as a white powder: 100-102 °C; IR (ATR): 1218, 1247, 1261, 1452, 1530, 1594, 1690, 1717, 1735, 2886, 2962, 3270, 3299 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.17 and 3.41 (br s, 2H, OH), 3.82 (dd, 2H, J = 5.6 and 3.6 Hz, CH_2), 3.90 (dd, 2H, J = 5.7 and 3.8 Hz, CH_2), 4.24-4.27 (m, 2H, CH_2), 4.38-4.41 (m, 2H, CH_2), 7.00 (td, 1H, J = 7.7 and 1.1 Hz), 7.49 (ddd, 1H, J = 8.7, 7.2 and 1.7 Hz), 8.01 (dd, 1H, J = 8.0 and 1.7 Hz), 8.31 (dd, 1H, J = 8.5 and 1.1 Hz), 10.35 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 60.7 (CH_2), 61.2 (CH_2), 66.8 (CH_2), 67.0 (CH_2), 114.9 (C), 119.0 and 119.1 (CH), 122.0 (CH), 131.2 (CH), 134.8 (CH), 141.2 and 141.4 (C), 153.9 (C, C=O), 168.1 and 168.2 (C, C=O). Crystal data for **3.** $C_{12}H_{15}NO_6$, M = 269.25, T = 150(2) K, monoclinic, P 2$_1$/c, a = 8.0584(8), b = 19.9778(15), c = 8.5074(9) Å, β = 111.608(3) °, V = 1273.3(2) Å^3, Z = 4, d = 1.404 g cm^{-3}, μ = 0.114 mm^{-1}. A final refinement on F^2 with 2905 unique intensities and 174 parameters converged at $\omega R(F^2)$ = 0.1356 ($R(F)$ = 0.0546) for 2187 observed reflections with $I > 2\sigma(I)$. CCDC 1898463.

6-Butyl-6-hydroxy-6*H*-indolo[2,1-*b*]quinazolin-12-one (4b).

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The white solid was isolated as a by-product in an attempt to deprotometallate **1-H** using lithium 2,2,6,6-tetramethylpiperidine. It was identified by comparison of its NMR data with those described previously:[56b] ^{1}H NMR ($CDCl_3$) δ 0.73 (t, 3H, *J* = 7.3 Hz, CH_3), 0.82-0.89 (m, 2H, CH_2), 1.16-1.21 (m, 2H, CH_2), 2.20-2.36 (m, 2H, CH_2), 4.02 (br s, 1H, OH), 7.16 (td, 1H, *J* = 7.4 and 1.4 Hz), 7.23 (td, 1H, *J* = 7.8 and 1.8 Hz), 7.48 (ddd, 1H, *J* = 8.2, 6.7 and 1.7 Hz), 7.51-7.54 (m, 1H), 7.74 (td, 1H, *J* = 7.4 and 1.5 Hz), 7.77-7.80 (m, 1H), 8.23 (dd, 1H, *J* = 7.9 and 1.4 Hz), 8.30 (dd, 1H, *J* = 7.5 and 1.4 Hz); ^{13}C NMR ($CDCl_3$) δ 13.8 (CH_3), 22.6 (CH_2), 25.5 (CH_2), 39.6 (CH_2), 78.4 (CH), 116.4 (CH), 121.3 (C), 123.7 (CH), 127.1 (CH), 127.2 (CH), 127.3 (CH), 127.7 (CH), 129.9 (CH), 132.8 (C), 134.5 (CH), 138.3 (C), 147.2 (C), 159.9 (C), 160.9 (C). Crystal data for **4b.** $C_{19}H_{18}N_2O_2$, *M* = 306.35, *T* = 150(2) K, triclinic, *P* -1, *a* = 9.5244(18), *b* = 11.009(2), *c* = 16.485(3) Å, *α* = 72.146(6), *β* = 84.597(7), *γ* = 69.590(6) °, *V* = 1541.8(5) Å^3, *Z* = 4, *d* = 1.320 g cm^{-3}, *μ* = 0.087 mm^{-1}. A final refinement on F^2 with 6873 unique intensities and 424 parameters converged at ω*R*(F^2) = 0.1969 (*R*(*F*) = 0.0938) for 5521 observed reflections with $I > 2\sigma(I)$. CCDC 1898464.

Acknowledgements

We thank the Centre National de la Recherche Scientifique (CNRS) and the Université de Rennes 1. We acknowledge Fonds Européen de Développement Régional (FEDER) funds (D8 VENTURE Bruker AXS diffractometer). This research has been partly performed as part of the CNRS Projet International de Coopération Scientifique (PICS) project "Bimetallic synergy for the functionalization of heteroaromatics". Z. F. thanks Dr. A. ElDouhaibi and Dr. M. Hamze for their collaboration to this study.

Keywords: isatin; tryptanthrin; heterocycle; iodine; proteasome

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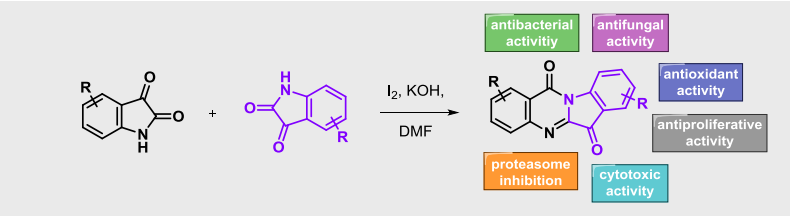
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FULL PAPER



A straightforward and versatile synthetic approach to tryptanthrins is developed. Starting from isatins, through addition of iodine and potassium hydroxide, the method is shown to be an efficient route to these indoloquinazoline alkaloids, whose *in vitro* bioactivities are investigated.

Bioactive heterocycles

Rim Amara, Haçan Awad, Diana Chaker, Ghenia Bentabed-Ababsa, Frédéric Lassagne, William Erb, Floris Chevallier, Thierry Roisnel, Vincent Dorcet, Ziad Fajloun,* Joëlle Vidal* and Florence Mongin**

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Conversion of isatins to tryptanthrins, heterocycles endowed with a myriad of bioactivities

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