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Original article

Synthesis and cytotoxic activity of 2-acyl-1*H*-indole-4,7-diones on human cancer cell lines

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Abstract

Synthesis and cytotoxic activity of a series of 2-acyl-1*H*-indole-4,7-diones on human cancer cell lines are described. Due to close structural relationship to 2-acylindoles, potent inhibitors of tubulin polymerization, the mode of action of these novel compounds has been investigated. Cytotoxicity, the influence on tubulin polymerization, and cell cycle dependent cytotoxicity on colon carcinoma cells by investigation of RKO exo p27 versus RKO $p27^{kip1}$ cells are described. IC_{50} values of arrested versus proliferating cells differ only in a range of two to fourfold and therefore cellular targets, predominantly relevant for mitotic progression, are excluded. As shown by the significant difference in the IC_{90} values on different tumor cell lines, the investigated compounds seem to act selectively on mammary and renal cancer cells.

Keywords: Indole-4,7-diones; Cytotoxic activity; Cell cycle specificity; Tubulin

1. Introduction

Reflecting the diversity in structure, synthetic and naturally occurring indoles exhibit antibacterial, fungicide, cytotoxic and antiproliferative activity. We recently reported on two classes of 2-indolylmethanones **1** and **2**, being potent inhibitors of receptor tyrosine kinases [1] and highly potent antiproliferative agents by acting as destabilizing tubulin inhibitors with anti-angiogenic potential [2] (Fig. 1).

Moreover, scientists have noted on a large number of anticancer agents containing chinoide structures [3–6], including the 2-acetyl-naphtho[2,3-*b*]furan-4,9-dione (**3**) [7], a naturally occurring potent inhibitor of proliferation in a human keratinocyte cell system [8], being promising candidates for the treatment of proliferative disorders [9]. Therefore, in search for new, simple chinoide systems with potential pharmacological value, we prepared a series of 2-acyl-1*H*-indole-4,7-diones, structurally related to **3** and investigated the biological properties of these systems, combining the structural features of the 2-indolyl-methanone and a chinoide system.

2. Results and discussion

2.1. Chemistry

According to Scheme 1 the desired 2-acyl-1*H*-indole-4,7-diones were prepared from commercially accessible 2,5-dimethoxybenzaldehyde **(5)** employing the strategy of Beneteau and Besson [10], which includes nitration of the aldehyde **5**, Henry reaction of **6** and hydrogenolytic ring closure. After protection of 4,7-dimethoxyindol **(8)** with benzene sulfonyl chloride, lithiation of **9** was performed with n-BuLi at –78 °C in THF. Position and amount of metallation (≥90%) was proved by quenching the 2-indolyl-lithium with MeOD and ^{1}H-NMR spectroscopy of the resulting 1-benzenesulfonyl-2-deutero-4,7-dimethoxy-1*H*-indole **(9a)**. Reaction of the result-

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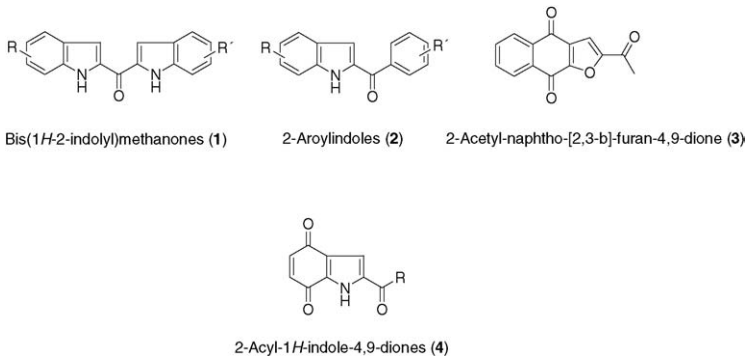


Fig. 1. Chemical structures of various methanone systems exhibiting antiproliferative activity. Dependent on the structural features, inhibition of growth factor receptors with intrinsic tyrosine kinase **(1)** or inhibition of tubulin assembling **(2)** are predominantly responsible for the antiproliferative activity. The structurally related chinoide furan derivative **(3)** was found to be a potent inhibitor of human keratinocyte growth.

ing 2-lithiated indole derivative with the respective carboxylic acid chloride yielded the C-2 acylated indoles **10** in good yields. The chinoide system **11** was generated by oxidation of the dimethoxy indoles **10** using ceric ammonium nitrate (CAN) [11] in acetonitrile/water. Removal of the protection group with tetrabutylammonium fluoride (TBAF) in THF [1] furnished the 1*H*-indole-4,7-diones **12**. Instead of benzoic acid chlorides (Scheme 1) also acetic acid anhydride and 4-morpholinecarbonyl chloride were employed.

The N-protected indol-4,7-diones **11** as well as their unprotected analogues **12** were candidates for a pharmacological in vitro profiling to evaluate cytotoxicity, effects on tubulin polymerization and specificity towards mitotic cells.

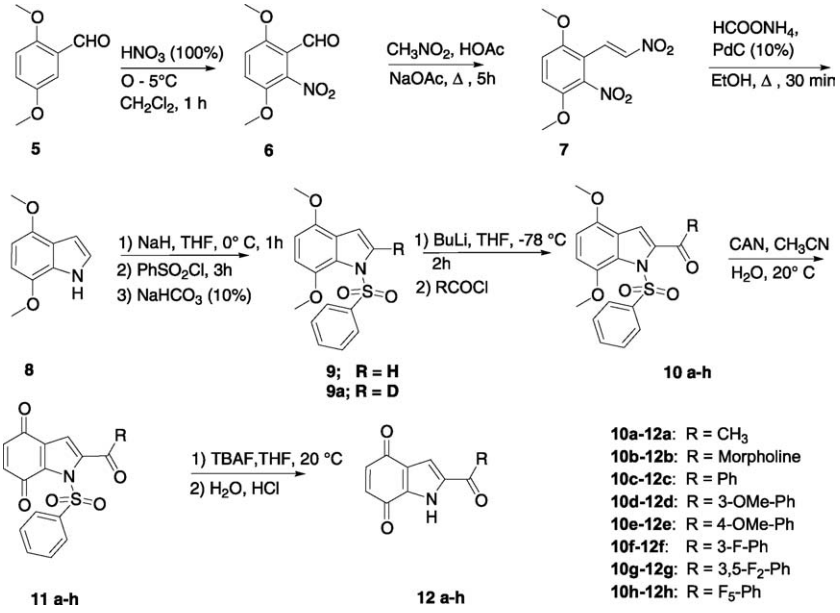
2.2. Biological results

In vitro activity on human HeLa cervical carcinoma cells was investigated using the Alamar Blue assay as a fast and simple method for the determination of cellular metabolic activity [12,13] (Table 1).

The investigated chinones exhibit significant cytotoxicity with IC_{50} values ranging from 0.80 to 3.20 μM. The benzene sulfonyl-group in position 1 is well tolerated and weakly influences the cytotoxic potential. Different substituents in the methanone substructure from methyl **(12a)** to morpholine **(12b)** and substituted phenyl (**12c**–**12h**) increase the cytotoxic potential up to threefold activity only. This indicates the chinoide structure to be the most important pharmacophore in these compounds. Patterns of substitution in the phenyl system affect the biological activity only weakly.

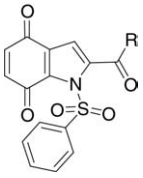
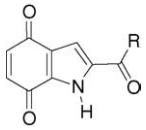
As already mentioned, the structurally related 2-acylindoles are effective antiproliferative agents by acting as destabilizing tubulin inhibitors [2]. By investigation of bovine brain tubulin polymerization in vitro using the naphthol blue assay as described [14] some 2-substituded 1*H*-indole-4,7-diones showed moderate tubulin inhibitory activity. Compounds **11a**, **11b**, **11f**, **11h**, **12a** and **12b** were characterized by IC_{50} values of 32–50 μM.

Tubulin inhibitors interfere with the M-phase of the cell cycle by binding to tubulin, inducing a mitotic catastrophy.



Scheme 1. Synthesis of 2-substituded 1*H*-indole-4,7-diones.

Table 1
Cytotoxicity (IC_{50} values) of 2-substituted 1*H*-indole-4,7-diones on HeLa cervical carcinoma cells

Number	IC_{50} (μM)	Number	IC_{50} (μM)	R=
11a	2.97	**12a**	2.92	CH_3
11b	2.98	**12b**	1.59	Morpholine
11c	3.20	**12c**	2.44	Ph
11d	1.98	**12d**	3.08	3-OMe-Ph
11e	1.35	**12e**	2.15	4-OMe-Ph
11f	2.53	**12f**	0.80	3-FPh
11g	1.79	**12g**	2.70	3,5-F_2Ph
11h	1.45	**12h**	1.03	2,3,4,5,6-F_5Ph

For evaluation of a mitosis dependent mode of action, selected compounds with an IC_{50} < 1.5 μM in HeLa cell cytotoxicity assay and weak activity towards tubulin in vitro were tested in the human colon carcinoma cell line RKO exo p27 with inducible expression of the cell division cycle kinase inhibitor $p27^{kip1}$ [15]. Induction of $p27^{kip1}$ arrests proliferating RKO cells in the G1 phase of the cell division cycle, thus inducing complete resistance to anti-mitotic agents like taxol as a stabilizing tubulin inhibitor [16].

As demonstrated in Fig. 2, the proliferating cell line RKO exo p27 showed insensitivity to taxol, used as the standard, up to 33 μM. In contrast, proliferating RKO exo p27 cells without $p27^{kip}$ expression were highly sensitive with an IC_{50} of 4 nM (Fig. 2). The investigated chinoide analogues did not display a significant cell cycle dependent cytotoxicity. The respective IC_{50} values of arrested versus proliferating cells are summarized in Table 2 and differ only in a range of two to fourfold as also observable for standard chemotherapeutic drugs lacking cell cycle specificity as cisplatin, e.g.

2.3. Selectivity of inhibition on the growth of human tumor cell lines

To investigate cytotoxicity of selected compounds, namely the N-phenylsulfonated chinones **11a** and **11e** as well as their deprotected analogues (**12a**, **12e**) were studied using a panel of different human tumor cell lines including a gastric xenograft cell line (GXF 251L), adeno (LXFA 629L) and large (LXFL 529L) cell lung cancer, mammary (MACL MCF7, MAXF 401NL), melanoma (MEXF 462 NL and 514L), prostate (PRCL PC3M), renal (RXF 486L and 944L) and an uterus body cell line (UXF 1138L). IC_{70} and IC_{90} values were determined in a screening from XTT proliferation assays after incubation with test compound for 48 h as described [2]. Independent from substitution of the chinoide methanone—methyl or 4-methoxyphenyl—as well as from N-phenylsulfonation all investigated compounds stopped tumor growth of mammary and renal cells at concentrations of 3 μg ml^{-1}, exhibiting IC_{90} values <3 μg ml^{-1}. Remaining high potency in melanoma xenograft models also was observed for **12a** and **12e**, exhibiting IC_{70} values <3 μg ml^{-1} in MEXF 462 NL and 514L cell lines. In contrast, the IC_{90} values for inhibition of tumor

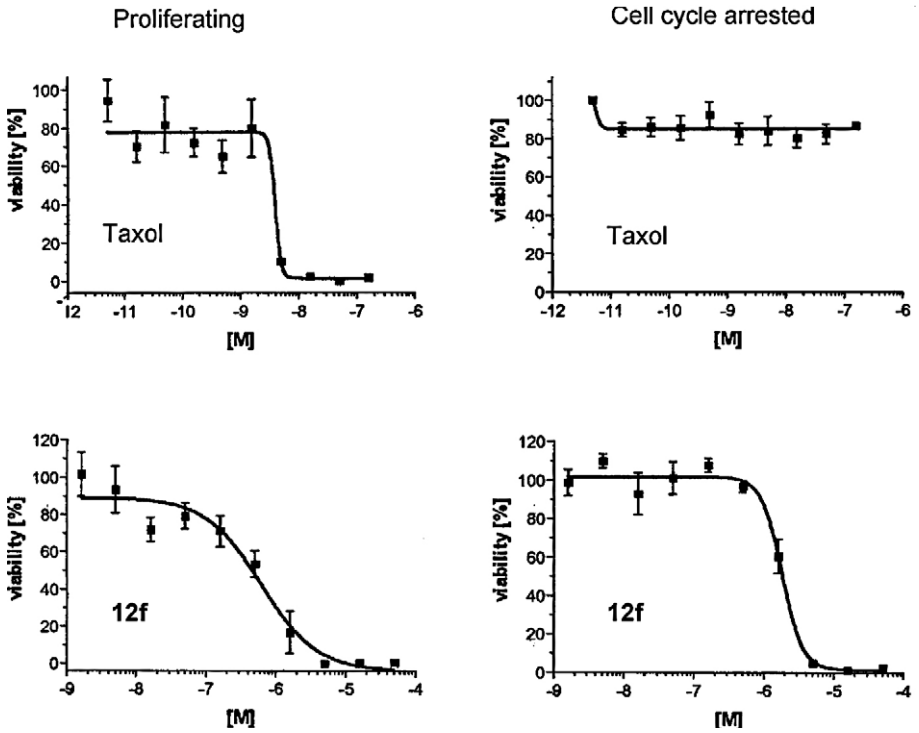
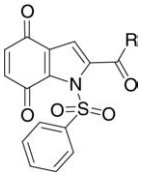
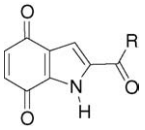


Fig. 2. Concentration dependent viability of RKO exo p27 colon carcinoma cells proliferating (left) and non-proliferated (right) by $p27^{kip1}$ induced cell cycle arrest. The cells treated with taxol (top) are only sensitive in a proliferative state without expression of $p27^{kip}$ (IC_{50} = 4 nM). Compound **12f** exhibits cytotoxic potential independent on the proliferative status of the RKO exo p27 cells (bottom).

Table 2
Cytotoxicity (IC_{50} values) on proliferating RKO exo p27 and non-proliferating cells with expression of $p27^{kip1}$





Number	IC_{50} Alamar Blue assay (μM)		Number	IC_{50} Alamar Blue assay (μM)		
	Proliferating	Arrested (+$p27^{kip1}$)		Proliferating	Arrested (+$p27^{kip1}$)	R=
11e	1.37	2.18	**12e**	–	–	4-OMe-Ph
11f	–	–	**12f**	0.61	1.80	3-FPh
11h	0.47	2.09	**12h**	0.52	1.88	2,3,4,5,6-F_5-Ph
Taxol	0.004	>0.1				

growth of gastric, lung, prostate and uterus cell lines differed in general at least a 10-fold in potency (IC_{90} > 30 μg ml^{-1}).

2.4. Conclusion

The 2-acyl-1*H*-indole-4,7-diones represent a novel series of chinoide compounds exhibiting significant cytotoxic activity towards human cancer cell lines represented by the RKO colon and HeLa cervical carcinoma models. Considering the moderate tubulin polymerization interference as well as the lack of correlation between the IC_{50} values of tubulin inhibition and cytotoxicity of the investigated compounds, tubulin polymerization seems not to be the main cellular target responsible for cytotoxicity—in contrast to the structurally related 2-aroylindoles **2**, lacking a chinoide system.

In general chinoide agents were considered to exhibit cytotoxic activity by DNA intercalation [3,5], interference with topoisomerase II DNA-complexes as well as by generating free radicals or reactive oxygen species through redox cycling [6]. Our data as presented in this study do not rule out the possibility that 2-acyl-1*H*-indole-4,7-diones might act by one of these mechanisms. The lack of significant cell cycle specificity suggests a more common mode of interference with cell viability and a target(s) irrelevant for mitotic progression.

As shown by the significant difference in the IC_{90} values on different tumor cell lines, the investigated compounds seem to act selectively on mammary and renal cancer cells.

3. Experimental section

3.1. Biological methods

3.1.1. Tubulin polymerization assay

The assay was performed as described [14]. Bovine brain tubulin heterodimers (5 μg $μl^{-1}$; 50 μg per assay), provided by Cytoskeleton/TEBU (MAP-rich, order No. ML-113F), were incubated with test compounds in PEM buffer pH 6.6 containing 1 mM GTP in a total volume of 100 μl at 37 °C for 1 h. Concentration dependent inhibition of GTP/heat induced tubulin polymerization was calculated using the GraphPad Prism software. Colchicine or Vincristine were included as positive controls.

3.1.2. Cell viability assay

Metabolic activity correlating with cell proliferation/viability was quantified using the Alamar Blue assay as described [13] and HeLa (ATCC CCL2) cervical as well as RKO exo p27 colon carcinoma cell lines [15]. The compounds were dissolved in dimethylsulfoxide (DMSO; 20 mM) and subsequently diluted in semi-logarithmic steps. DMSO dilutions were further diluted 1:100 into Dulbecco's modified Eagle's medium (DMEM) containing 10% inactivated fetal calf serum (FCSi) to a final concentration twice as high as the final concentration in the test. RKO exo p27 or HeLa cells were seeded into 96 well flat bottom plates at a density of 5000 cells or 1000 cells per well, respectively, in a volume of 50 μl per well. To determine the effect on arrested RKO exo p27 cells, these cells were seeded at a density of 15,000 cells per well and pretreated with 10 μM Ponasterone A. Twenty-four hours after seeding, 50 μl each of the compound dilutions in DMEM were added per well with 1% DMSO treated cells as a control. After incubation with the compounds for 72 h at 37 °C in a humidified atmosphere containing 5% CO_2, 10 μl Alamar Blue solution (Biosource) were added and the fluorescence measured (ex 544 nm, em 590 nm). For calculation of cell viability, the emission value from untreated cells was set as 100% viability and the emission rates of treated cells were set in relation to the values of untreated cells. The corresponding IC_{50} values were determined from the concentration–effect-curves using the program GraphPad Prism.

3.2. Chemistry

3.2.1. General

^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Bruker Avance 300 spectrometer at 300 K, using TMS as an internal standard. AA′BB′ and AA′XX′ spectra as well as AB-spectra are characterized by the center of the respective AA′, BB′ or XX′ part. For AB-spectra the coupling constants are given in addition. Elemental analyses were performed by

the Analytical Laboratory, University of Regensburg. Melting points were determined with a Büchi Melting Point B-545 device. IR spectra (KBr or pure solid) were measured with a Bruker Tensor 27 spectrometer, MS spectra were measured with a Finnigan MAT 95 (EI, 70 eV). All reactions were carried out under nitrogen.

*3.2.2. 1-Benzenesulfonyl-4,7-dimethoxy-1*H*-indole **(9)***

During 20 min, NaH (2.37 g, 59.3 mmol) (60% in paraffin) was added to a stirred solution of 4,7-dimethoxy-1*H*-indole **(8)** (10.0 g; 56.4 mmol) in 200 ml of anhydrous THF at 0 °C. After stirring at room temperature for 1 h, benzenesulfonyl chloride (7.59 ml, 59.3 mmol) was added slowly. The reaction mixture was stirred for additional 3 h, poured into 500 ml of 10% aq $NaHCO_3$ and extracted with ether (3 × 250 ml). The combined organic layers were dried (Na_2SO_4), and the solvent was removed under reduced pressure to give a beige solid. Recrystallization from ethanol afforded **9** as colorless crystals. Yield: 15.43 g (82%), m.p.: 105.1–105.4 °C. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 3.54 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 6.65 (d, 1H, ^3J = 8.6 Hz, aromat.), 6.71 (d, 1H, ^3J = 8.6 Hz, aromat.), 6.78 (d, 1H, ^3J = 3.8 Hz, aromat.), 7.59–7.74 (m, 3H, aromat.), 7.79 (d, 1H, ^3J = 3.8 Hz, aromat.), 7.84–7.87 (m, 2H, aromat.). EI-MS (70 eV) *m/z* (%): 317 (94) $[M]^{+\bullet}$, 176 (100), 148 (60), 146 (58), 133 (79). Anal. ($C_{16}H_{15}NO_4S$) Calc. C 60.55, H 4.76, N 4.41. Found: C 60.51, H 4.89 N 4.53.

*3.2.3. 1-Benzenesulfonyl-2-deutero-4,7-dimethoxy-1*H*-indole **(9a)***

Lithiation of **9** and work up of **9a** were performed as described for **10a**–**10h**. The lithiated species was quenched with CD_3OD, and ^{1}H-NMR measurements were performed to determine the amount of H/D-exchange (>90%). ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 3.54 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 6.65 (d, 1H, ^3J = 8.6 Hz, aromat.), 6.71 (d, 1H, ^3J = 8.6 Hz, aromat.), 6.78 (s, 1H, aromat.), 7.59–7.74 (m, 3H, aromat.), 7.84–7.87 (m, 2H, aromat.).

*3.2.4. (1-Benzenesulfonyl-4,7-dimethoxy-1*H*-indol-2-yl)-ones (**10a**–**10h**)*

To a stirred solution of **9** (2.50 g; 7.88 mmol) in 50 ml of dry THF, n-butyllithium (5.41 ml 1.6 M in hexane; 8.66 mmol) was added at –78 °C. The solution was stirred at this temperature for 2 h, until the indole-2-yl lithium precipitates. The respective carbonic acid chloride (8.66 mmol) was added—in case of solid carboxylic acid chlorides a solution in THF (10.0 ml), pre-cooled to –78 °C—all at once. The mixture was stirred for additional 2 h and allowed to reach room temperature over night. Na_2CO_3 solution (200 ml; 2%) was added to the stirred solution, the precipitating product removed by filtration, purified (cc SiO_2, CH_2Cl_2) and crystallized from ethanol.

*3.2.4.1. 1-(1-Benzenesulfonyl-4,7-dimethoxy-1*H*-indol-2-yl)-ethanone **(10a)**.* Instead of the carbonic acid chloride acetic acid anhydride was used for condensation. Yield: 2.00 g (70%) colorless crystals, m.p.: 168.8–168.9 °C. IR (solid): 1689 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 2.62 (s, 3H, CH_3), 3.61 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.77 (d, 1H, ^3J = 8.7 Hz, aromat.), 6.95 (d, 1H, ^3J = 8.7 Hz, aromat.), 7.69 (s, 1H, aromat.), 7.71–7.78 (m, 3H, aromat.), 8.31–8.35 (m, 2H, aromat.). EI-MS (70 eV) *m/z* (%): 359 (23) $[M]^{+\bullet}$, 218 (98), 188 (42), 160 (18), 148 (88), 77 (33), 43 (100). Anal. ($C_{18}H_{17}NO_5S$) Calc. C 60.16, H 4.77, N 3.90. Found: C 59.80, H 4.89 N 3.43.

*3.2.4.2. (1-Benzenesulfonyl-4,7-dimethoxy-1*H*-indol-2-yl)-morpholin-4-yl-methanone **(10b)**.* Yield: 2.34 g (69%) colorless crystals, m.p.: 187.9–188.0 °C. IR (KBr): 2978, 1633 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 3.57 (s, 3H, OCH_3), 3.66 (s, br., 8H), 3.83 (s, 3H, OCH_3), 6.74 (d, 1H, ^3J = 8.7 Hz, aromat.), 6.83 (d, 1H, ^3J = 8.7 Hz, aromat.), 6.95 (s, 1H, aromat.), 7.70–7.78 (m, 3H, aromat.), 8.28–8.32 (m, 2H, aromat.). EI-MS (70 eV) *m/z* (%): 330 (14) $[M]^{+\bullet}$, 289 (13), 204 (100), 176 (24), 77 (8). Anal. ($C_{21}H_{22}N_2O_6S$) Calc. C 58.59, H 5.15, N 6.51. Found: C 58.41, H 5.42, N 6.10.

*3.2.4.3. (1-Benzenesulfonyl-4,7-dimethoxy-1*H*-indol-2-yl)-phenyl-methanone **(10c)**.* Yield: 2.82 g (85%) light yellowish crystals, m.p.: 182.0–184.6 °C. IR (KBr): 3068, 1658 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 3.62 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.80 (d, 1H, ^3J = 8.7 Hz, aromat.), 6.96 (d, 1H, ^3J = 8.7 Hz, aromat.), 7.11 (s, 1H, aromat.), 7.60–7.71 (m, 2H, aromat.), 7.72–7.79 (m; 4H, aromat.), 8.02–8.05 (m, 2H, aromat.), 8.27–8.30 (m, 2H, aromat.). EI-MS (70 eV) *m/z* (%): 421 (11) $[M]^{+\bullet}$, 280 (40), 265 (12), 105 (100), 77 (40). Anal. ($C_{23}H_{19}NO_5S$) Calc. C 65.55, H 4.54, N 3.32. Found: C 65.69, H 4.72, N 3.21.

*3.2.4.4. (1-Benzenesulfonyl-4,7-dimethoxy-1*H*-indol-2-yl)-(3-methoxyphenyl)-methanone **(10d)**.* Yield: 2.85 g (80%) light yellowish crystals, m.p.: 101.5–102.2 °C. IR (solid): 1660 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 3.62 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.80 (d, 1H, ^3J = 8.7 Hz, aromat.), 6.96 (d, 1H, ^3J = 8.7 Hz, aromat.), 7.12 (s, 1H, aromat.), 7.32–7.36 (m, 1H, aromat.), 7.49–7.86 (m, 6H, aromat.), 8.27–8.31 (m, 2H, aromat.). Anal. ($C_{24}H_{21}NO_6S$) Calc. C 63.85, H 4.69, N 3.10. Found: C 63.80, H 4.81, N 2.84.

*3.2.4.5. 1-(1-Benzenesulfonyl-4,7-dimethoxy-1*H*-indol-2-yl)-(4-methoxyphenyl)-methanone **(10e)**.* Yield: 2.03 g (57%), m.p.: 161.1–161.7 °C. IR (Solid): 1669 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 3.34 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.79 (d, 1H, ^3J = 8.8 Hz, aromat.), 6.94 (d, 1H, ^3J = 8.7 Hz, aromat.), 7.06 (s, 1H, aromat.), 7.16 (d, 2H, ^3J = 8.8 Hz, aromat.), 7.70–7.82 (m, 3H, aromat.), 8.04 (d, 2H, ^3J = 8.8 Hz, aromat.), 8.32 (d, 2H, ^3J = 8.0 Hz, aromat.). EI-MS (70 eV) *m/z* (%): 451 (35) $[M]^{+\bullet}$, 310 (100). Anal. ($C_{24}H_{21}NO_6S$) Calc. C 63.85, H 4.69, N 3.10. Found: C 64.51, H 4.99, N 2.83.

*3.2.4.6. (1-Benzenesulfonyl-4,7-dimethoxy-1*H*-indol-2-yl)-(3-fluorophenyl)-methanone **(10f)**.* Yield: 2.25 g (65%), m.p.: 172.5–172.6 °C. IR (KBr): 1655 cm^{-1}. ^{1}H-NMR (DMSO-

[D_6]): δ (ppm) = 3.62 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.81 (d, 1H, ^3J = 8.8 Hz, aromat.), 6.98 (d, 1H, ^3J = 8.8 Hz, aromat.), 7.22 (s, 1H, aromat.), 7.60–7.84 (m, 6H, aromat.), 7.90 (d, 1H, ^3J = 7.4 Hz, aromat.), 8.29 (d, 2H, ^3J = 7.6 Hz, aromat.). EI-MS (70 eV) *m/z* (%): 439 (33) $[M]^{+\bullet}$, 298 (78), 123 (100). Anal. ($C_{23}H_{18}FNO_5S$) Calc. C 62.86, H 4.13, N 3.19. Found: C 62.77, H 4.28, N 3.17.

*3.2.4.7. (1-Benzenesulfonyl-4,7-dimethoxy-1*H*-indol-2-yl)-(3,5-difluorophenyl)-methanone **(10g)**.* Yield: 2.94 g (82%) yellow crystals, m.p.: 159.0–159.1 °C. IR (KBr): 1655 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 3.62 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.81 (d, 1H, ^3J = 8.7 Hz, aromat.), 6.99 (d, 1H, ^3J = 8.7 Hz, aromat.), 7.33 (s, 1H, aromat.), 7.68–7.82 (m, 6H, aromat.), 8.26–8.30 (m, 2H, aromat.). EI-MS (70 eV) *m/z* (%): 457 (50) $[M]^{+\bullet}$, 316 (86), 141 (100). Anal. ($C_{23}H_{17}F_2NO_5S$) Calc. C 60.39, H 3.75, N 3.06. Found: C 60.80, H 3.69, N 2.84.

*3.2.4.8. (1-Benzenesulfonyl-4,7-dimethoxy-1*H*-indol-2-yl)-(pentafluorophenyl)-methanone **(10h)**.* Yield: 1.70 g (42%) yellow crystals, m.p.: 203.4–203.7 °C. IR (KBr): 1680 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 3.64 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.83 (d, 1H, ^3J = 8.8 Hz, aromat.), 7.07 (d, 1H, ^3J = 8.8 Hz, aromat.), 7.72–7.84 (m, 4H, aromat.), 8.32–8.36 (m, 2H, aromat.). EI-MS (70 eV) *m/z* (%): 511 (24) $[M]^{+\bullet}$, 370 (56), 195 (100). Anal. ($C_{23}H_{13}F_5NO_5S$) Calc. C 54.02, H 2.76, N 2.74. Found: C 54.09, H 2.85, N 2.76.

*3.2.5. (1-Benzenesulfonyl-1*H*-indole-4,7-dione-2-yl)-ones **(11a–11h)***

To a stirred solution of **10a–10h** (3.00 mmol) in 30 ml of CH_3CN, ceric(IV)ammonium nitrate (4.93 g in 9 ml H_2O; 9.00 mmol) was added at 20 °C. The solution/suspension was stirred at room temperature for 30 min, diluted with water (200 ml), and the precipitated yellow to orange product was removed by filtration and purified by cc (SiO_2, CH_2Cl_2). Crystallization from CH_2Cl_2 by addition of hexane and removement of a part of the solvent under reduced pressure yielded the desired products.

*3.2.5.1. 2-Acetyl-1-benzenesulfonyl-1*H*-indole-4,7-dione **(11a)**.* Yield: 0.75 g (76%) yellow crystals, m.p.: 130.9–131.4 °C. IR (KBr): 1710, 1671 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 2.67 (s, 3H, CH_3), 6.85 (s, 2H, quinone), 7.58 (s, 1H, pyrrole), 7.75–7.90 (m, 3H, aromat.), 8.46–8.50 (m, 2H, aromat.). EI-MS (70 eV) *m/z* (%): 329 (3) $[M]^{+\bullet}$, 265 (57), 77 (100). Anal. ($C_{16}H_{11}NO_5S$) Calc. C 58.35, H 3.37, N 4.25. Found: C 57.98, H 3.59, N 4.28.

*3.2.5.2. 1-Benzenesulfonyl-2-(morpholine-4-carbonyl)-1*H*-indole-4,7-dione **(11b)**.* Yield: 1.07 g (89%) yellow crystals, m.p.: 215.2–215.7 °C. IR (KBr): 1671, 1638 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 3.50 (s br, 2H, CH_2), 3.67 (s br, 6H, CH_2), 6.74 (d, 1H, ^3J = 10.2 Hz, quinone), 6.89 (d, 1H, ^3J = 10.2 Hz, quinone), 6.99 (s, 1H, pyrrole), 7.72–7.87 (m, 3H, aromat.), 8.41–8.44 (m, 2H, aromat.). Anal. ($C_{19}H_{16}N_2O_6S$) Calc. C 56.99, H 4.03, N 7.00. Found: C 56.81, H 3.80, N 6.90.

*3.2.5.3. 1-Benzenesulfonyl-2-benzoyl-1*H*-indole-4,7-dione **(11c)**.* Yield: 1.08 g (92%) yellow crystals, m.p.: 184.5–186 °C. IR (solid): 1668, 1658 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 6.82 (d, 1H, ^3J = 10.3 Hz, quinone), 6.85 (d, 1H, ^3J = 10.3 Hz, quinone), 7.11 (s, 1H, pyrrole), 7.60–7.90 (m, 6H, aromat.), 7.98–8.02 (m, 2H, aromat.), 8.33–8.37 (m, 2H, aromat.). EI-MS (70 eV) *m/z* (%): 391 (34) $[M]^{+\bullet}$, 250 (61), 77 (100). Anal. ($C_{21}H_{13}NO_5S$) Calc. C 64.44, H 3.35, N 3.58. Found: C 64.42, H 3.42, N 3.49.

*3.2.5.4. 1-Benzenesulfonyl-2-(3-methoxybenzoyl)-1*H*-indole-4,7-dione **(11d)**.* Yield: 1.06 g (84%) orange crystals, m.p.: 195.1–196.2 °C. IR (solid): 1663 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 3.85 (s, 3H, OCH_3), 6.81 (d, 1H, ^3J = 10.3 Hz, quinone), 6.85 (d, 1H, ^3J = 10.3 Hz, quinone), 7.11 (s, 1H, pyrrole), 7.33–7.39 (m, 1H, aromat.), 7.36–7.58 (m, 3H, aromat.), 7.74–7.90 (m, 3H, aromat.), 8.33–8.38 (m, 2H, aromat.). EI-MS (70 eV) *m/z* (%): 421 (14) $[M]^{+\bullet}$, 280 (93), 77 (100). Anal. ($C_{22}H_{15}NO_6S$) Calc. C 62.70, H 3.59, N 3.32. Found: C 62.47, H 3.51, N 2.95.

*3.2.5.5. 1-Benzenesulfonyl-2-(4-methoxybenzoyl)-1*H*-indole-4,7-dione **(11e)**.* Yield: 0.87 g (69%) yellow crystals, m.p.: 153.3–155.4 °C. IR (KBr): 1673 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 3.90 (s, 3H, OCH_3), 6.81 (d, 1H, ^3J = 10.3 Hz, quinone), 6.84 (d, 1H, ^3J = 10.3 Hz, quinone), 7.06 (s, 1H, pyrrole), 7.15 (d, 2H, ^3J = 7 Hz, aromat.), 7.75–7.96 (m, 3H, aromat.), 7.99 (d, 2H, ^3J = 7 Hz, aromat.), 8.37–8.41 (m, 2H, aromat.). EI-MS (70 eV) *m/z* (%): 421 (7) $[M]^{+\bullet}$, 280 (100). Anal. ($C_{22}H_{15}NO_6S$) Calc. C 62.70, H 3.59, N 3.32. Found: C 62.73, H 3.89, N 3.58.

*3.2.5.6. 1-Benzenesulfonyl-2-(3-fluorobenzoyl)-1*H*-indole-4,7-dione **(11f)**.* Yield: 0.95 g (77%) yellow crystals, m.p.: 191.9–192.0 °C. IR (KBr): 1670 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 6.78–6.86 (m, 2H), 7.31 (s, 1H, pyrrole), 7.53–7.99 (m, 7H, aromat.), 8.31–8.37 (m, 2H, aromat.). EI-MS (70 eV) *m/z* (%): 409 (6) $[M]^{+\bullet}$, 269 (100). Anal. ($C_{21}H_{12}FNO_5S$) Calc. C 61.61, H 2.95, N 3.42. Found: C 61.32, H 3.85, N 3.39.

*3.2.5.7. 1-Benzenesulfonyl-2-(3,5-difluorobenzoyl)-1*H*-indole-4,7-dione **(11g)**.* Yield: 0.95 g (96%) orange crystals, m.p.: 216.0–216.5 °C. IR (solid): 1673 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 6.81 (d, 1H, ^3J = 10.3 Hz, quinone), 6.84 (d, 1H, ^3J = 10.3 Hz, quinone), 7.26 (s, 1H, pyrrole), 7.70–7.90 (m, 6H, aromat.), 8.60–8.65 (m, 2H, aromat.). EI-MS (70 eV) *m/z* (%): 427 (3) $[M]^{+\bullet}$, 287 (100). Anal. ($C_{21}H_{11}F_2NO_5S$) Calc. C 59.02, H 2.59, N 3.28. Found: C 58.75, H 2.90, N 3.22.

*3.2.5.8. 1-Benzenesulfonyl-2-pentafluorobenzoyl-1*H*-indole-4,7-dione **(11h)**.* Yield: 1.00 g (69%) orange solid, m.p.: 143.1–144.9 °C. IR (KBr): 1677, 1652 cm^{-1}. ^{1}H-NMR

(DMSO-[D_6]): δ (ppm) = 6.82 (d, 1H, ^3J = 10.4 Hz, quinone), 6.87 (d, 1H, ^3J = 10.4 Hz, quinone),7.28–7.34 (m, 3H, aromat.), 7.45 (s, 1H, pyrrole), 7.56–7.67 (m, 2H, aromat.). EI-MS (70 eV) *m/z* (%): 481 (2) $[M]^{+\bullet}$, 417 (32), 141 (58), 77 (100). Anal. ($C_{21}H_8F_5NO_5S$) Calc. C 52.40, H 1.68, N 2.91. Found: C 52.37, H 1.79, N 2.94.

*3.2.6. 1-(1*H*-indole-4,7-dione-2-yl)-ones (**12a–12h**)*

To a stirred solution of **11a–11h** (1.50 mmol) in 15 ml of THF TBAF trihydrate (0.52 g, 1.65 mmol) was added at 20 °C. The dark red solution was stirred at room temperature for 10 min, diluted with water (100 ml), the solution/suspension acidified with hydrochloric acid and extracted with CH_2Cl_2 (3 × 50 ml). The combined organic layers were dried (Na_2SO_4) and purified by cc (SiO_2, CH_2Cl_2, ethyl acetate 8:1). Crystallization from CH_2Cl_2 by addition of hexane and removement of a part of the solvent under reduced pressure, yielding the products as orange crystals.

*3.2.6.1. 2-Acetyl-1*H*-indole-4,7-dione **(12a)**.* Yield: 0.07 g (26%), m.p.: 189.9–190.5 °C. IR (KBr): 1653 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 3.35 (s, 3H, CH_3), 6.76 (d, 1H, ^3J = 10.6 Hz, quinone), 6.79 (d, 1H, ^3J = 10.6 Hz, quinone), 7.36 (s, 1H, pyrrole), 13.4 (s, 1H, NH). EI-MS (70 eV) *m/z* (%):189 (86) $[M]^{+\bullet}$, 174 (100). Anal. ($C_{10}H_7NO_3$) Calc. C 63.49, H 3.73, N 7.40. Found: C 62.98, H 3.98, N 7.39.

*3.2.6.2. 2-(Morpholine-4-carbonyl)-1*H*-indole-4,7-dione **(12b)**.* Yield: 0.32 g (83%), m.p.: 230.6–230.7 °C. IR (KBr): 1655 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 3.61 (s, br., 8H, CH_2), 6.69 (d, 1H, ^3J = 10.3 Hz, quinone), 6.73 (d, 1H, ^3J = 10.3 Hz, quinone), 6.80 (s, 1H, pyrrole), 13.2 (s, 1H, NH). EI-MS (70 eV) *m/z* (%): 260 (90) $[M]^{+\bullet}$, 174 (100). Anal. ($C_{13}H_{12}N_2O_4$) Calc. C 60.00, H 4.65, N 10.76. Found: C 59.94, H 4.88, N 10.78.

*3.2.6.3. 2-Benzoyl-1*H*-indole-4,7-dione **(12c)**.* Yield: 0.24 g (65%), m.p.: 180.2–180.4 °C. IR (KBr): 1663, 1635 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 6.79 (d, 1H, ^3J = 10.3 Hz, quinone), 6.83 (d, 1H, ^3J = 10.3 Hz, quinone), 7.03 (s, 1H, pyrrole), 7.57–7.63 (m, 2H, aromat.), 7.69–7.74 (m, 1H, aromat.) 7.87–7.91 (m, 2H, aromat.), 13.71 (s, 1H, NH). EI-MS (70 eV) *m/z* (%): 251 (100) $[M]^{+\bullet}$. Anal. ($C_{15}H_9NO_3$) Calc. C 71.71, H 3.61, N 5.57. Found: C 71.32, H 3.60, N 5.45.

*3.2.6.4. 2-(3-Methoxybenzoyl)-1*H*-indole-4,7-dione **(12d)**.* Yield: 0.26 g (61%), m.p.: 186.0–188.0 °C. IR (Solid): 1662, 1637 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 3.84 (s, 3H, OCH_3), 6.78 (d, 1H, ^3J = 10.3 Hz, quinone), 6.82 (d, 1H, ^3J = 10.3 Hz, quinone), 7.03 (s, 1H, pyrrole), 7.25–7.29 (m, 1H, aromat.), 7.34–7.36 (m, 1H, aromat.) 7.44–7.54 (m, 2H, aromat.), 13.65 (s, 1H, NH). EI-MS (70 eV) *m/z* (%): 281 (100) $[M]^{+\bullet}$. Anal. ($C_{16}H_{11}NO_4$) C 68.33, H 3.94, N 4.98. Found: C 68.20, H 3.83, N 4.71.

*3.2.6.5. 2-(4-Methoxybenzoyl)-1*H*-indole-4,7-dione **(12e)**.* Yield: 0.27 g (65%), m.p.: 224.4–224.5 °C. IR (KBr): 1677, 1667 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 3.87 (s, 3H, OCH_3), 6.78 (d, 1H, ^3J = 10.3 Hz, quinone), 6.82 (d, 1H, ^3J = 10.3 Hz, quinone), 7.02 (s, 1H, pyrrole), 7.12 (d, 2H, ^3J = 8.9 Hz, aromat.), 7.92 (d, 2H, ^3J = 8.9 Hz, aromat.), 13.59 (s, 1H, NH). EI-MS (70 eV) *m/z* (%): 281 (100) $[M]^{+\bullet}$. Anal. ($C_{16}H_{11}NO_4$) C 68.33, H 3.94, N 4.98. Found: C 68.01, H 3.89, N 4.79.

*3.2.6.6. 2-(3-Fluorobenzoyl)-1*H*-indole-4,7-dione **(12f)**.* Yield: 0.35 g (87%), m.p.: 198.0–198.5 °C (decomp.). IR (KBr): 1668, 1635 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 6.80 (d, 1H, ^3J = 10.3 Hz, quinone), 6.84 (d, 1H, ^3J = 10.3 Hz, quinone), 7.09 (s, 1H, pyrrole), 7.53–7.76 (m, 4H, aromat.), 13.75 (s, 1H, NH). EI-MS (70 eV) *m/z* (%): 269 (100) $[M]^{+\bullet}$. Anal. ($C_{15}H_8FNO_3$) C 66.92, H 3.00, N 5.20. Found: C 66.82, H 3.08, N 5.27.

*3.2.6.7. 2-(3,5-Difluorobenzoyl)-1*H*-indole-4,7-dione **(12g)**.* Yield: 0.15 g (35%), m.p.: 234.2–234.8 °C (decomp.). IR (solid): 1660, 1644 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 6.80 (d, 1H, ^3J = 10.3 Hz, quinone), 6.84 (d, 1H, ^3J = 10.3 Hz, quinone), 7.14 (s, 1H, pyrrole), 7.50–7.67 (m, 3H, aromat.), 13.77 (s, 1H, NH). EI-MS (70 eV) *m/z* (%): 287 (100) $[M]^{+\bullet}$. Anal. ($C_{15}H_7F_2NO_3$) C 62.73, H 2.46, N 4.88. Found: C 62.80, H 2.80, N 4.93.

*3.2.6.8. 2-Pentafluorobenzoyl-1*H*-indole-4,7-dione **(12h)**.* Yield: 0.23 g (45%), m.p.: 185.9–186 °C. IR (KBr): 1661 cm^{-1}. ^{1}H-NMR (DMSO-[D_6]): δ (ppm) = 6.82 (d, 1H, ^3J = 10.3 Hz, quinone), 6.87 (d, 1H, ^3J = 10.3 Hz, quinone), 7.45 (s, 1H, pyrrole), 14.08 (s, 1H, NH). EI-MS (70 eV) *m/z* (%): 341 (100) $[M]^{+\bullet}$. Anal. ($C_{15}H_4F_5NO_3$) C 52.80, H 1.18, N 4.11. Found: C 52.83, H 1.51, N 4.14.

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