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Synthesis and photodynamic activity of new 5-[(*E*)-2-(3-alkoxy-1-phenyl-1*H*-pyrazol-4-yl)ethenyl]-2-phenyl-3*H*-indoles

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Abstract

A series of new indole-pyrazole hybrids **8a–m** were synthesized through the palladium-catalyzed ligandless Heck coupling reaction from easily accessible unsubstituted, methoxy- or fluoro-substituted 4-ethenyl-1*H*-pyrazoles and 5-bromo-3*H*-indoles. These compounds exerted cytotoxicity to melanoma G361 cells when irradiated with blue light (414 nm) and no cytotoxicity in the dark at concentrations up to 10 µM, prompting us to explore their photodynamic effects. The photodynamic properties of the example compound **8d** were further investigated in breast cancer MCF-7 cells. Evaluation revealed comparable anticancer activities of **8d** in both breast and melanoma cancer cell lines within the submicromolar range. The treatment induced a massive generation of reactive oxygen species, leading to different types of cell death depending on the compound concentration and the irradiation intensity.

KEYWORDS
cytotoxicity, indole, photodynamic effect, pyrazole, reactive oxygen species

1 | INTRODUCTION

Heterocycles are fundamental scaffolds in both natural and synthetic compounds and are of particular importance in the field of medicinal chemistry. The indole nucleus, in particular, governs a wide spectrum of biological activities and serves as a structural cornerstone in several pharmaceuticals currently available in the market.[1–5] Design of hybrid molecules, that is, molecules in which two or more different pharmacophore scaffolds are connected via covalent bonds or a linker,[6]

Gabrielė Varvuolytė and Eva Řezníčková contributed equally to this study.

especially those based on the molecular hybridization of indole with other nitrogen-containing heterocyclic moieties,[7,8] has proven to be a successful strategy to discover both single- and multitargeted agents.[9–11]

Indole-based hybrids are present in many pharmacologically active substances (Figure 1). For instance, a notable example includes the indole-pyrimidine hybrid **I**, named osimertinib, sold under the brand name Tagrisso™, which is used to treat non-small-cell lung cancer.[12,13] Indole-imidazole hybrid **II** demonstrated good inhibitory activity against methicillin-resistant *Staphylococcus aureus*,[14] whereas an indole-triazole hybrid **III** targets tubulin, inhibits its polymerization, and causes apoptosis in HeLa cells.[15] Indole-oxadiazole hybrid **IV** protected Friedreich's ataxia fibroblasts against buthionine sulfoximine-induced glutathione depletion and increased the survival of *Caenorhabditis elegans* exposed to juglone-induced oxidative stress.[16] A potent *h*HDAC6 inhibitor with indole-pyridine core **V** was proposed as a potential pharmacological tool for idiopathic pulmonary fibrosis treatment.[17] Moreover, an indoline imidazolium salt **VI** displayed selective cytotoxic activity toward cancer cell lines and induced the G2/M phase cell-cycle arrest and apoptosis.[18] Indole-thiazole hybrid **VII** and its analogs were designed as antiparasitic agents against *Trypanosoma brucei brucei* and *Trypanosoma brucei gambiense* strains.[19] When it comes to indole-pyrazole hybrids, a number of such molecules were revealed to possess kinase inhibitory, antioxidant, anti-inflammatory, and antimicrobial activities.[20] Indole-3-pyrazole carboxamide analog **VIII** has shown strong activity against liver cancer cell lines, with moderate inhibition of tubulin polymerization as well,[21] while benzo[*e*]indole-pyrazole hybrid **IX** was reported to possess high cytotoxicity to human colorectal carcinoma HCT 116 cell line, likely through DNA minor groove binding.[22]

In our previous work, we reported an ethenyl π-bridge-possessing 2-phenyl-3*H*-indole and pyrazole hybrid **X**, which showed blue light-inducible photodynamic activity against G361 melanoma cells and caused cell death through the production of reactive oxygen species (ROS) and extensive DNA damage.[23] To assess if the photodynamic activity of this type of the compounds could be improved, in this work we ought to focus on structural modifications of compound **X**, namely, the introduction of electron-donating and withdrawing substituents, variation of the length of the alkoxy groups at the 3-position of the pyrazole ring, and replacement of methyl groups with cycloalkyl groups in the 3-position of the indole fragment.

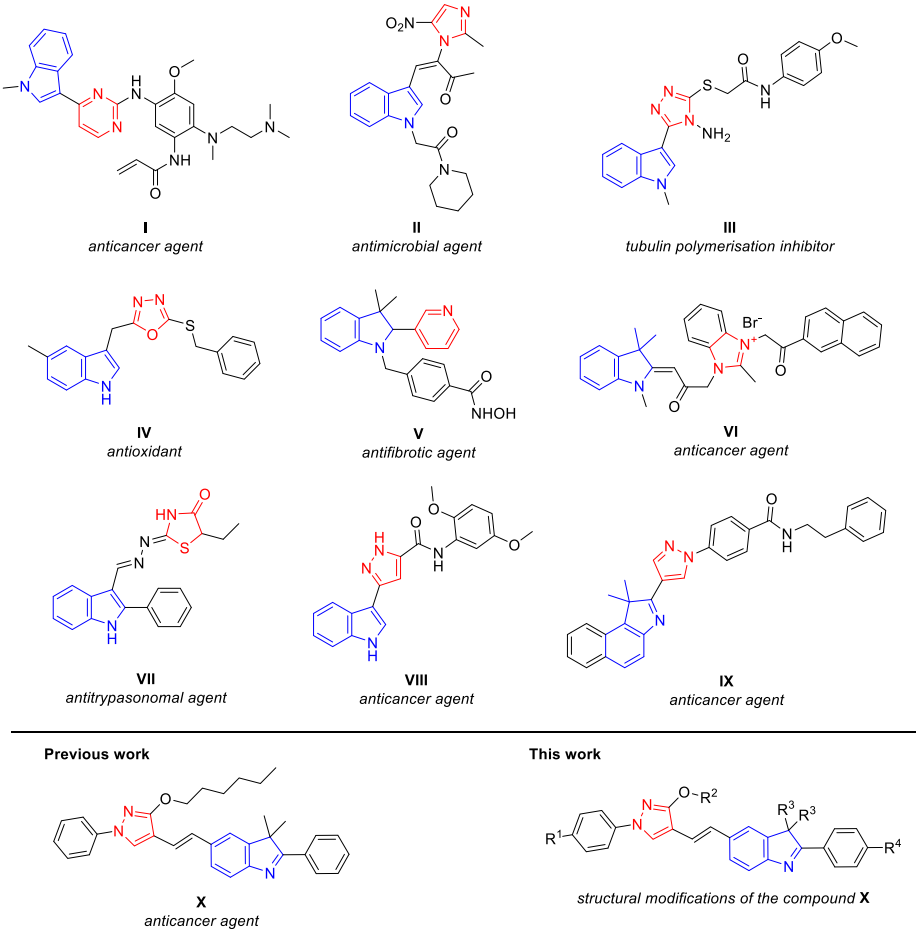


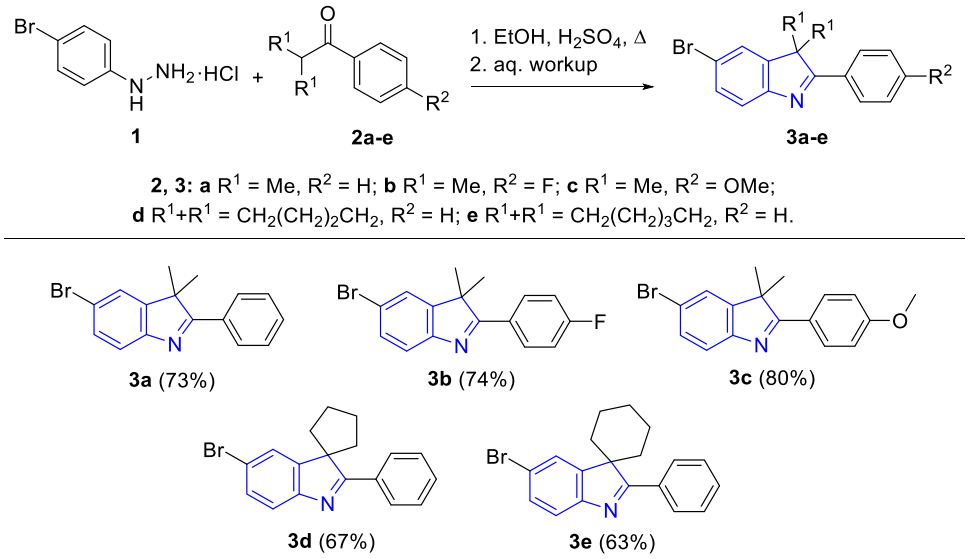
FIGURE 1 Examples of biologically active indole-heterocycle hybrids **I–X**.

2 | RESULTS AND DISCUSSION

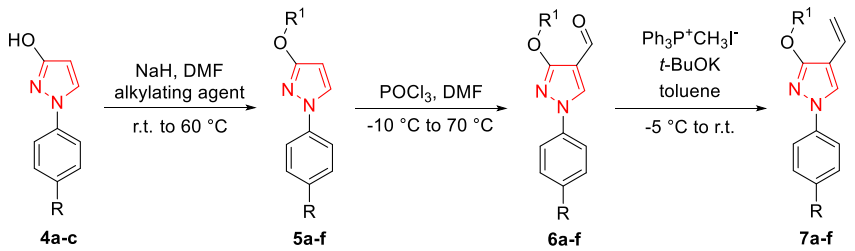
2.1 | Synthesis

While 3,3-dimethyl-, or spiro(cycloalkyl)-substituted derivatives of 2-phenyl-3*H*-indole can be synthesized through nucleophilic aromatic substitution reaction of 2-(methoxyphenyl)acetonitriles and lithium reagents,[24] typically, the method of Fischer indole synthesis from easily accessible phenylhydrazines and ketones is employed.[25] The Fischer indolization reaction can be conducted in neat acetic acid at reflux temperature,[26] in boiling ethanol with a catalytic amount of concentrated sulfuric acid,[27] with *p*-toluenesulfonic acid as a catalyst either on its own[28] or alongside zinc chloride,[29,30] and upon microwave irradiation, with water as a "green" solvent and sulfuric acid as catalyst.[31] In this work, 5-bromo-3,3-dimethyl-3*H*-indoles **3a–c** were obtained from 4-bromophenylhydrazine hydrochloride **1** and appropriate phenylketones **2a–c** via the Fischer indole synthesis in ethanol, with a catalytic amount of concentrated sulfuric acid (Scheme 1). While ketones **2a,b** were commercially available, **2c** was readily prepared through Friedel–Crafts acylation of anisole with isobutyryl chloride.[32] Moreover, since several spiro(cycloalkyl)compounds, including spiro(cycloalkyl)indoles, have been demonstrated to possess various biological activities, including significant activity against cancer cells,[33–37] we ought to incorporate spiro (cycloalkyl) fragments into our pyrazole-indole derivatives. For this, the same Fischer indole synthesis reaction was employed to obtain 5′-bromo-2′-phenylspiro[cyclopentane-1,3′-indole] (**3d**) and 5′-bromo-2′-phenylspiro[cyclohexane-1,3′-indole] (**3e**), from corresponding cycloalkylphenylketones **2d–e** in moderate yields.

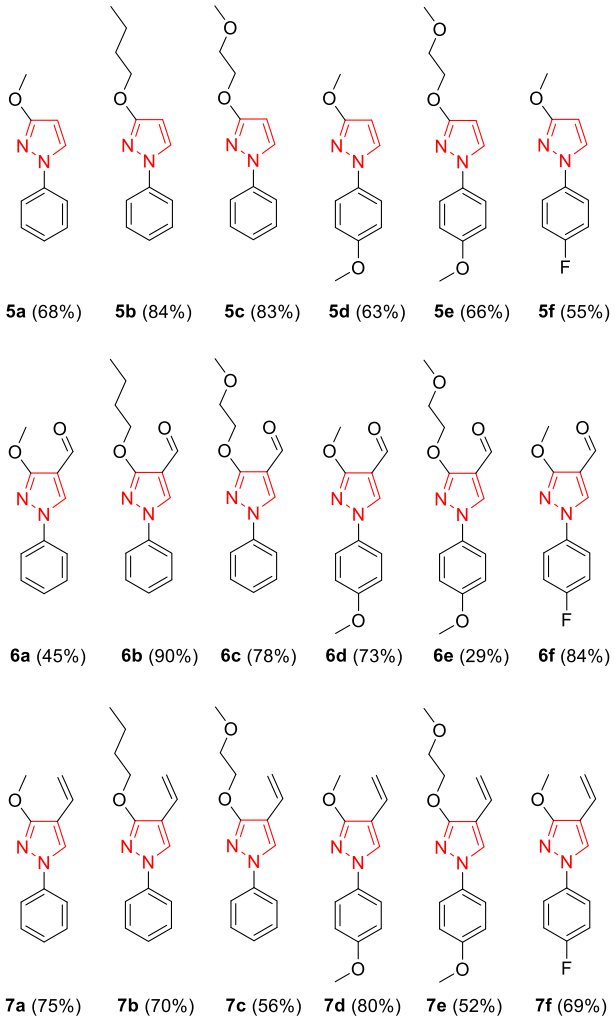
Recently, several fluorinated, pyrazole-containing compounds have been reported as potential photosensitizers for photodynamic treatment of melanoma, some of them possessing IC_{50} values in the nanomolar range.[38–40] Introduction of strongly electronegative fluorine atoms can fine-tune the lipophilicity and pK_a values of the compounds resulting in superior biological properties, compared with their nonfluorinated analogs, due to the so-called "magic fluorine effect". Moreover, replacing metabolically labile hydrogen atoms with fluorine could increase the metabolic stability of oxidizable alkenes, phenyl, and heterocyclic rings.[41–43] When it comes to the optical properties of fluorinated compounds, for example, fluorescent 3*H*-indole (hemi)cyanines, upon inclusion of fluorine a bathochromic shift in absorption and fluorescence maxima is expected, and, regarding the biological properties, fluorine should improve the selectivity in cell targeting.[44] Incorporation of alkoxy groups, for example, methoxyethoxy-, can help to increase the solubility of flat aromatic compounds in dimethylsulfoxide and water due to reduced π–π stacking.[45] Consequently, fluorine and alkoxy groups of varying lengths were introduced into the pyrazole fragment of our target hybrid molecules. 1-Phenyl-,[46] 1-(4-methoxyphenyl-)[47] and 1-(4-fluorophenyl)-3-hydroxy-1*H*-pyrazoles[48] **4a–c** were *O*-alkylated with either iodomethane, iodobutane, or 2-bromoethylmethylether via the Williamson ether synthesis, in the presence of sodium hydride as a base, to yield alkoxypyrazoles **5a–f**. Then, the 3-alkoxy-1*H*-pyrazoles **5a–f** were subjected to the Vilsmeier–Haack formylation reaction to give a set of new and previously reported pyrazole-4-carbaldehydes **6a–f**.[49,50] Noteworthy, the low yield of **6e** was caused by predominantly occuring dual functionalization of **5e**, leading to 3-(2-chloroethoxy)-1-(4-methoxyphenyl)-1*H*-pyrazole-4-carbaldehyde as the major product of the reaction.[51] Wittig olefination reaction of pyrazole-4-carbaldehydes **6a–f** with in situ generated methylenetriphenylphosphorane[23] proceeded smoothly to give the desired 4-ethenyl-1*H*-pyrazoles **7a–f** (Scheme 2). Successful formation of terminal alkenes from pyrazole carbaldehydes can be confirmed by ^{13}C NMR and DEPT-135 data, as a negative signal of a methylene carbon $=CH_2$ appears at 112.7–113.3 ppm in the DEPT-135 spectrum, and the signal of the carbonyl carbon –CHO (183.1–183.6 ppm) disappears in the ^{13}C NMR spectrum.



SCHEME 1 Synthesis of starting 5-bromo-2-phenyl-3*H*-indoles **3a–e**.



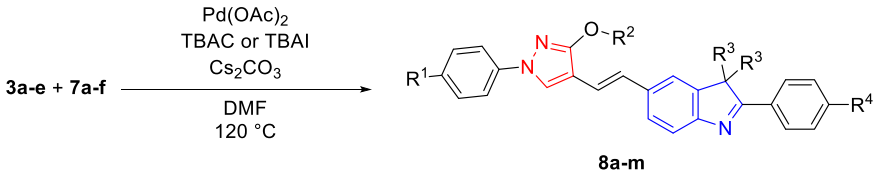
4: **a** R = H; **b** R = OMe; **c** R = F. **5, 6, 7:** **a** R = H, R^1 = Me; **b** R = H, R^1 = Bu; **c** R = H, R^1 = CH_2CH_2OMe; **d** R = OMe, R^1 = Me; **e** R = OMe, R^1 = CH_2CH_2OMe; **f** R = F, R^1 = Me.



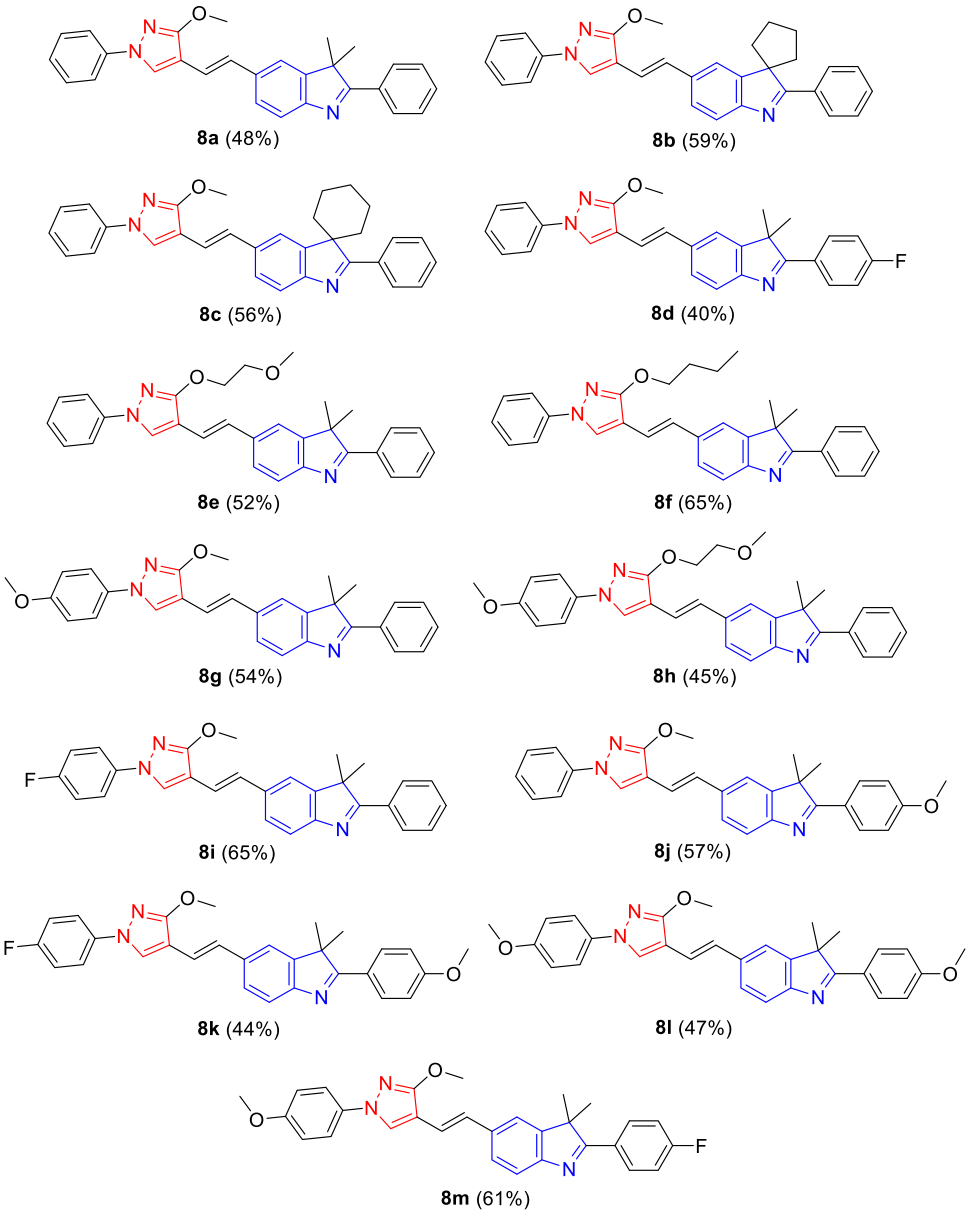
SCHEME 2 Synthesis of starting 4-ethenyl-1*H*-pyrazoles **7a–f**.

Lastly, ethenylpyrazoles **7a–f** and 3*H*-indoles **3a–e** were subjected to the ligand-free Heck reaction[52] in the presence of a tetrabutylammonium salt as a phase-transfer catalyst giving rise to fluorescent pyrazole-indole hybrids **8a–m** in satisfactory yields (40%–65%) (Scheme 3).

The formation of new 5-[(*E*)-2-(3-alkoxy-1-phenyl-1*H*-pyrazol-4-yl)ethenyl]-2-phenyl-3*H*-indoles **8a–m** was confirmed through detailed analysis of their spectroscopic data. Key information for structure elucidation was obtained from NMR spectral data using a combination of standard and advanced NMR spectroscopy techniques, such as ^{1}H-^{13}C HMBC (heteronuclear multiple bond correlation), ^{1}H-^{13}C HSQC (heteronuclear single quantum correlation), ^{1}H-^{13}C H2BC (heteronuclear 2-bond correlation), ^{1}H-^{15}N LR-HSQMBC (long-range heteronuclear single quantum multiple bond correlation), ^{1}H-^{15}N HMBC, ^{1}H-^{1}H correlation spectroscopy, ^{1}H-^{1}H total correlation spectroscopy, ^{1}H-^{1}H nuclear overhauser effect spectroscopy, and 1,1-ADEQUATE (adequate sensitivity double-quantum spectroscopy) experiments. Data analysis showed that the chemical shift values were highly consistent within a series of compounds, thus validating the shifts for each position.



8: a $R^1 = R^4 = H$, $R^2 = R^3 = Me$; **b** $R^1 = R^4 = H$, $R^2 = Me$; $R^3+R^3 = CH_2(CH_2)_2CH_2$; **c** $R^1 = R^4 = H$, $R^2 = Me$; $R^3+R^3 = CH_2(CH_2)_3CH_2$; **d** $R^1 = H$, $R^2 = R^3 = Me$, $R^4 = F$; **e** $R^1 = R^4 = H$, $R^2 = CH_2CH_2OMe$; $R^3 = Me$; **f** $R^1 = R^4 = H$, $R^2 = Bu$, $R^3 = Me$; **g** $R^1 = OMe$, $R^2 = R^3 = Me$, $R^4 = H$; **h** $R^1 = OMe$, $R^2 = CH_2CH_2OMe$, $R^3 = Me$, $R^4 = H$; **i** $R^1 = F$, $R^2 = R^3 = Me$, $R^4 = H$; **j** $R^1 = H$, $R^2 = R^3 = Me$, $R^4 = OMe$; **k** $R^1 = F$, $R^2 = R^3 = Me$, $R^4 = OMe$; **l** $R^1 = R^4 = OMe$, $R^2 = R^3 = Me$; **m** $R^1 = OMe$, $R^2 = R^3 = Me$, $R^4 = F$.



SCHEME 3 Synthesis of compounds **8a–m**.

The aforementioned styryl-like compounds represent a biheterocyclic system in which a 3-alkoxy-1-phenyl-1*H*-pyrazol-4-yl moiety is connected to the 3*H*-indole ring through an ethene bridge unit. The *E*-configuration of the ethene double bond unequivocally follows from the magnitude of the vicinal coupling between the olefinic protons H_a (δ 6.93–6.98 ppm) and H_b (δ 7.13–7.22 ppm), which exhibited an AB-spin system and appeared as two sets of doublets ($^3J_{Ha,\ Hb}$ = 16.3–16.4 Hz). The ^1H-^{13}C HSQC spectrum indicated that these protons have a

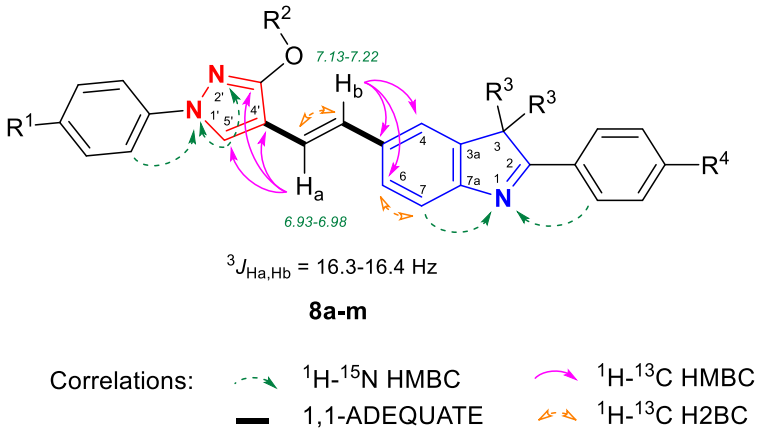


FIGURE 2 Relevant ^{1}H-^{13}C HMBC, ^{1}H-^{15}N HMBC, ^{1}H-^{13}C H2BC, and 1,1-ADEQUATE, correlations of compounds **8a–m**. adequate ADEQUATE, sensitivity double-quantum spectroscopy; H2BC, heteronuclear 2-bond correlation; HMBC, heteronuclear multiple bond correlation.

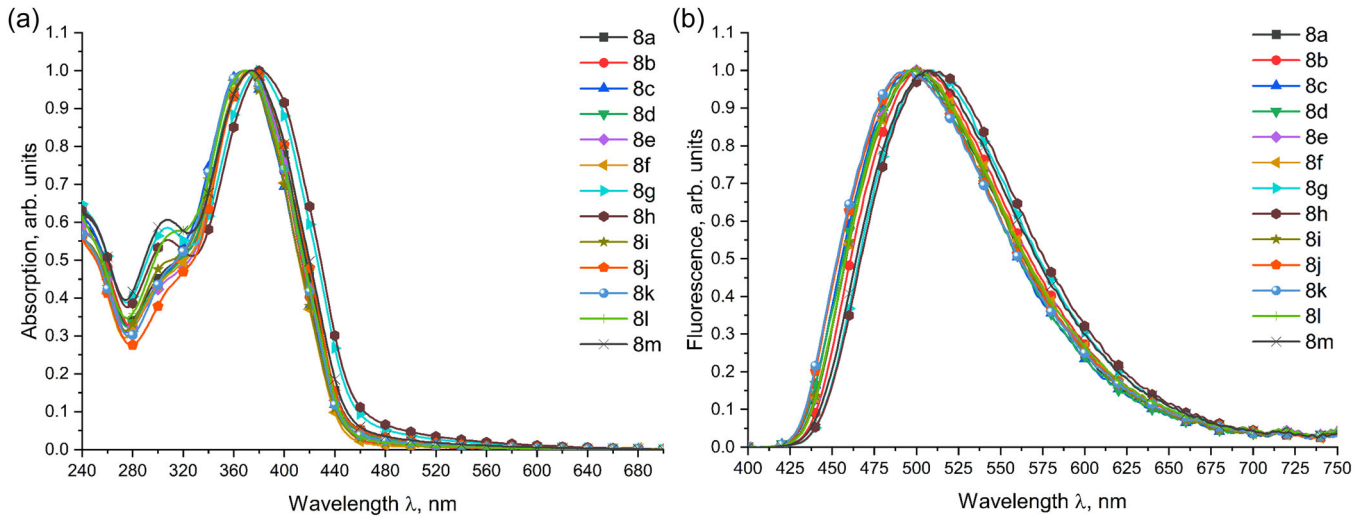


FIGURE 3 (a) UV–Vis absorption spectra of compounds **8a–m** in water. (b) Fluorescence emission spectra (λ_{ex} = 380 nm) of compounds **8a–m** in water.

one-bond correlation with the ethene bridge carbons CH_a (δ 116.1–116.8 ppm) and CH_b (δ 127.3–128.2 ppm), respectively.

This finding, together with data from 1,1-ADEQUATE and ^{1}H-^{13}C H2BC experiments, allowed us to unambiguously assign the adjacent pyrazole C-4′ (δ 107.7–108.7 ppm) and indole C-5 (δ 135.1–136.2 ppm) quaternary carbon signals. Moreover, this was further supported by the ^{1}H-^{13}C HMBC spectral data, which showed strong long-range correlations between the olefinic proton H_a and the pyrazole ring C-3′, C-4′, and C-5′ carbon atoms. Meanwhile, in the case of the olefinic proton H_b, strong long-range HMBC correlations with indole C-4, C-5, and C-6 carbon atoms were observed, thus affirming the connection between these different heterocyclic moieties via ethene bridge (Figure 2).

The ^{19}F NMR spectra of 4-fluorophenyl moiety containing compounds **8d**, **8i**, **8k**, and **8m** revealed that chemical shifts were in a range from δ −109.4 to −117.6 ppm, which is in good agreement with the data reported in the literature.[53]

The skeleton of new pyrazole-indole hybrids **8a–m** contains three nitrogen atoms. The ^{15}N NMR spectroscopic data were obtained via long-range ^{1}H-^{15}N HMBC and HSQMBC correlations and showed highly consistent chemical shift values for the aforementioned compounds. For instance, the pyrazole ring proton 5-H′ exhibited long-range correlations with neighboring N-1′ "pyrrole-like" (from δ −188.6 to −190.9 ppm) and N-2′ "pyridine-like" (from δ −118.6 to −120.3 ppm) nitrogen atoms, whereas indole ring proton 7-H exhibited a strong three-bond correlation with indole N-1 nitrogen atom, resonating in a range from δ −67.4 to −77.2 ppm.

2.2 | Optical properties

To determine the wavelength of light, at which our pyrazole-indole hybrids **8a–m** should be irradiated for the investigation of their photodynamic activity, the UV–Vis absorption and fluorescence properties were measured in water (Figure 3; Supporting Information S2: Table S1). The excitation wavelength λ_{ex} was set to 380 nm. The investigated compounds **8a–m** had their UV–Vis absorption maxima in a range of 369–381 nm. Compounds **8g** and **8h**, which contain a methoxy group in the pyrazole phenyl ring showed bathochromically shifted absorption maxima (380; 381 nm), compared with their counterparts **8a**,**e**

with unsubstituted phenyls (374 nm). Also, it should be noted that the compounds **8g** and **8h** had the most red-shifted emission among the tested compounds, 509 and 508 nm in aqueous solutions, respectively. All compounds have large Stokes shifts in water (118–132 nm); with compound **8m** possessing the largest Stokes shift.

Notably, in tetrahydrofuran solutions (Supporting Information S2: Figure S1 and Table S2), all of the investigated compounds showed high fluorescence quantum yields Φ_f, from 66% to 80%, at the excitation wavelength of 380 nm. The UV–Vis absorption maxima for compounds **8a–m** in tetrahydrofuran (THF) were measured to be in the range of 368–375 nm, and fluorescence emission maxima at 462–480 nm.

2.3 | Biology

Despite the compounds' ability to absorb both UV and visible blue light, we opted for a 414 nm emitting LED source for photodynamic experiments[54] due to the adverse effects of UV and near-UV light on cells.[55] To assess the photodynamic effects of the synthesized compounds, G361 cells were treated with compounds for 4 h and subsequently exposed to blue light, at a total irradiation dose of 10 J/cm^2. Cell viability was quantified 72 h after irradiation by an MTT assay, revealing photodynamic properties of the compounds. Importantly, no cytotoxicity was observed in the absence of light at concentrations up to 10 μM (Table 1). Prepared compounds display potency comparable to previously identified derivative **X** (EC_{50} = 0.262 μM[23]) and about fivefold higher potency than porphyrin derivative 5,10,15,20-tetrakis(1-methyl-4-pyridinio)porphyrin tetra(*p*-toluensulfonate) (TMPyP) used as a reference photosensitizer.[56]

Photodynamic therapy (PDT) is typically used to treat skin cancers, such as basal cell carcinoma and squamous cell carcinoma,[57] as well as lung, esophageal, cervical, and bladder cancers,[58] among others. Intriguingly, recent studies demonstrate that PDT is also emerging as a promising prospect in treating breast cancer,[59,60] which is of particular importance in cases of multidrug resistance.[61] The example compound **8d** was further evaluated for its cytotoxic properties in combination with different light irradiation doses (2, 6, and 10 J/cm^2) not only against G361 cells but also against the MCF-7 breast adenocarcinoma cell line. Porphyrin derivative TMPyP was used as a reference photosensitizer; data are available from the Supporting Information (Supporting Information S2: Table S3). While **8d** without irradiation showed no significant signs of toxicity up to a concentration of 10 μM, in combination with 10 J/cm^2 blue light irradiation it showed EC_{50} values of 0.5 μM and 0.2 μM after subsequent 24 h and 72 h incubations, respectively (Table 2). The experiments also confirmed expected dose–response effects, indicating a clear correlation between the irradiation dose and the cytotoxic efficacy of compound **8d**, and demonstrating that higher light doses enhance the photodynamic activity. Thus, the EC_{50} values obtained for MCF-7 cells varied in a comparable concentration range as for G361 melanoma cells.

During PDT, potent photosensitizers can generate high levels of reactive oxygen species (ROS), which can initiate the targeted destruction of cancer cells. Therefore, the ability of **8d** to induce ROS formation in MCF-7 cells was evaluated using different fluorescent probes. 5-(And-6)-chloromethyl-2,7-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) and dihydrorhodamine 123 (DHR123) are typically used as general oxidative stress indicators. As expected, increasing concentrations of **8d** without irradiation did not increase ROS levels. On the other hand, blue light irradiation caused a massive induction of ROS

TABLE 1 Cytotoxicity of compounds **8a-m** in G361 cells.

	EC_{50} (μM)	
Compound	**Dark**	**Light (414 nm, 10 J/cm^2)**
8a	>10	0.142 ± 0.012
8b	>10	0.138 ± 0.004
8c	>10	0.241 ± 0.106
8d	>10	0.174 ± 0.015
8e	>10	0.145 ± 0.006
8f	>10	0.255 ± 0.078
8g	>10	0.196 ± 0.098
8h	>10	0.136 ± 0.013
8i	>10	0.244 ± 0.098
8j	>10	0.144 ± 0.007
8k	>10	0.153 ± 0.001
8l	>10	0.155 ± 0.008
8m	>10	0.179 ± 0.044
TMPyP	>10	0.956 ± 0.365

Abbreviation: TMPyP, 5,10,15,20-tetrakis(1-methyl-4-pyridinio)porphyrin tetra(p-toluensulfonate).

TABLE 2 Cytotoxicity of **8d** in G361 and MCF-7 cell lines.

		Cmpd. 8d: EC_{50} (μM)			
Cell line	**Incubation**	**Dark**	**2 J/cm^2**	**6 J/cm^2**	**10 J/cm^2**
G361	24 h	>10	1.215 ± 0.132	0.424 ± 0.072	0.228 ± 0.016
	72 h	>10	1.013 ± 0.198	0.355 ± 0.077	0.174 ± 0.015
MCF-7	24 h	>10	1.044 ± 0.091	0.395 ± 0.067	0.248 ± 0.024
	72 h	>10	0.934 ± 0.151	0.315 ± 0.023	0.193 ± 0.001

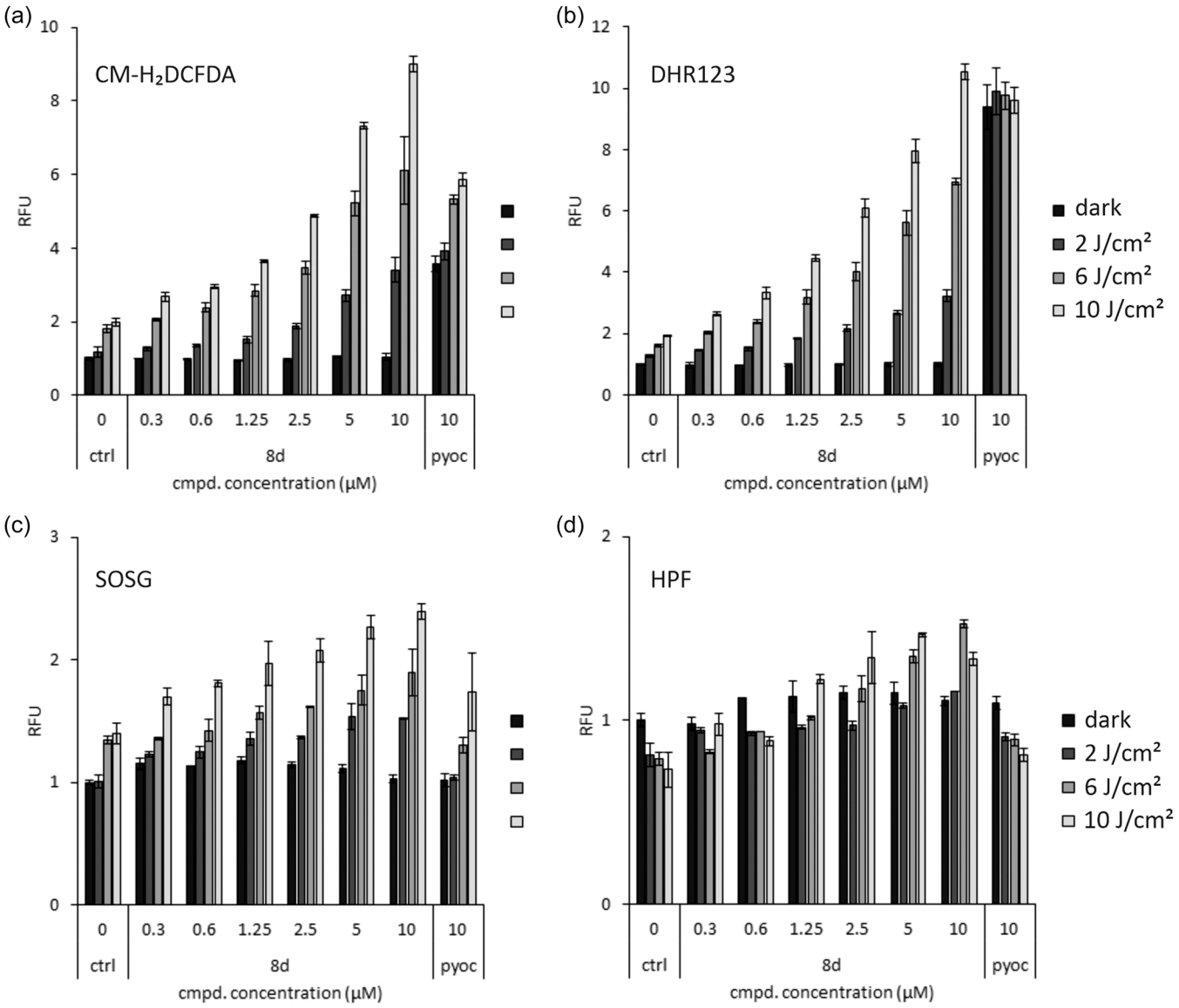


FIGURE 4 Analysis of reactive oxygen species (ROS) production using (a) CM-H_2DCFDA, (b) DHR123, (c) Singlet oxygen sensor green (SOSG), and (d) hydroxyphenyl fluorescein (HPF) probes in MCF-7 cells treated with **8d** and irradiated with increasing energy doses of blue light. Relative fluorescence units (RFU) were normalized to the untreated control sample kept in the dark. Pyocyanin was used as a control.

levels that was both concentration and energy level dependent (Figure 4a, b). Furthermore, the effects caused by **8d** were comparable to those induced by pyocyanin, used as a standard ROS inducer.[62,63] To further demonstrate that the measured effects were related to increased ROS levels, MCF-7 cells were pretreated with *N*-acetylcysteine, a ROS inhibitor, which significantly attenuated the ROS-inducing effects of **8d** as well as pyocyanin (Supporting Information S2: Figure S2). Singlet oxygen sensor green (SOSG) probe signal exhibited a similar trend in treated cells, although the increase in fluorescence was not as pronounced (Figure 4c). The signal from hydroxyphenyl fluorescein (HPF), used for the quantification of hydroxyl radicals, also revealed a slight but clearly dose-dependent increase (Figure 4d). Therefore, the generation of ROS is proposed as the primary mechanism of photocytotoxic activity of **8d**, as these species were detected after irradiation even in its solution in plain water (Supporting Information S2: Figure S3). In a parallel experiment, we analyzed the photochemical stability of **8d** under the conditions used for cell treatment, and the results indicate that the compound undergoes unspecified light-induced chemical changes, potentially leading to cytotoxic effects (Supporting Information S2: Figure S4).

ROS generation induced by PDT can lead to the initiation of different types of cell death. Microscopic observation of cells during incubation with **8d** revealed very rapid morphological changes in the cells within hours after irradiation. While lower concentrations of **8d** caused cell rounding and their detachment from the culture plate surface, higher concentrations induced condensation of cellular content and significant cell swelling (Figure 5).

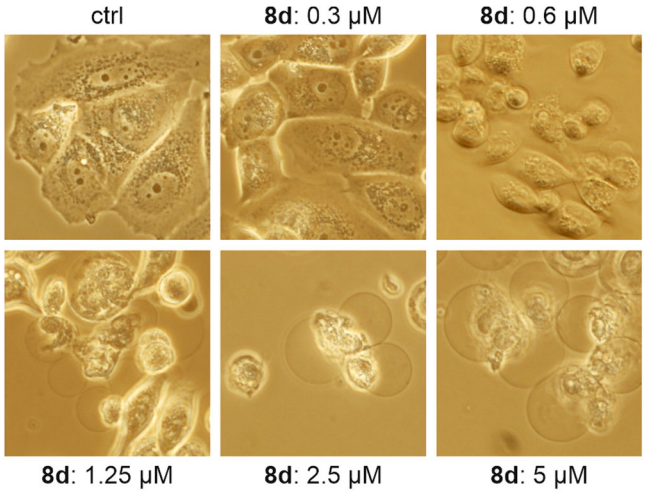


FIGURE 5 Cell morphology of MCF-7 cells treated with increasing concentrations of **8d** for 4 h and incubated for further 20 h after blue light irradiation at 10 J/cm^2.

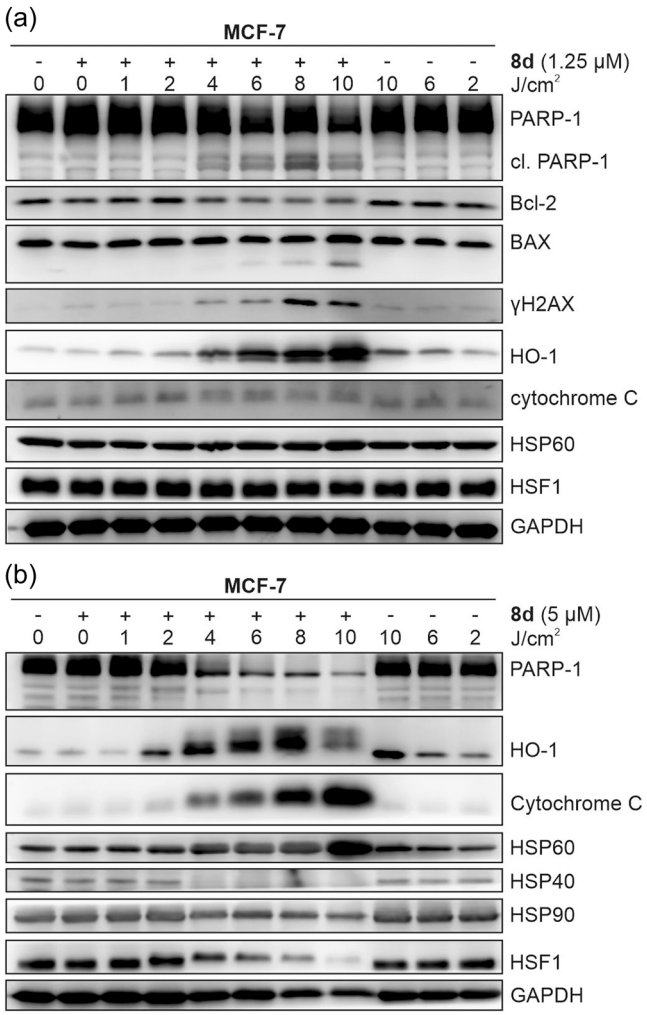


FIGURE 6 Analysis of protein expression in MCF-7 cells treated with **8d** and exposed to blue light. (a) MCF-7 cells were treated with 1.25 μM concentration of **8d** for 4 h, irradiated, and then incubated for another 20 h. (b) MCF-7 cells were treated with 5 μM concentration of **8d** for 4 h, irradiated, and then incubated for another 4 h. GAPDH was used as a control for equal protein loading.

The difference between the effects induced by different concentrations of **8d** was also observed at the protein level. A more proapoptotic effect was observed after exposure to a lower concentration of **8d** (1.25 μM) and a longer incubation period, where we observed energy level-dependent cleavage of the protein Poly (ADP-Ribose) Polymerase 1 (PARP-1), a common marker of apoptosis. This was accompanied by reduced levels of the antiapoptotic protein Bcl-2 and cleavage of the proapoptotic protein BAX, whose 18 kDa fragment[64] is associated with stress-induced activation of apoptosis (Figure 6a). In addition, compound **8d** in combination with blue light irradiation induced an increase in the phosphorylation of histone H2A.X at S139 (γH2AX), indicating DNA damage. Moreover, increased levels of HO-1 protein, which is upregulated in response to oxidative stress, independently confirmed the ROS-mediated mechanism of action of **8d** (Figure 4).

On the other hand, the application of 5 μM concentration of **8d** in combination with increasing energy doses of blue light rapidly reduced the level of total PARP-1 protein. Together with the complete absence of the 89 kDa cleavage fragment of PARP-1 (Figure 6b) and the dramatic morphological changes mentioned above (Figure 5), the results suggest a mechanism of cell death other than apoptosis[65,66] and indicate the switch from apoptosis to programmed necrosis with increasing concentration of compound as well as the intensity of irradiation.

Moreover, 5 μM concentration of **8d** induced a dramatic increase in cytochrome C and HSP60 levels, which was not as evident when 1.25 μM concentration of **8d** was used. In contrast, reduced levels of HSP40 and HSP90 were observed. Cytochrome C and HSP60 are localized in the mitochondrial matrix,[67,68] whereas HSP90 and HSP40 are localized in the cytoplasm or cytoplasm and nucleus, respectively.[69,70] These results suggest that **8d** in combination with light affects the integrity of the mitochondrial outer membrane. The proteins are subsequently released from the mitochondrial intermembrane space, probably increasing their yield in the whole cell lysates. This result, together with the knowledge of common PDT-induced mitochondrial damage, led us to assess mitochondrial integrity after **8d**-PDT. To visualize the mitochondria, we used stably transfected MCF-7 cells expressing the mitochondrial SSBP1 protein fused to GFP. While we observed a typical tubular mitochondrial network in **8d**-untreated cells that were irradiated at 10 J/cm^2, treatment of the cells with 5 μM of **8d** in combination with irradiation (414 nm, 10 J/cm^2) induced rapid mitochondrial disruption (Figure 7).

Notably, mitochondrial SSBP1 has been previously implicated in cellular protection against proteotoxic stress and other adverse conditions. Its role extends to potentiating stress-induced heat shock factor 1 (HSF1) transcriptional activity. HSF1, a key player in maintaining cellular proteostasis, also responds to various insults, including oxidative stress. The activation of HSF1 in PDT-treated cells was demonstrated previously.[71] To investigate this further, we

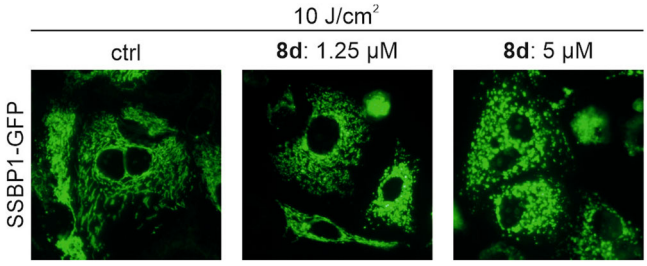


FIGURE 7 Mitochondrial integrity in MCF-7 cells expressing mitochondrial SSBP1 fused with GFP treated with **8d** for 4 h and incubated for a further 2 h after blue light irradiation at 10 J/cm^2.

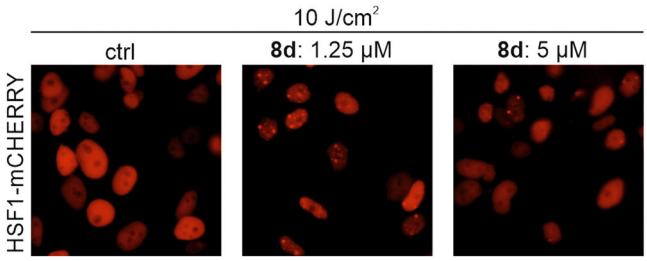


FIGURE 8 HSF1 nuclear foci formation in MCF-7 cells expressing HSF1 fused with mCHERRY treated with **8d** for 4 h and incubated for a further 2 h after blue light irradiation at 10 J/cm^2. HSF1, heat shock factor 1.

utilized MCF-7 cells stably expressing HSF1 fused with the fluorescent label mCHERRY. Our evaluation focused on the formation of HSF1 nuclear foci.[72] Indeed, compound **8d** in combination with 10 J/cm^2 induced their formation which was more evident when a lower concentration of **8d** was used (Figure 8). We hypothesize that this effect is probably related to the energy-dependent degradation of the HSF1 protein, as evidenced from immunoblotting results (Figure 6b).

3 | CONCLUSION

In this manuscript, we describe a set of new 5-[(*E*)-2-(3-alkoxy-1-phenyl-1*H*-pyrazol-4-yl)ethenyl]-3*H*-indoles prepared from easily accessible 4-ethenyl-1*H*-pyrazoles and 5-bromo-3*H*-indoles via a sequence of the Williamson ether synthesis, Vilsmeier–Haack formylation, Wittig olefination and ligand-free Heck reaction. Derivatives with modifications of the two phenyl groups on the pyrazole and indole rings were prepared to explore their photodynamic properties. These compounds were not cytotoxic to melanoma G361 cells in the dark at concentrations up to 10 μM but exerted cytotoxicity when irradiated with blue light (414 nm). All compounds exhibited similar potency, and the effects of substituents designed to modulate the electronic densities of the two aromatic rings by adding electron-withdrawing and donating groups were marginal. Furthermore, the photodynamic properties of the compound **8d** were investigated in breast cancer MCF-7 cells. Evaluation revealed comparable anticancer activities of **8d** in both breast and melanoma cancer cell lines within submicromolar range. Treatment with **8d** induced a massive generation of reactive oxygen species, leading to different types of cell death depending on the concentration of compound and irradiation intensity. The data obtained with the new compounds enrich our understanding of their light-induced cytotoxicity and provide a more comprehensive analysis of their potential as photodynamic therapy agents.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

All chemicals and solvents were purchased from commercial suppliers and used without further purification unless otherwise specified. Before use, dimethylformamide and toluene were stored over molecular sieves (4 Å). The ^{1}H, ^{13}C, ^{15}N NMR spectra were recorded in $CDCl_3$ solutions at 25°C on a Bruker Avance III 700 (700 MHz for ^{1}H, 176 MHz for ^{13}C, 71 MHz for ^{15}N) spectrometer equipped with a 5 mm TCI ^{1}H-^{13}C/^{15}N/D z-gradient cryoprobe. The ^{19}F NMR spectra (376 MHz) were obtained on a Bruker Avance III 400 spectrometer. The chemical shifts, expressed in ppm, were relative to tetramethylsilane (TMS). Fourier-transform infrared spectroscopy (FT-IR) spectra were collected using the attenuated total reflectance (ATR) method on a Bruker Vertex 70 v spectrometer with an integrated Platinum ATR accessory. The melting points of crystalline compounds were determined in open capillary tubes with a Buchi M-565 apparatus (temperature gradient–2°C/min) and are uncorrected. High-resolution mass spectrometry (HRMS) spectra were obtained in ESI mode on a Bruker MicrOTOF-Q III spectrometer. All optical measurements were performed under ambient conditions. The UV-Vis spectra of 10^{-4} M solutions of the compounds in water and THF were recorded on a Shimadzu 2600 UV–Vis spectrophotometer. The fluorescence spectra were recorded on an FLS920 fluorescence spectrofluorometer from Edinburgh Instruments. The fluorescence quantum yields were estimated from dilute THF solutions by an absolute method using the Edinburgh Instruments integrating sphere excited with an Xe lamp. Optical densities of the sample solutions were ensured to be below 0.1 to avoid reabsorption effects. All reactions were performed under an argon atmosphere with magnetic stirring. Reaction progress was monitored by TLC analysis on Macherey-Nagel™ ALUGRAM® Xtra SIL G/UV$_{254}$ plates. TLC plates were visualized with UV light (wavelengths 254 and 365 nm). Compounds were purified by flash chromatography in a glass column (stationary phase–silica gel 60, 0.063–0.200 mm, 70–230 mesh ASTM, Merck).

The InChI codes of the investigated compounds, together with some biological activity data, are provided as Supporting Information (Supporting Information S1).

4.1.2 | General procedure I for the synthesis of compounds **3a-e**

4-Bromophenylhydrazine hydrochloride (**1**) (4.5 mmol, 1 eq.) and appropriate ketone **2a–e** (6.75 mmol, 1.5 eq) were dissolved in ethanol (10 mL), and the reaction mixture was refluxed for 3 h. Then, the solution of sulfuric acid in ethanol (11 mL, 1/10 *v/v*) was added dropwise. The reaction was stirred at reflux temperature for 24 h. The mixture was cooled, poured into distilled water (150 mL), and extracted with dichloromethane (4 × 25 mL). The organic layer was dried with anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluent–EtOAc/Hex 1/12 *v/v*).

5-Bromo-3,3-dimethyl-2-phenyl-3*H*-indole (**3a**): Previously reported by Lackner et al.[73] Synthesized according to the General procedure I from 2-methyl-1-phenylpropan-1-one (**2a**) (1 mL, 6.75 mmol). Beige solid; yield 73% (981 mg). Obtained spectral data matches the previously reported data.

5-Bromo-2-(4-fluorophenyl)-3,3-dimethyl-3*H*-indole (**3b**): Synthesized according to the General procedure I from 1-(4-fluorophenyl)-2-methylpropan-1-one (**2b**) (1.1 mL, 6.75 mmol). Yellowish solid; yield 74% (1050 mg); m.p. 140–141°C; R_f = 0.66 (EtOAc/Hex 1/6, *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.14 (dd, *J* = 8.9, 5.4 Hz, 2H, C-Ph 2,6-H), 7.53 (d, *J* = 8.2 Hz, 1H, Ind 7-H), 7.49–7.45 (m, 2H, Ar-H), 7.20–7.14 (m, 2H, Ar-H), 1.57 (s, 6H, 3-($C\underline{H}_3$)$_2$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 182.3 (Ind C-2), 164.3 (d, $^1J_{CF}$ = 252.6 Hz, C-Ph C-4), 151.9, 149.5, 130.9, 130.5 (d, $^3J_{CF}$ = 8.5 Hz, C-Ph C-2, C-6), 129.1 (d, $^4J_{CF}$ = 3.3 Hz, C-Ph C-1), 124.5, 122.2, 119.6, 115.8 (d, $^2J_{CF}$ = 21.5 Hz, C-Ph C-3, C-5), 53.9 (Ind C-3), 24.6 (Ind 3-($\underline{C}H_3$)$_2$). IR (ν_{max}, cm^{-1}): 3079, 2979, 2965, 2909, 2866 (CH_{arom}, CH_{aliph}), 1597, 1502, 1459, 1405, 1390, 1330, 1292, 1248, 1221, 1213, 1149, 1089, 1009, 994, 832, 812, 781 (C=C, CH_3 bending, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{16}H_{14}BrFN$, 318.0288; found, 318.0288.

5-Bromo-2-(4-methoxyphenyl)-3,3-dimethyl-3*H*-indole (**3c**): Synthesized according to the General procedure I from 1-(4-methoxyphenyl)-2-methylpropan-1-one (**2c**) (1193 mg, 6.75 mmol). Beige solid; yield 80% (1187 mg); m.p. 124–125°C; R_f = 0.51 (EtOAc/Hex 1/4, *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.14–8.09 (m, 2H, C-Ph 2,6-H), 7.50 (d, *J* = 8.1 Hz, 1H, Ind 7-H), 7.48– 7.42 (m, 2H, Ar-H), 7.02–6.97 (m, 2H, Ar-H), 3.88 (s, 3H, –O$\underline{C}H_3$), 1.58 (s, 6H, Ind 3-($C\underline{H}_3$)$_2$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 182.9 (Ind C-2), 161.8, 152.2, 149.6, 130.8, 130.2, 125.4, 124.4, 121.8, 118.9, 114.1, 55.4 (–O$\underline{C}H_3$), 53.7 (Ind C-3), 24.9 (Ind 3-($\underline{C}H_3$)$_2$). IR (ν_{max}, cm^{-1}): 3082, 3058, 3004, 2981, 2965, 2936, 2913, 2866, 2834, 2760, 2715 (CH_{arom}, CH_{aliph}), 1600, 1574, 1504, 1453, 1411, 1386, 1336, 1304, 1251, 1210, 1162, 1114, 1039, 828, 806, 775 (C=C, CH_2 bending, C–O, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{17}H_{17}BrNO$, 330.0488; found, 330.0489.

5′-Bromo-2′-phenylspiro[cyclopentane-1,3′-indole] (**3d**): Synthesized according to the General procedure I from cyclopentyl(phenyl) methanone (**2d**) (1.1 mL, 6.75 mmol). Tan solid; yield 67% (975 mg); m.p. 150–151°C; R_f = 0.58 (EtOAc/Hex 1/6 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.08–8.04 (m, 2H, C-Ph 2,6-H), 7.56–7.44 (m, 6H, Ar-H), 2.46–2.39 (m, 2H, Cp $C\underline{H}_2$), 2.24–2.15 (m, 4H, Cp ($C\underline{H}_2$)$_2$), 1.96–1.89 (m, 2H, Cp $C\underline{H}_2$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 183.0, 152.2, 152.0, 132.3, 130.7, 130.4, 128.7, 128.3, 124.4, 121.9, 119.4, 63.6, 36.8 ($\underline{C}H_2$), 27.5 ($\underline{C}H_2$). IR (ν_{max}, cm^{-1}): 3089, 3056, 3026, 2965, 2872 (CH_{arom}, CH_{aliph}), 1574, 1517, 1490, 1441, 1413, 1337, 1249, 1211, 1117, 878, 865, 819, 773 (C=C, CH_2 bending, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{18}H_{17}BrN$, 326.0539; found, 326.0539.

5′-Bromo-2′-phenylspiro[cyclohexane-1,3′-indole] (**3e**): Synthesized according to the General procedure I from cyclohexyl(phenyl) methanone (**2e**) (1264 mg, 6.75 mmol). Yellowish-white solid; yield 63% (985 mg); m.p. 158–159°C; R_f = 0.61 (EtOAc/Hex 1/6 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.04 (dd, *J* = 6.7, 2.8 Hz, 2H, C-Ph 2,6-H), 7.94 (s, 1H, Ar-H), 7.57 (d, *J* = 8.1 Hz, 1H, Ar-H, Ind 7′-H), 7.52 (dd, *J* = 8.2, 1.9 Hz, 1H, Ind 6′-H), 7.50–7.45 (m, 3H, Ar-H), 2.29 (td, *J* = 13.5, 4.5 Hz, 2H, CHex $C\underline{H}_2$), 2.04 (d, *J* = 13.3 Hz, 1H, CHex $C\underline{H}_2$), 1.94 (qt, *J* = 13.6, 3.9 Hz, 2H, CHex $C\underline{H}_2$), 1.85 (d, *J* = 14.2 Hz, 2H, CHex $C\underline{H}_2$), 1.51 (qt, *J* = 13.4, 4.1 Hz, 1H, CHex $C\underline{H}_2$), 1.44 (d, *J* = 13.7 Hz, 2H, CHex $C\underline{H}_2$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 184.2, 152.8, 148.2, 133.5, 130.7, 130.2, 128.6, 128.5, 127.5, 122.5, 118.7, 58.9, 31.0 ($\underline{C}H_2$), 25.0 ($\underline{C}H_2$), 21.7 ($\underline{C}H_2$). IR (ν_{max}, cm^{-1}): 3089, 3018, 2949, 2944, 2921, 2862, 2851 (CH_{arom}, CH_{aliph}), 1568, 1521, 1494, 1457, 1437, 1411, 1332, 1254, 1223, 1178, 1057, 988, 895, 865, 810, 764 (C=C, CH_2 bending, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{19}H_{19}BrN$, 340.0695; found, 340.0695.

4.1.3 | General procedure II for the synthesis of compounds **5a-f**

Appropriate 1*H*-pyrazol-3-ol **4a–c** (15 mmol, 1 eq.) was dissolved in dry dimethylformamide (15 mL) at r.t under Ar. Sodium hydride (60% in mineral oil, 18.75 mmol, 1.25 eq.) was added in portions, and the mixture was stirred for 20 min. at r.t. Then, an appropriate alkylating agent was added dropwise (22.5 mmol, 1.5 eq.) and the reaction temperature was subsequently raised to 60°C. The reaction mixture was stirred at 60°C for 24 h, diluted with 200 mL of distilled water, and extracted with ethyl acetate (4 × 50 mL). The organic layer was washed with brine (200 mL), dried with anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel.

3-Methoxy-1-phenyl-1*H*-pyrazole (**5a**): Previously reported by Kazlauskas et al.[49] Synthesized according to the General procedure II from 1-phenyl-1*H*-pyrazol-3-ol (**4a**) (2400 mg, 15 mmol) and iodomethane (2.5 mL, 22.5 mmol). Obtained spectral data matches the previously reported data.

3-Butoxy-1-phenyl-1*H*-pyrazole (**5b**): Synthesized according to the General procedure II from 1-phenyl-1*H*-pyrazol-3-ol (**4a**) (2400 mg, 15 mmol) and iodobutane (2.5 mL, 22.5 mmol). Column chromatography on silica gel (eluent–EtOAc/Hex 1/20 *v/v*). Yellowish liquid; yield 84% (2724 mg); R_f = 0.68 (EtOAc/Hex 1/6 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 7.72 (d, *J* = 2.6 Hz, 1H, Pyr 5-H), 7.60 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.41–7.37 (m, 2H, Ar-H), 7.19 (t, *J* = 7.4 Hz, 1H,

Ph 4-H), 5.88 (d, J = 2.6 Hz, 1H, Pyr 4-H), 4.24 (t, J = 6.6 Hz, 2H, $CH_3CH_2CH_2\underline{CH_2}O$–), 1.82–1.75 (m, 2H, $CH_3CH_2\underline{CH_2}CH_2O$–), 1.54–1.46 (m, 2H, $CH_3\underline{CH_2}CH_2CH_2O$–), 0.98 (t, J = 7.4 Hz, 3H, $\underline{CH_3}CH_2CH_2CH_2O$–). ^{13}C NMR (176 MHz, $CDCl_3$): δ 164.7, 140.2, 129.3, 127.5, 125.2, 117.8, 93.7, 69.0 ($\underline{CH_2}$), 31.3 ($\underline{CH_2}$), 19.2 ($\underline{CH_2}$), 13.9 ($\underline{CH_3}$). IR (ν_{max}, cm^{-1}): 3154, 3066, 2964, 2942, 2870 (CH_{arom}, CH_{aliph}), 1600, 1543, 1506, 1487, 1465, 1456, 1402, 1371, 1347, 1329, 1269, 1236, 1072, 1045, 1035, 1013, 983, 932, 903, 754, 738, 691 cm^{-1} (C=C, CH_3, CH_2 bending, C–O, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{13}H_{17}N_2O$, 217.1335; found, 217.1335.

3-(2-Methoxyethoxy)-1-phenyl-1*H*-pyrazole (**5c**): Previously reported by Urbonavičius et al.[50] Synthesized according to the General procedure II from 1-phenyl-1*H*-pyrazol-3-ol (**4a**) (2400 mg, 15 mmol) and 2-bromoethylmethylether (2.1 mL, 22.5 mmol). Column chromatography on silica gel (eluent–EtOAc/Hex 1/3 *v/v*). Yellowish liquid, yield 83% (2703 mg). Obtained spectral data matches the reported data.

3-Methoxy-1-(4-methoxyphenyl)-1*H*-pyrazole (**5d**): Synthesized according to the General procedure II from 1-(4-methoxyphenyl)-3-hydroxy-1*H*-pyrazole (**4b**) (2852 mg, 15 mmol) and iodomethane (1.4 mL, 22.5 mmol). Column chromatography on silica gel (eluent–EtOAc/Hex 1/6 *v/v*). Yellowish liquid; yield 63% (1919 mg). R_f = 0.43 (EtOAc/Hex 1/6 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 7.61 (d, J = 2.5 Hz, 1H, Pyr 5-H), 7.53–7.47 (m, 2H, Ph 2,6-H), 6.95–6.90 (m, 2H, Ph 3,5-H), 5.85 (d, J = 2.5 Hz, 1H, Pyr 4-H), 3.96 (s, 3H, Pyr 3-O$\underline{CH_3}$), 3.81 (s, 3H, Ph 4-O$\underline{CH_3}$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 164.9 (Pyr C-3), 157.5 (Ph C-4), 134.1 (Ph C-1), 127.8 (Pyr C-5), 119.7 (Ph C-2,6), 114.5 (Ph C-3,5), 92.6 (Pyr C-4), 56.4 (Pyr 3-O$\underline{CH_3}$), 55.5 (Ph 4-O$\underline{CH_3}$). IR (ν_{max}, cm^{-1}): 3149, 3132, 3019, 2978, 2967, 2938 (CH_{arom}, CH_{aliph}), 1546, 1517, 1489, 1456, 1417, 1388, 1255, 1234, 1028, 936, 827, 741 cm^{-1} (C=C, CH_3 bending, C–O, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{11}H_{13}N_2O_2$, 205.0972; found, 205.0972.

3-(2-Methoxyethoxy)-1-(4-methoxyphenyl)-1*H*-pyrazole (**5e**): Synthesized according to the General procedure II from 1-(4-methoxyphenyl)-3-hydroxy-1*H*-pyrazole (**4b**) (2852 mg, 15 mmol) and 2-bromoethylmethylether (2.1 mL, 22.5 mmol). Column chromatography on silica gel (eluent–EtOAc/Hex 1/3 *v/v*). Yellowish orange liquid; yield 66% (2438 mg). R_f = 0.31 (EtOAc/Hex 1/3 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 7.61 (d, J = 2.6 Hz, 1H, Pyr 5-H), 7.51–7.47 (m, 2H, Ph 2,6-H), 6.95–6.91 (m, 2H, Ph 3,5-H), 5.89 (d, J = 2.5 Hz, 1H, Pyr 4-H), 4.43–4.39 (m, 2H, $CH_3OCH_2\underline{CH_2}O$–), 3.82 (s, 3H, Ph 4-O$\underline{CH_3}$), 3.78–3.74 (m, 2H, $CH_3O\underline{CH_2}CH_2O$–), 3.45 (s, 3H, $\underline{CH_3}OCH_2CH_2O$–). ^{13}C NMR (176 MHz, $CDCl_3$): δ 164.0 (Pyr C-3), 157.5 (Ph C-4), 134.1 (Ph C-1), 127.7 (Pyr C-5), 119.6 (Ph C-2,6), 114.5 (Ph C-3,5), 93.3 (Pyr C-4), 71.1 ($CH_3O\underline{CH_2}CH_2O$-), 68.1 ($CH_3OCH_2\underline{CH_2}O$–), 59.1 ($\underline{CH_3}OCH_2CH_2O$–), 55.6 (Ph 4-O$\underline{CH_3}$). IR ($\nu_{max}$, cm^{-1}): 3134, 3118, 3019, 2999, 2966, 2934, 2885, 2841, 2820 (CH_{arom}, CH_{aliph}), 1541, 1515, 1448, 1377, 1301, 1241, 1077, 1024, 836, 768 (C=C, CH_3, CH_2 bending, C–O, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{13}H_{16}N_2NaO_3$, 271.1053; found, 271.1053.

1-(4-Fluorophenyl)-3-methoxy-1*H*-pyrazole (**5f**): Previously reported by Savickienė et al.[74] Synthesized according to the General procedure II from 1-(4-fluorophenyl)-1*H*-pyrazol-3-ol (**4c**) (2670 mg, 15 mmol) and iodomethane (1.43 mL, 22.5 mmol). Column chromatography on silica gel (eluent–EtOAc/Hex 1/15 *v/v*). Yellowish liquid; yield 55% (1577 mg). R_f = 0.61 (EtOAc/Hex 1/6 *v/v*). Obtained spectral data matches the reported data.

4.1.4 | General procedure III for the synthesis of compounds 6a–f

Phosphorus oxychloride (40 mmol, 4 eq.) was added dropwise into dry dimethylformamide (40 mmol, 4 eq.) under Ar at −10°C. The mixture was stirred at −10°C until the viscous, white Vilsmeier reagent was formed. Then, the appropriate 3-alkoxy-1*H*-pyrazole **5a–f** (10 mmol, 1 eq.) was dissolved in dry dimethylformamide (5 mL) and added dropwise into the Vilsmeier reagent at r.t. The reaction temperature was subsequently raised to 70°C and maintained for 24 h. The reaction mixture was chilled, poured into ice water (200 mL), and basified with solid Na_2CO_3 and NaOH (pH > 10). The resulting precipitate was filtered, washed with hot water and hexane, and dried to yield 3-alkoxy-1*H*-pyrazole-4-carbaldehydes **6a–f**. Compound **6e** was purified by column chromatography on silica gel (eluent–EtOAc/Hex 1/3 to 1/1 *v/v*).

3-Methoxy-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**6a**): Previously reported by Kazlauskas et al.[49] Synthesized according to the General procedure III from 3-methoxy-1-phenyl-1*H*-pyrazole (**5a**) (1742 mg, 10 mmol). Obtained spectral data matches the reported data.

3-Butoxy-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**6b**): Synthesized according to the General procedure III from 3-butoxy-1-phenyl-1*H*-pyrazole (**5b**) (2162 mg, 10 mmol). Off-white solid; yield 90% (2209 mg); m.p. 76-77°C; R_f = 0.39 (EtOAc/Hex 1/6 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 9.86 (s, 1H, –C$\underline{H}$O), 8.25 (s, 1H, Pyr 5-H), 7.64 (d, J = 7.5 Hz, 2H, Ar-H), 7.49–7.43 (m, 2H, Ar-H), 7.32 (t, J = 7.5 Hz, 1H, Ph 4-H), 4.40 (t, J = 6.6 Hz, 2H, $CH_3CH_2CH_2\underline{CH_2}O$–), 1.84 (m, 2H, $CH_3CH_2\underline{CH_2}CH_2O$–), 1.52 (h, J = 7.4 Hz, 2H, $CH_3\underline{CH_2}CH_2CH_2O$–), 1.00 (t, J = 7.4 Hz, 3H, $\underline{CH_3}CH_2CH_2CH_2O$–). ^{13}C NMR (176 MHz, $CDCl_3$): δ 183.5 (–$\underline{C}$HO), 164.1, 139.1, 129.6, 129.2, 127.2, 118.8, 111.5, 69.4 ($\underline{CH_2}$), 31.1 ($\underline{CH_2}$), 19.1 ($\underline{CH_2}$), 13.8 ($\underline{CH_3}$). IR (ν_{max}, cm^{-1}): 3124, 3101, 2953, 2930, 2873, 2839 (CH_{arom}, CH_{aliph}), 1674, 1598, 1566, 1504, 1474, 1416, 1372, 1348, 1223, 1205, 1033, 993, 942, 912, 863, 753 (C=O, C=C, CH_3, CH_2 bending, C–O, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{14}H_{16}N_2NaO_2$, 267.1104; found, 267.1104.

3-(2-Methoxyethoxy)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**6c**): Previously reported by Urbonavičius *et al.*[50] Synthesized according to the General procedure III from 3-(2-methoxyethoxy)-1-phenyl-1*H*-pyrazole (**5c**) (2183 mg, 10 mmol). Column chromatography on silica gel (eluent–EtOAc/Hex 1/3 *v/v*). White crystals, yield 78% (1915 mg). Obtained spectral data matches the reported data.

3-Methoxy-1-(4-methoxyphenyl)-1*H*-pyrazole-4-carbaldehyde (**6d**): Synthesized according to the General procedure III from

3-methoxy-1-(4-methoxyphenyl)-1*H*-pyrazole (**5d**) (2043 mg, 10 mmol). White solid; yield 73% (1691 mg); m.p. 126–127°C; R_f = 0.69 (EtOAc/Hex 1/1 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 9.84 (s, 1H, –CHO), 8.14 (s, 1H, Pyr 5-H), 7.57–7.53 (m, 2H, Ph 2,6-H), 6.99–6.95 (m, 2H, Ph 3,5-H), 4.08 (s, 3H, Pyr 3-OCH_3), 3.85 (s, 3H, Ph 4-OCH_3). ^{13}C NMR (176 MHz, $CDCl_3$): δ 183.1 (–CHO), 164.1 (Pyr C-3), 158.8 (Ph C-4), 132.7 (Ph C-1), 129.8 (Pyr C-5), 120.6 (Ph C-2,6), 114.7 (Ph C-3,5), 110.9 (Pyr C-4), 56.6 (Pyr 3-OCH_3), 55.6 (Ph 4-OCH_3). IR (ν_{max}, cm^{-1}): 3126, 3100, 3009, 2976, 2964, 2937, 2835, 2763 (CH_{arom}, CH_{aliph}), 1661, 1555, 1511, 1413, 1389, 1248, 1224, 1173, 1006, 825, 710 (C=O, C=C, CH_3 bending, C–O, C–N, C–H_{arom} oop bending). HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{12}H_{12}N_2NaO_3$, 255.0740; found, 255.0740.

3-(2-Methoxyethoxy)-1-(4-methoxyphenyl)-1*H*-pyrazole-4-carbaldehyde (**6e**): Synthesized according to the General procedure III from 3-(2-methoxyethoxy)-1-(4-methoxyphenyl)-1*H*-pyrazole (**5e**) (2482 mg, 10 mmol). White solid; yield 29% (790 mg); m.p. 111–112°C; R_f = 0.11 (EtOAc/Hex 1/3 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 9.87 (s, 1H, –CHO), 8.15 (s, 1H, Pyr 5-H), 7.55–7.52 (m, 2H, Ph 2,6-H), 6.99–6.95 (m, 2H, Ph 3,5-H), 4.56–4.53 (m, 2H, $CH_3OCH_2CH_2O$–), 3.84 (s, 3H, Ph 4-OCH_3), 3.83–3.80 (m, 2H, $CH_3OCH_2CH_2O$–), 3.47 (s, 3H, $CH_3OCH_2CH_2O$–). ^{13}C NMR (176 MHz, $CDCl_3$): δ 183.4 (–CHO), 163.7 (Pyr C-3), 158.8 (Ph C-4), 132.6 (Ph C-1), 129.0 (Pyr C-5), 120.5 (Ph C-2,6), 114.7 (Ph C-3,5), 110.9 (Pyr C-4), 70.7 ($CH_3OCH_2CH_2O$–), 68.7 ($CH_3OCH_2CH_2O$–), 59.2 ($CH_3OCH_2CH_2O$–), 55.6 (Ph 4-OCH_3). IR (ν_{max}, cm^{-1}): 3123, 3092, 3006, 2994, 2933, 2891, 2863, 2835, 2773, 2752 (CH_{arom}, CH_{aliph}), 1657, 1600, 1559, 1520, 1498, 1448, 1382, 1347, 1305, 1248, 1219, 1202, 1187, 1028, 832, 709 (C=O, C=C, CH_3, CH_2 bending, C–O, C–N, C–H_{arom} oop bending). HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{14}H_{16}N_2NaO_4$, 299.1002; found, 299.1003.

1-(4-Fluorophenyl)-3-methoxy-1*H*-pyrazole-4-carbaldehyde (**6f**): Previously reported by Savickienė et al.[74] Synthesized according to the General procedure III from 1-(4-fluorophenyl)-3-methoxy-1*H*-pyrazole (**5f**) (1921 mg, 10 mmol). White solid; yield 84% (1850 mg); m.p. 164–165°C; R_f = 0.24 (EtOAc/Hex 1/6 *v/v*). Obtained spectral data matches the reported data.

4.1.5 | General procedure IV for the synthesis of compounds 7a–f

Into the suspension of methyltriphenylphosphonium iodide (5 mmol, 1.25 eq.) in dry toluene (50 mL), potassium *tert*-butoxide (10 mmol, 1.5 eq.) was added in one portion at −5°C under Ar. The mixture was stirred at the same temperature for 15 min, and then removed from the ice bath and stirred at r.t. until viscous yellow phosphonium ylide formed. Subsequently, the reaction mixture was cooled to 0°C and the suspension of appropriate pyrazole-4-carbaldehyde **6a–f** (4 mmol, 1 eq.) in dry toluene (25 mL) was added dropwise. After the addition of aldehyde, the reaction temperature was maintained at 0°C for 1 h and then stirred at r.t. for another 1 h. Upon completion, the reaction was quenched with saturated ammonium chloride solution (150 mL) and the toluene layer was dried with anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (eluent–EtOAc/Hex 1/15 *v/v*).

4-Ethenyl-3-methoxy-1-phenyl-1*H*-pyrazole (**7a**): Synthesized according to the General procedure IV from 3-methoxy-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**6a**) (809 mg, 4 mmol). Yellowish oil; yield 75% (597 mg); R_f = 0.76 (EtOAc/Hex 1/3 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): *δ* 7.73 (s, 1H, Pyr 5-H), 7.58 (d, *J* = 7.8 Hz, 2H, Ph 2,6-H), 7.43–7.36 (m, 2H, Ph 3,5-H), 7.19 (t, *J* = 7.4 Hz, 1H, Ph 4-H), 6.51 (dd, *J* = 17.7, 11.3 Hz, 1H, Pyr 4-CH=CH_2), 5.69 (dd, *J* = 17.7, 1.6 Hz, 1H, Pyr 4-CH=CH_2), 5.15 (dd, *J* = 11.3, 1.6 Hz, 1H, Pyr 4-CH=CH_2), 4.05 (s, 3H, –OCH_3). ^{13}C NMR (176 MHz, $CDCl_3$): *δ* 162.5 (Pyr C-3), 140.0 (Ph C-1), 129.3 (Ph C-3,5), 125.23 (Pyr C-5), 125.19 (Ph C-4), 125.0 (Pyr 4-CH=CH_2), 117.6 (Ph C-2,6), 113.1 (Pyr 4-CH=CH_2), 108.4 (Pyr C-4), 56.2 (–OCH_3). IR (ν_{max}, cm^{-1}): 3102, 3071, 3050, 3014, 2977, 2945, 2896, 2870, 2815 (CH_{arom}, CH_{aliph}), 1637, 1599, 1566, 1501, 1462, 1428, 1414, 1398, 1247, 1204, 1010, 940, 897, 808, 751, 647 (C=C, C–O, C–N, CH_3 bending, CH_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{12}H_{13}N_2O$, 201.1022; found, 201.1020.

3-Butoxy-4-ethenyl-1-phenyl-1*H*-pyrazole (**7b**): Synthesized according to the General procedure IV from 3-butoxy-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**6b**) (977 mg, 4 mmol). Yellowish oil; yield 70% (681 mg); R_f = 0.66 (EtOAc/Hex 1/6 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): *δ* 7.72 (s, 1H, Pyr 5-H), 7.57 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.42–7.36 (m, 2H, Ar-H), 7.18 (t, *J* = 7.4 Hz, 1H, Ph 4-H), 6.52 (dd, *J* = 17.7, 11.3 Hz, 1H, Pyr 4-CH=CH_2), 5.71 (dd, *J* = 17.7, 1.8 Hz, 1H, Pyr 4-CH=CH_2), 5.14 (dd, *J* = 11.3, 1.8 Hz, 1H, Pyr 4-CH=CH_2), 4.35 (t, *J* = 6.5 Hz, 2H, $CH_3CH_2CH_2CH_2O$–), 1.85–1.79 (m, 2H, $CH_3CH_2CH_2CH_2O$–), 1.52 (h, *J* = 7.4 Hz, 2H, $CH_3CH_2CH_2CH_2O$–), 0.99 (t, *J* = 7.4 Hz, 3H, $CH_3CH_2CH_2CH_2O$–). ^{13}C NMR (176 MHz, $CDCl_3$): *δ* 162.2, 140.0, 129.3, 125.16, 125.10, 125.06, 117.6, 113.0 (Pyr 4-CH=CH_2), 108.5, 68.7 (CH_2), 31.3 (CH_2), 19.3 (CH_2), 13.9 (CH_3). IR (ν_{max}, cm^{-1}): 3100, 3051, 3014, 2958, 2935, 2873 (CH_{arom}, CH_{aliph}), 1638, 1599, 1564, 1501, 1465, 1427, 1245, 1205, 1056, 1032, 939, 897, 807, 751 (C=C, CH_3, CH_2 bending, C–O, C–N, CH_{arom}, CH_{alkene} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{15}H_{19}N_2O$, 243.1492; found 243.1492.

4-Ethenyl-3-(2-methoxyethoxy)-1-phenyl-1*H*-pyrazole (**7c**): Synthesized according to the General procedure IV from 3-(2-methoxyethoxy)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**6c**) (985 mg, 4 mmol). Colorless oil; yield 56% (552 mg); R_f = 0.67 (EtOAc/Hex 1/3 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): *δ* 7.74 (s, 1H, Pyr 5-H), 7.59–7.55 (m, 2H, Ph 2,6-H), 7.41–7.36 (m, 2H, Ph 3,5-H), 7.20–7.15 (m, 1H, Ph 4-H), 6.52 (dd, *J* = 17.7, 11.3 Hz, 1H, Pyr 4-CH=CH_2), 5.70 (dd, *J* = 17.7, 1.7 Hz, 1H, Pyr 4-CH=CH_2), 5.15 (dd, *J* = 11.3, 1.7 Hz, 1H, Pyr 4-CH=CH_2), 4.53–4.48 (m, 2H, $CH_3OCH_2CH_2O$–), 3.83–3.78 (m, 2H, $CH_3OCH_2CH_2O$–), 3.46 (s, 3H, –OCH_3). ^{13}C NMR (176 MHz, $CDCl_3$): *δ* 161.8 (Pyr C-3), 139.9 (Ph C-1), 129.3 (Ph C-3,5), 125.2 (Ph C-4), 125.0 (Pyr C-5), 124.9 (Pyr 4-CH=CH_2), 117.6 (Ph C-2, C-6), 113.2 (Pyr 4-CH=CH_2), 108.6 (Pyr C-4), 71.0 ($CH_3OCH_2CH_2O$–), 68.2 ($CH_3OCH_2CH_2O$–), 59.1 (–OCH_3). IR (ν_{max}, cm^{-1}): 3101, 3070, 3051, 3014, 2982, 2929, 2883, 2815

(CH_{arom}, CH_{aliph}), 1638, 1599, 1565, 1499, 1449, 1353, 1246, 1202, 1127, 1045, 1030, 990, 942, 899, 851, 810, 752, 735 (C=C, CH_3, CH_2 bending, C–O, C–N, C–H_{arom} oop bending). HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{14}H_{16}N_2NaO_2$, 267.1104; found, 267.1104.

4-Ethenyl-3-methoxy-1-(4-methoxyphenyl)-1*H*-pyrazole (**7d**): Synthesized according to the General procedure IV from 3-methoxy-1-(4-methoxyphenyl)-1*H*-pyrazole-4-carbaldehyde (**6d**) (929 mg, 4 mmol). White solid; yield 80% (735 mg); m.p. 58–59°C; R_f = 0.62 (EtOAc/Hex 1/3 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): *δ* 7.62 (s, 1H, Pyr 5-H), 7.50–7.46 (m, 2H, Ph 2,6-H), 6.95–6.90 (m, 2H, Ph 3,5-H), 6.50 (dd, *J* = 17.7, 11.3 Hz, 1H, Pyr 4-C<u>H</u>=CH_2), 5.65 (dd, *J* = 17.7, 1.8 Hz, 1H, Pyr 4-CH=C<u>H</u>$_2$), 5.12 (dd, *J* = 11.3, 1.8 Hz, 1H, Pyr 4-CH=C<u>H</u>$_2$), 4.03 (s, 3H, Pyr 3-OC<u>H</u>$_3$), 3.81 (s, 3H, Ph 4-OC<u>H</u>$_3$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 162.3 (Pyr C-3), 157.4 (Ph C-4), 133.9 (Ph C-1), 125.3 (Pyr C-5), 125.1 (Pyr 4-<u>C</u>H=CH_2), 119.4 (Ph C-2,6), 114.5 (Ph C-3,5), 112.7 (Pyr 4-CH=<u>C</u>H$_2$), 107.7 (Pyr C-4), 56.2 (Pyr 3–O<u>C</u>H$_3$), 55.6 (Ph 4-O<u>C</u>H$_3$). IR (ν_{max}, cm^{-1}): 3154, 3133, 3101, 3055, 3013, 2960, 2946, 2911, 2897, 2869, 2837 (CH_{arom}, CH_{aliph}), 1635, 1563, 1508, 1461, 1445, 1414, 1401, 1297, 1241, 1029, 1008, 995, 940, 900, 824, 735 (C=C, CH_3 bending, C–O, C–N, C–H_{arom} oop bending). HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{13}H_{14}N_2NaO_2$, 253.0947; found, 253.0946.

4-Ethenyl-3-(2-methoxyethoxy)-1-(4-methoxyphenyl)-1*H*-pyrazole (**7e**): Synthesized according to the General procedure IV from 3-(2-methoxyethoxy)-1-(4-methoxyphenyl)-1*H*-pyrazole-4-carbaldehyde (**6e**) (1120 mg, 4 mmol). White solid; yield 52% (570 mg); m.p. 83–84°C; R_f = 0.53 (EtOAc/Hex 1/3 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): *δ* 7.63 (s, 1H, Pyr 5-H), 7.49–7.44 (m, 2H, Ph 2,6-H), 6.95–6.89 (m, 2H, Ph 3,5-H), 6.52 (dd, *J* = 17.7, 11.3 Hz, 1H, Pyr 4-C<u>H</u>=CH_2), 5.67 (dd, *J* = 17.7, 1.7 Hz, 1H, Pyr 4-CH=C<u>H</u>$_2$), 5.12 (dd, *J* = 11.3, 1.7 Hz, 1H, Pyr 4-CH=C<u>H</u>$_2$), 4.51–4.46 (m, 2H, CH_3OCH_2-C<u>H</u>$_2$O-), 3.84–3.78 (m, 5H, CH_3OCH_2<u>C</u>H$_2$O– and Ph 4-OC<u>H</u>$_3$), 3.45 (s, 3H, C<u>H</u>$_3$OCH$_2$CH$_2$O–). ^{13}C NMR (176 MHz, $CDCl_3$): *δ* 161.5 (Pyr C-3), 157.4 (Ph C-4), 133.8 (Ph C-1), 125.1 (Pyr C-5), 125.0 (Pyr 4-<u>C</u>H=CH_2), 119.4 (Ph C-2,6), 114.5 (Ph C-3,5), 112.7 (Pyr 4-CH=<u>C</u>H$_2$), 107.9 (Pyr C-4), 71.1 (CH_3O<u>C</u>H$_2$CH$_2$O–), 68.2 (CH_3OCH_2-<u>C</u>H$_2$O–), 59.1 (<u>C</u>H$_3$OCH$_2$CH$_2$O–), 55.6 (Ph 4-O<u>C</u>H$_3$). IR (ν_{max}, cm^{-1}): 3084, 3012, 2990, 2955, 2930, 2887, 2845, 2833, 2818 (CH_{arom}, CH_{aliph}), 1633, 1558, 1501, 1441, 1424, 1350, 1302, 1243, 1198, 1180, 1125, 1103, 1062, 1048, 1026, 994, 943, 913, 831, 813, 737 (C=C, CH_3, CH_2 bending, C–O, C–N, C–H_{arom} oop bending). HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{15}H_{18}N_2NaO_3$, 297.1210; found, 297.1212.

4-Ethenyl-1-(4-fluorophenyl)-3-methoxy-1*H*-pyrazole (**7f**): Synthesized according to the General procedure IV from 1-(4-fluorophenyl)-3-methoxy-1*H*-pyrazole-4-carbaldehyde (**6f**) (883 mg, 4 mmol). Yellowish oil; yield 69% (600 mg); R_f = 0.71 (EtOAc/Hex 1/6 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): *δ* 7.65 (s, 1H, Pyr 5-H), 7.55–7.50 (m, 2H, Ar-H), 7.12–7.06 (m, 2H, Ar-H), 6.50 (dd, *J* = 17.7, 11.3 Hz, 1H, Pyr 4-C<u>H</u>=CH_2), 5.68 (dd, *J* = 17.7, 1.8 Hz, 1H, Pyr 4-CH=C<u>H</u>$_2$), 5.15 (dd, *J* = 11.3, 1.8 Hz, 1H, Pyr 4-CH=C<u>H</u>$_2$), 4.03 (s, 3H, –O<u>C</u>H$_3$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 162.6, 160.4 (d, $^1J_{CF}$ = 244.5 Hz, Ph C-4), 136.4 (d, $^4J_{CF}$ = 2.9 Hz, Ph C-1), 125.3, 124.9, 119.3 (d, $^3J_{CF}$ = 8.4 Hz, Ph C-2,6), 116.1 (d, $^2J_{CF}$ = 22.9 Hz, Ph C-3,5), 113.3, 108.4, 56.2 (–O<u>C</u>H$_3$). IR (ν_{max}, cm^{-1}): 3102, 3020, 2990, 2951 (CH_{arom}, CH_{aliph}), 1633, 1563, 1508, 1460, 1442, 1412, 1246, 1223, 1207, 1155, 1099, 1030, 1008, 992, 938, 905, 833, 819, 738 (C=C, CH_3 bending, C–O, C–N, CH_{arom}, CH_{alkene} oop bending). HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{12}H_{12}FN_2O$, 219.0928; found 219.0928.

4.1.6 | General procedure V for the synthesis of compounds 8a–m

Appropriate 4-ethenyl-1*H*-pyrazole **7a–f** (1 mmol, 1 eq.) and 5-bromo-3*H*-indole **3a–e** (1.25 mmol, 1.25 eq.) were dissolved in dry dimethylformamide (2 mL) under Ar. Then, cesium carbonate (1.5 mmol, 1.5 eq.), appropriate tetrabutylammonium halide (1.5 mmol, 1.5 eq.), and palladium(II) acetate (10–20 mol%, 0.1–0.2 mmol, 0.1–0.2 eq.) were added and the reaction mixture was stirred at 120°C for 24–48 h. Upon completion, the reaction was cooled to r.t., poured into brine (100 mL), and extracted with ethyl acetate (4 × 25 mL). Organic layers were combined, washed with brine (100 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel.

5-[(*E*)-2-(3-(Methoxy)-1-phenyl-1*H*-pyrazol-4-yl)ethenyl]-3,3-dimethyl-2-phenyl-3*H*-indole (**8a**): Synthesized according to the General procedure V from 4-ethenyl-3-methoxy-1-phenyl-1*H*-pyrazole (**7a**) (211 mg, 1 mmol) and 5-bromo-3,3-dimethyl-2-phenyl-3*H*-indole (**3a**) (375 mg, 1.25 mmol) using tetrabutylammonium iodide (TBAI) (554 mg) and 20 mol% of palladium(II) acetate. The reaction was conducted for 48 h. Column chromatography on silica gel (eluent–EtOAc/Hex 1/6 *v/v*). Greenish yellow amorphous material; yield 48% (203 mg); R_f = 0.43 (EtOAc/Hex 1/3 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.15 (dd, *J* = 7.5, 2.2 Hz, 2H, C-Ph 2,6-H), 7.86 (s, 1H, Pyr 5-H), 7.66–7.60 (m, 3H, N-Ph 2,6-H, Ind 7-H), 7.51–7.38 (m, 7H, N-Ph 3,5-H, Ind 4,6-H, C-Ph 3,4,5-H), 7.20 (t, *J* = 7.4 Hz, 1H, N-Ph 4-H), 7.17 (d, *J* = 16.3 Hz, 1H, Pyr–CH=C<u>H</u>–Ind), 6.96 (d, *J* = 16,4 Hz, 1H, Pyr–C<u>H</u>=CH–Ind), 4.13 (s, 3H, –O<u>C</u>H$_3$), 1.62 (s, 6H, Ind 3-(C<u>H</u>$_3$)$_2$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 182.9 (Ind C-2), 162.5 (Pyr C-3), 152.5 (Ind C-7a), 148.2 (Ind C-3a), 139.9 (N-Ph C-1), 136.1 (Ind C-5), 133.3 (C-Ph C-1), 130.5 (C-Ph C-4), 129.4 (N-Ph C-3,5), 128.6 (C-Ph C-3,5), 128.3 (C-Ph C-2,6), 127.9 (Pyr–CH=<u>C</u>H–Ind), 126.1 (Ind C-6), 125.2 (N-Ph C-4), 125.0 (Pyr C-5), 120.9 (Ind C-7), 118.2 (Ind C-4), 117.5 (N-Ph C-2,6), 116.6 (Pyr–<u>C</u>H=CH–Ind), 108.4 (Pyr C-4), 56.3 (-O<u>C</u>H$_3$), 53.4 (Ind C-3), 24.9 (Ind 3-(<u>C</u>H$_3$)$_2$). ^{15}N NMR (71 MHz, $CDCl_3$): δ –70.3 (Ind N-1), –120.1 (Pyr N-2), –188.9 (Pyr N-1). IR (ν_{max}, cm^{-1}): 3048, 3019, 3007, 2966, 2943, 2928, 2865, 2814 (CH_{arom}, CH_{aliph}), 1638, 1598, 1566, 1502, 1460, 1442, 1406, 1344, 1250, 1222, 1201, 1053, 1013, 961, 941, 820, 775, 752 (C=C, CH_3 bending, C–O, C–N, C–H_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{28}H_{26}N_3O$, 420.2070; found, 420.2070.

5′-[(*E*)-2-(3-Methoxy-1-phenyl-1*H*-pyrazol-4-yl)ethenyl]-2′-phenylspiro[cyclopentane-1,3′-indole] (**8b**): Synthesized according to the General procedure V from 4-ethenyl-3-methoxy-1-phenyl-1*H*-pyrazole (**7a**) (200 mg, 1 mmol) and 5′-bromo-2′-phenylspiro [cyclopentane-1,3′-indole] (**3d**) (425 mg, 1.25 mmol) using

tetrabutylammonium chloride (TBAC) (416 mg) and 10 mol% of palladium(II) acetate. The reaction was conducted for 24 h. Column chromatography on silica gel (eluent–EtOAc/Hex 1/9 *v/v*). Yellow solid; yield 59% (264 mg); m.p. = 165–166°C; R_f = 0.26 (EtOAc/Hex 1/6 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.09 (dd, *J* = 7.5, 2.2 Hz, 2H, C-Ph 2,6-H), 7.86 (s, 1H, Pyr 5-H), 7.62 (d, *J* = 8.4 Hz, 3H, N-Ph 2,6-H, Ind 7′-H), 7.50–7.40 (m, 7H, Ind 4′,6′-H, C-Ph 3,4,5-H, N-Ph 3,5-H), 7.20 (t, *J* = 7.4 Hz, 1H, N-Ph 4-H), 7.17 (d, *J* = 16.3 Hz, 1H, Pyr–CH=CH–Ind), 6.94 (d, *J* = 16.3 Hz, 1H, Pyr–CH=CH–Ind), 4.13 (s, 3H, –OCH_3), 2.48–2.41 (m, 2H, Cp 2,5-H), 2.31–2.18 (m, 4H, Cp 3,4-CH_2), 2.01–1.94 (m, 2H, Cp 2,5-H). ^{13}C NMR (176 MHz, $CDCl_3$): δ 182.4 (Ind C-2′), 162.5 (Pyr C-3), 152.4 (Ind C-7a′), 150.9 (Ind C-3a′), 139.9 (N-Ph C-1), 136.1 (Ind C-5′), 132.8 (C-Ph C-1), 130.4 (C-Ph C-4), 129.4 (N-Ph C-3,5), 128.6 (C-Ph C-3,5), 128.2 (C-Ph C-2,6), 128.0 (Pyr–CH=CH–Ind), 125.5 (Ind C-6′), 125.2 (N-Ph C-4), 125.0 (Pyr C-5), 120.7 (Ind C-7′), 118.4 (Ind C-4′), 117.5 (N-Ph C-2,6), 116.4 (Pyr–CH=CH–Ind), 108.5 (Pyr C-4), 63.1 (Ind C-1,3′), 56.3 (–OCH$_3$), 37.0 (Cp C-2,5), 27.7 (Cp C-3,4). ^{15}N NMR (71 MHz, $CDCl_3$): δ −67.4 (Ind N-1), −120.1 (Pyr N-2), −189.0 (Pyr N-1). IR (ν_{max}, cm^{-1}): 3044, 3012, 2955, 2940, 2868, 2811 (CH_{arom}, CH_{aliph}), 1637, 1596, 1565, 1502, 1462, 1441, 1402, 1248, 1201, 1053, 1016, 962, 939, 817, 748, 711 (C=C, CH_3, CH_2 bending, C–O, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{30}H_{28}N_3O$, 446.2227; found, 446.2227.

5′-[(*E*)-2-(3-Methoxy-1-phenyl-1*H*-pyrazol-4-yl)ethenyl]-2′-phenylspiro[cyclohexane-1,3′-indole] (**8c**): Synthesized according to the General procedure V from 4-ethenyl-3-methoxy-1-phenyl-1*H*-pyrazole (**7a**) (200 mg, 1 mmol) and 5′-bromo-2′-phenylspiro [cyclohexane-1,3′-indole] (**3e**) (425 mg, 1.25 mmol) using TBAC (416 mg) and 10 mol% of palladium(II) acetate. The reaction was conducted for 24 h. Column chromatography on silica gel (eluent–EtOAc/Hex 1/9 *v/v*). Yellow amorphous material; yield 56% (256 mg); R_f = 0.34 (EtOAc/Hex 1/6 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.07 (dd, *J* = 7.7, 1.9 Hz, 2H, C-Ph 2,6-H), 7.87 (d, *J* = 2.7 Hz, 2H, Pyr 5-H, C-Ph 4-H), 7.67 (d, *J* = 8.0 Hz, 1H, Ind 7′-H), 7.63 (d, *J* = 7.4 Hz, 2H, N-Ph 2,6-H), 7.52 (dd, *J* = 8.0, 1.6 Hz, 1H, Ind 6′-H), 7.50–7.44 (m, 3H, Ar-H, Ind 4′-H, C-Ph 3,5-H), 7.44–7.39 (m, 2H, N-Ph 3,5-H), 7.23–7.17 (m, 2H, Pyr–CH=CH–Ind, N-Ph 4-H), 6.94 (d, *J* = 16.3 Hz, 1H, Pyr–CH=CH–Ind), 4.14 (s, 3H, –OCH_3), 2.32 (td, *J* = 13.5, 4.5 Hz, 2H, CHex 2,6-H), 2.13–2.03 (m, 3H, CHex 3,4,5-H), 1.90–1.83 (m, 2H, CHex 3,5-H), 1.59–1.50 (m, 1H, CHex 4-H), 1.48 (d, *J* = 14.9 Hz, 2H, CHex 2,6-H). ^{13}C NMR (176 MHz, $CDCl_3$): δ 183.5 (Ind C-2′), 162.5 (Pyr C-3), 153.2 (Ind C-7a′), 146.9 (Ind C-3a′), 139.9 (N-Ph C-1), 135.1 (Ind C-5′), 133.9 (C-Ph C-1), 129.9 (C-Ph C-4), 129.4 (N-Ph C-3,5), 128.6 (C-Ph C-2,6), 128.4 (C-Ph C-3,5), 128.2 (Pyr–CH=CH–Ind), 125.5 (Ind C-6′), 125.2 (N-Ph C-4), 125.1 (Pyr C-5), 122.1 (C-Ph C-4), 121.3 (Ind C-7′), 117.5 (N-Ph C-2,6), 116.6 (Pyr–CH=CH–Ind), 108.5 (Pyr C-4), 58.3 (CHex C-1,3′), 56.3 (–OCH$_3$), 31.2 (CHex C-2,6), 25.2 (CHex C-4), 21.8 (CHex C-3,5). ^{15}N NMR (71 MHz, $CDCl_3$): δ −68.1 (Ind N-1), −120.0 (Pyr N-2), −189.0 (Pyr N-1). IR (ν_{max}, cm^{-1}): 3050, 2939, 2864, 2814 (CH_{arom}, CH_{aliph}), 1638, 1598, 1566, 1502, 1460, 1403, 1247, 1229, 1200, 1054, 1014, 961, 941, 821, 752 (C=C, CH_3, CH_2 bending, C–O, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{31}H_{30}N_3O$, 460.2383; found, 460.2383.

2-(4-Fluorophenyl)-5-[(*E*)-2-(3-methoxy-1-phenyl-1*H*-pyrazol-4-yl)ethenyl]-3,3-dimethyl-3*H*-indole (**8d**): Synthesized according to the General procedure V from 4-ethenyl-3-methoxy-1-phenyl-1*H*-pyrazole (**7a**) (200 mg, 1 mmol) and 5-bromo-2-(4-fluorophenyl)-3,3-dimethyl-3*H*-indole (**3b**) (398 mg, 1.25 mmol) using TBAC (416 mg) and 10 mol% of palladium(II) acetate. The reaction was conducted for 24 h. Column chromatography on silica gel (eluent–EtOAc/Hex 1/6 *v/v*). Yellow amorphous material; yield 40% (175 mg); R_f = 0.39 (EtOAc/Hex 1/6 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.15 (dd, *J* = 8.9, 5.4 Hz, 2H, C-Ph 2,6-H), 7.83 (s, 1H, Pyr 5-H), 7.64–7.58 (m, 3H, N-Ph 2,6-H, Ind 7-H), 7.45–7.43 (m, 2H, Ind 4,6-H), 7.41–7.38 (m, 2H, N-Ph 3,5-H), 7.21–7.12 (m, 4H, C-Ph 3,5-H, N-Ph 4-H, Pyr–CH=CH–Ind), 6.95 (d, *J* = 16.4 Hz, 1H, Pyr–CH=CH–Ind), 4.11 (s, 3H, –OCH_3), 1.58 (s, 6H, Ind 3-(CH_3)$_2$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 181.6 (Ind C-2), 164.1 (d, $^1J_{CF}$ = 251.8 Hz, C-Ph C-4), 162.5 (Pyr C-3), 152.3 (Ind C-7a), 148.0 (Ind C-3a), 139.8 (N-Ph C-1), 136.1 (Ind C-5), 130.3 (d, $^3J_{CF}$ = 8.4 Hz, C-Ph C-2,6), 129.6 (d, $^4J_{CF}$ = 3.3 Hz, C-Ph C-1), 129.4 (N-Ph C-3,5), 127.8 (Pyr–CH=CH–Ind), 126.1 (Ind C-6), 125.2 (N-Ph C-4), 125.0 (Pyr C-5), 120.8 (Ind C-7), 118.2 (Ind C-4), 117.5 (N-Ph C-2,6), 116.6 (Pyr–CH=CH–Ind), 115.7 (d, $^2J_{CF}$ = 21.5 Hz, C-Ph C-3,5), 108.4 (Pyr C-4), 56.3 (–OCH$_3$), 53.2 (Ind C-3), 24.8 (Ind 3-(CH_3)$_2$). ^{15}N NMR (71 MHz, $CDCl_3$): δ −70.8 (Ind N-1), −120.1 (Pyr N-2), −188.9 (Pyr N-1). ^{19}F NMR (376 MHz, $CDCl_3$): δ −109.4. IR (ν_{max}, cm^{-1}): 3047, 2977, 2929, 2868 (CH_{arom}, CH_{aliph}), 1639, 1600, 1570, 1502, 1463, 1403, 1218, 1151, 1055, 1002, 966, 940, 839, 821, 750 (C=C, CH_3 bending, C–O, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{28}H_{25}FN_3O$, 438.1976; found, 438.1976.

5-{(*E*)-2-[3-(2-Methoxyethoxy)-1-phenyl-1*H*-pyrazol-4-yl] ethenyl}-3,3-dimethyl-2-phenyl-3*H*-indole (**8e**): Synthesized according to the General procedure V from 4-ethenyl-3-(2-methoxyethoxy)-1-phenyl-1*H*-pyrazole (**7c**) (244 mg, 1 mmol) and 5-bromo-3,3-dimethyl-2-phenyl-3*H*-indole (**3a**) (439 mg, 1.25 mmol) using TBAI (554 mg) and 20 mol% of palladium(II) acetate. The reaction was conducted for 48 h. Column chromatography on silica gel (eluent–EtOAc/Hex 1/6 *v/v*). Yellow-orange amorphous material; yield 52% (242 mg); R_f = 0.36 (EtOAc/Hex 1/3 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.15 (dd, *J* = 7.6, 2.0 Hz, 2H, C-Ph 2,6-H), 7.87 (s, 1H, Pyr 5-H), 7.64 (d, *J* = 7.9 Hz, 1H, Ind 7-H), 7.62–7.59 (m, 2H, N-Ph 2,6-H), 7.51–7.44 (m, 4H, Ind 4-H, C-Ph 3,4,5-H), 7.45–7.39 (m, 3H, Ind 6-H, N-Ph 3,4,5-H), 7.20 (t, *J* = 7.4 Hz, 1H, N-Ph 4-H), 7.17 (d, *J* = 16.3 Hz, 1H, Pyr–CH=CH–Ind), 6.98 (d, *J* = 16.4 Hz, 1H, Pyr–CH=CH–Ind), 4.61–4.54 (m, 2H, $CH_3OCH_2CH_2O$–), 3.90–3.86 (m, 2H, $CH_3OCH_2CH_2O$–), 3.51 (s, 3H, –OCH_3), 1.63 (s, 6H, Ind 3-(CH_3)$_2$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 182.9 (Ind C-2), 161.8 (Pyr C-3), 152.5 (Ind C-7a), 148.2 (Ind C-3a), 139.9 (N-Ph C-1), 136.1 (Ind C-5), 133.3 (C-Ph C-1), 130.5 (C-Ph C-4), 129.4 (N-Ph C-3,5), 128.6 (C-Ph C-3,5), 128.3 (C-Ph C-2,6), 127.9 (Pyr–CH=CH–Ind), 126.3 (Ind C-6), 125.3 (N-Ph C-4), 124.7 (Pyr C-5), 120.9 (Ind C-7), 118.1 (Ind C-4), 117.5 (N-Ph C-2,6), 116.5 (Pyr–CH=CH–Ind), 108.7 (Pyr C-4),

71.0 ($CH_3O\underline{C}H_2CH_2O$–), 68.3 ($CH_3OCH_2\underline{C}H_2O$–), 59.2 (–$O\underline{C}H_3$), 53.4 (Ind C-3), 24.9 (Ind 3-($\underline{C}H_3)_2$). ^{15}N NMR (71 MHz, $CDCl_3$): δ −70.4 (Ind N-1), −119.6 (Pyr N-2), −188.6 (Pyr N-1). IR (ν_{max}, cm^{-1}): 3050, 2964, 2927, 2878, 2815 (CH_{arom}, CH_{aliph}), 1638, 1598, 1566, 1500, 1461, 1445, 1407, 1355, 1250, 1236, 1199, 1126, 1043, 962, 942, 919, 821, 776, 753, 690 (C=C, CH_3, CH_2 bending, C–O, C–N, C–H_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{30}H_{30}N_3O_2$, 464.2333; found, 464.2334.

5-[(*E*)-2-(3-Butoxy-1-phenyl-1*H*-pyrazol-4-yl)ethenyl]-3,3-dimethyl-2-phenyl-3*H*-indole (**8f**): Synthesized according to the General procedure V from 3-butoxy-4-ethenyl-1-phenyl-1*H*-pyrazole (**7b**) (240 mg, 1 mmol) and 5-bromo-3,3-dimethyl-2-phenyl-3*H*-indole (**3a**) (375 mg, 1.25 mmol) using TBAC (416 mg) and 10 mol% of palladium(II) acetate. The reaction was conducted for 24 h. Column chromatography on silica gel (eluent–EtOAc/Hex 1/9 *v/v*). Yellow-orange amorphous material; yield 65% (297 mg); R_f = 0.34 (EtOAc/Hex 1/6 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.15 (dd, *J* = 7.6, 2.1 Hz, 2H, C-Ph 2,6-H), 7.84 (s, 1H, Pyr 5-H), 7.65 (d, *J* = 7.8 Hz, 1H, Ind 7-H), 7.63–7.59 (m, 2H, N-Ph 2,6-H), 7.51–7.42 (m, 5H, C-Ph 3,4,5-H, Ind 4,6-H), 7.43–7.38 (m, 2H, N-Ph 3,5-H), 7.22–7.17 (m, 2H, Pyr–CH=C<u>H</u>–Ind, N-Ph 4-H), 6.96 (d, *J* = 16.3 Hz, 1H, Pyr–C<u>H</u>=CH–Ind), 4.42 (t, *J* = 6.6 Hz, 2H, $CH_3CH_2CH_2C\underline{H}_2O$–), 1.93–1.86 (m, 2H, $CH_3CH_2\underline{C}H_2CH_2O$–), 1.63 (s, 6H, Ind 3-($\underline{C}H_3)_2$), 1.58 (h, *J* = 7.4 Hz, 2H, $CH_3\underline{C}H_2CH_2CH_2O$–), 1.04 (t, *J* = 7.4 Hz, 3H, $\underline{C}H_3CH_2CH_2CH_2O$–). ^{13}C NMR (176 MHz, $CDCl_3$): δ 182.9 (Ind C-2), 162.2 (Pyr C-3), 152.4 (Ind C-7a), 148.2 (Ind C-3a), 139.9 (N-Ph C-1), 136.2 (Ind C-5), 133.3 (C-Ph C-1), 130.5 (C-Ph C-4), 129.4 (N-Ph C-3,5), 128.6 (C-Ph C-3,5), 128.2 (C-Ph C-2,6), 127.8 (Pyr–CH=<u>C</u>H–Ind), 126.2 (Ind C-6), 125.1 (N-Ph C-4), 124.8 (Pyr C-5), 120.9 (Ind C-7), 118.0 (Ind C-4), 117.5 (N-Ph C-2,6), 116.8 (Pyr–<u>C</u>H=CH–Ind), 108.6 (Pyr C-4), 68.9 ($CH_3CH_2CH_2\underline{C}H_2O$–), 53.4 (Ind C-3), 31.3 ($CH_3CH_2\underline{C}H_2CH_2O$–), 24.9 (Ind 3-($\underline{C}H_3)_2$), 19.3 ($CH_3\underline{C}H_2CH_2CH_2O$–), 13.9 ($\underline{C}H_3CH_2CH_2CH_2O$–). ^{15}N NMR (71 MHz, $CDCl_3$): δ −70.3 (Ind N-1), −119.6 (Pyr N-2), −189.0 (Pyr N-1). IR (ν_{max}, cm^{-1}): 3050, 3004, 2958, 2930, 2871 (CH_{arom}, CH_{aliph}), 1737, 1639, 1599, 1565, 1501, 1462, 1407, 1374, 1248, 1238, 1226, 1200, 1053, 961, 939, 821, 775, 752 (C=C, CH_3, CH_2 bending, C–O, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{31}H_{32}N_3O$, 462.2540; found, 462.2540.

5-{(*E*)-2-[3-Methoxy-1-(4-methoxyphenyl)-1*H*-pyrazol-4-yl]ethenyl}-3,3-dimethyl-2-phenyl-3*H*-indole (**8g**): Synthesized according to the General procedure V from 4-ethenyl-3-methoxy-1-(4-methoxyphenyl)-1*H*-pyrazole (**7d**) (231 mg, 1 mmol) and 5-bromo-3,3-dimethyl-2-phenyl-3*H*-indole (**3a**) (375 mg, 1.25 mmol) using TBAI (554 mg) and 20 mol% of palladium(II) acetate. The reaction was conducted for 48 h. Column chromatography on silica gel (eluent–EtOAc/Hex 1/6 *v/v*). Yellow amorphous material; yield 54% (242 mg); R_f = 0.27 (EtOAc/Hex 1/3 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.15 (dd, *J* = 7.6, 2.1 Hz, 2H, C-Ph 2,6-H), 7.76 (s, 1H, Pyr 5-H), 7.63 (d, *J* = 7.9 Hz, 1H, Ind 7-H), 7.55–7.51 (m, 2H, N-Ph 2,6-H), 7.52–7.42 (m, 5H, Ind 4,6-H, C-Ph 3,4,5-H), 7.14 (d, *J* = 16.3 Hz, 1H, Pyr–CH=C<u>H</u>–Ind), 6.98–6.93 (m, 3H, N-Ph 3,5-H, Pyr–C<u>H</u>=CH–Ind), 4.11 (s, 3H, Pyr 3-O<u>C</u>H_3), 3.82 (s, 3H, N-Ph 4-O<u>C</u>H_3), 1.63 (s, 6H, Ind 3-($\underline{C}H_3)_2$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 182.9 (Ind C-2), 162.3 (Pyr C-3), 157.5 (N-Ph C-4), 152.4 (Ind C-7a), 148.2 (Ind C-3a), 136.2 (Ind C-5), 133.8 (N-Ph C-1), 133.3 (C-Ph C-1), 130.4 (C-Ph C-4), 128.6 (C-Ph C-3,5), 128.3 (C-Ph C-2,6), 127.4 (Pyr–CH=<u>C</u>H–Ind), 126.0 (Ind C-6), 125.1 (Pyr C-5), 120.9 (Ind C-7), 119.4 (N-Ph C-2,6), 118.1 (Ind C-4), 116.7 (Pyr–<u>C</u>H=CH–Ind), 114.6 (N-Ph C-3,5), 107.8 (Pyr C-4), 56.3 (Pyr 3-O<u>C</u>H_3), 55.6 (N-Ph 4-O<u>C</u>H_3), 53.4 (Ind C-3), 24.9 (Ind 3-($\underline{C}H_3)_2$). ^{15}N NMR (71 MHz, $CDCl_3$): δ −70.4 (Ind N-1), −119.2 (Pyr N-2), −189.3 (Pyr N-1). IR (ν_{max}, cm^{-1}): 3054, 3002, 2963, 2930, 2865, 2834 (CH_{arom}, CH_{aliph}), 1636, 1564, 1510, 1460, 1443, 1412, 1301, 1243, 1171, 1055, 1030, 1014, 963, 943, 823, 775 (C=C, CH_3 bending, C–O, C–N, C–H_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{29}H_{28}N_3O_2$, 450.2176; found, 450.2176.

5-{(*E*)-2-[3-(2-Methoxyethoxy)-1-(4-methoxyphenyl)-1*H*-pyrazol-4-yl]ethenyl}-3,3-dimethyl-2-phenyl-3*H*-indole (**8h**): Synthesized according to the General procedure V from 4-ethenyl-3-(2-methoxyethoxy)-1-(4-methoxyphenyl)-1*H*-pyrazole (**7e**) (274 mg, 1 mmol) and 5-bromo-3,3-dimethyl-2-phenyl-3*H*-indole (**3a**) (375 mg, 1.25 mmol) using TBAI (554 mg) and 20 mol% of palladium (II) acetate. The reaction was conducted for 48 h. Column chromatography on silica gel (eluent–EtOAc/Hex 1/3 *v/v*). Yellow-orange amorphous material; yield 45% (222 mg); R_f = 0.20 (EtOAc/Hex 1/3 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.15 (dd, *J* = 7.5, 2.2 Hz, 2H, C-Ph 2,6-H), 7.77 (s, 1H, Pyr 5-H), 7.63 (d, *J* = 8.0 Hz, 1H, Ind 7-H), 7.54–7.45 (m, 6H, N-Ph 2,6-H, Ind 4-H, C-Ph 3,4,5-H), 7.42 (dd, *J* = 8.0, 1.7 Hz, 1H, Ind 6-H), 7.13 (d, *J* = 16.3 Hz, 1H, Pyr–CH=C<u>H</u>–Ind), 6.97 (d, *J* = 16.4 Hz, 1H, Pyr–C<u>H</u>=CH–Ind), 6.96–6.94 (m, 2H, N-Ph 3,5-H), 4.59–4.53 (m, 2H, $CH_3OCH_2C\underline{H}_2O$–), 3.89–3.85 (m, 2H, $CH_3OC\underline{H}_2CH_2O$–), 3.83 (s, 3H, N-Ph 4-O<u>C</u>H_3), 3.51 (s, 3H, $C\underline{H}_3OCH_2CH_2O$-), 1.63 (s, 6H, Ind 3-($\underline{C}H_3)_2$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 182.8 (Ind C-2), 161.5 (Pyr C-3), 157.5 (N-Ph C-4), 152.4 (Ind C-7a), 148.2 (Ind C-3a), 136.2 (Ind C-5), 133.8 (N-Ph C-1), 133.4 (C-Ph C-1), 130.4 (C-Ph C-4), 128.6 (C-Ph C-3,5), 128.3 (C-Ph C-2,6), 127.4 (Pyr–CH=<u>C</u>H–Ind), 126.2 (Ind C-6), 124.8 (Pyr C-5), 120.9 (Ind C-7), 119.4 (N-Ph C-2,6), 118.0 (Ind C-4), 116.7 (Pyr–<u>C</u>H=CH–Ind), 114.5 (N-Ph C-3,5), 108.0 (Pyr C-4), 71.1 ($CH_3O\underline{C}H_2CH_2O$–), 68.3 ($CH_3OCH_2\underline{C}H_2O$–), 59.2 ($\underline{C}H_3OCH_2$-$CH_2O$–), 55.6 (N-Ph 4-O<u>C</u>H_3), 53.4 (Ind C-3), 24.9 (Ind 3-($\underline{C}H_3)_2$). ^{15}N NMR (71 MHz, $CDCl_3$): δ −70.4 (Ind N-1), −118.6 (Pyr N-2), −188.9 (Pyr N-1). IR (ν_{max}, cm^{-1}): 3055, 2962, 2928, 2881, 2834, 2815 (CH_{arom}, CH_{aliph}), 1637, 1563, 1512, 1461, 1444, 1405, 1387, 1355, 1344, 1301, 1244, 1198, 1172, 1126, 1043, 1030, 963, 945, 919, 824, 776, 695 (C=C, CH_3, CH_2 bending, C–O, C–N, C–H_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{31}H_{32}N_3O_3$, 494.2438; found, 494.2440.

5-{(*E*)-2-[1-(4-Fluorophenyl)-3-methoxy-1*H*-pyrazol-4-yl]ethenyl}-3,3-dimethyl-2-phenyl-3*H*-indole (**8i**): Synthesized according to the General procedure V from 4-ethenyl-1-(4-fluorophenyl)-3-methoxy-1*H*-pyrazole (**7f**) (217 mg, 1 mmol) and 5-bromo-3,3-dimethyl-2-phenyl-3*H*-indole (**3a**) (375 mg, 1.25 mmol) using TBAC (416 mg) and 10 mol% of palladium(II) acetate. The reaction was conducted for 24 h. Column chromatography on silica gel

(eluent–EtOAc/Hex 1/6 *v/v*). Yellow amorphous material; yield 65% (282 mg); R_f = 0.34 (EtOAc/Hex 1/6 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.15 (dd, *J* = 7.4, 2.4 Hz, 2H, C-Ph 2,6-H), 7.78 (s, 1H, Pyr 5-H), 7.64 (d, *J* = 7.9 Hz, 1H, Ind 7-H), 7.60–7.55 (m, 2H, N-Ph 2,6-H), 7.51–7.47 (m, 3H, C-Ph 3,4,5-H), 7.46–7.43 (m, 2H, Ind 4,6-H), 7.16 (d, *J* = 16.3 Hz, 1H, Pyr–CH=CH–Ind), 7.15–7.09 (m, 2H, N-Ph 3,5-H), 6.95 (d, *J* = 16.3 Hz, 1H, Pyr–CH=CH–Ind), 4.11 (s, 3H, -OCH$_3$), 1.62 (s, 6H, Ind 3-(CH$_3$)$_2$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 182.9 (Ind C-2), 162.5 (Pyr C-3), 160.4 (d, $^1J_{CF}$ = 244.6 Hz, N-Ph C-4), 152.5 (Ind C-7a), 148.2 (Ind C-3a), 136.3 (d, $^4J_{CF}$ = 2.9 Hz, N-Ph C-1), 136.0 (Ind C-5), 133.3 (C-Ph C-1), 130.5 (C-Ph C-4), 128.6 (C-Ph C-3,5), 128.3 (C-Ph C-2,6), 128.0 (Pyr–CH=CH–Ind), 126.1 (Ind C-6), 125.1 (Pyr C-5), 120.9 (Ind C-7), 119.2 (d, $^3J_{CF}$ = 8.3 Hz, N-Ph C-2, C-6), 118.2 (Ind C-4), 116.4 (Pyr–CH=CH–Ind), 116.1 (d, $^2J_{CF}$ = 23.1 Hz, N-Ph C-3, C-5), 108.5 (Pyr C-4), 56.3 (–OCH$_3$), 53.4 (Ind C-3), 24.9 (Ind 3-(CH$_3$)$_2$). ^{15}N NMR (71 MHz, $CDCl_3$): δ −70.4 (Ind N-1), −119.6 (Pyr N-2), −190.8 (Pyr N-1). ^{19}F NMR (376 MHz, $CDCl_3$): δ −117.5. IR (ν_{max}, cm^{-1}): 3095, 2984, 2944, 2868 (CH_{arom}, CH_{aliph}), 1638, 1569, 1505, 1461, 1442, 1413, 1404, 1241, 1212, 1023, 966, 944, 837, 823, 778 (C=C, CH_3 bending, C–O, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{28}H_{25}FN_3O$, 438.1976; found, 438.1976.

2-(4-Methoxyphenyl)-5-[(*E*)-2-(3-methoxy-1-phenyl-1*H*-pyrazol-4-yl)ethenyl]-3,3-dimethyl-3*H*-indole (**8j**): Synthesized according to the General procedure V from 4-ethenyl-3-methoxy-1-phenyl-1*H*-pyrazole (**7a**) (200 mg, 1 mmol) and 5-bromo-2-(4-methoxyphenyl)-3,3-dimethyl-3*H*-indole (**3c**) (413 mg, 1.25 mmol) using TBAC (416 mg) and 10 mol% of palladium(II) acetate. The reaction was conducted for 24 h. Column chromatography on silica gel (eluent–EtOAc/Hex 1/6 to 1/4 *v/v*). Yellow solid; yield 57% (254 mg); m.p. = 203–204°C; R_f = 0.26 (EtOAc/Hex 1/4 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.14 (d, *J* = 8.9 Hz, 2H, C-Ph 2,6-H), 7.85 (s, 1H, Pyr 5-H), 7.64–7.60 (m, 2H, N-Ph 2,6-H), 7.60 (d, *J* = 7.8 Hz, 1H, Ind 7-H), 7.46–7.40 (m, 4H, N-Ph 3,5-H, Ind 4,6-H), 7.23–7.18 (m, 1H, N-Ph 4-H), 7.17 (d, *J* = 16.3 Hz, 1H, Pyr–CH=CH–Ind), 7.01–6.98 (m, 2H, C-Ph 3,5-H), 6.95 (d, *J* = 16.3 Hz, 1H, Pyr–CH=CH–Ind), 4.12 (s, 3H, Pyr 3-OCH$_3$), 3.87 (s, 3H, C-Ph 4-OCH$_3$), 1.61 (s, 6H, Ind 3-(CH$_3$)$_2$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 182.4 (Ind C-2), 162.5 (Pyr C-3), 161.5 (C-Ph C-4), 152.7 (Ind C-7a), 148.1 (Ind C-3a), 139.9 (N-Ph C-1), 135.6 (Ind C-5), 130.0 (C-Ph C-2,6), 129.4 (N-Ph C-3,5), 128.0 (Pyr–CH=CH–Ind), 126.1 (Ind C-6), 126.0 (C-Ph C-1), 125.2 (N-Ph C-4), 124.9 (Pyr C-5), 120.4 (Ind C-7), 118.1 (Ind C-4), 117.5 (N-Ph C-2,6), 116.3 (Pyr–CH=CH–Ind), 114.0 (C-Ph C-3,5), 108.5 (Pyr C-4), 56.3 (Pyr 3-OCH$_3$), 55.4 (C-Ph 4-OCH$_3$), 53.1 (Ind C-3), 25.1 (Ind 3-(CH$_3$)$_2$). ^{15}N NMR (71 MHz, $CDCl_3$): δ −76.9 (Ind N-1), −120.3 (Pyr N-2), −189.1 (Pyr N-1). IR (ν_{max}, cm^{-1}): 3004, 2981, 2966, 2944, 2928, 2865, 2839 (CH_{arom}, CH_{aliph}), 1633, 1596, 1567, 1500, 1456, 1415, 1396, 1304, 1246, 1204, 1183, 1161, 1053, 1029, 1011, 970, 938, 893, 838, 824, 751 (C=C, CH_3 bending, C–O, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{29}H_{28}N_3O_2$, 450.2176; found, 450.2178.

5-{(*E*)-2-[1-(4-Fluorophenyl)-3-methoxy-1*H*-pyrazol-4-yl]ethenyl}-2-(4-methoxyphenyl)-3,3-dimethyl-3*H*-indole (**8k**): Synthesized according to the General procedure V from 4-ethenyl-1-(4-fluorophenyl)-3-methoxy-1*H*-pyrazole (**7f**) (218 mg, 1 mmol) and 5-bromo-2-(4-methoxyphenyl)-3,3-dimethyl-3*H*-indole (**3c**) (413 mg, 1.25 mmol) using TBAC (416 mg) and 10 mol% of palladium(II) acetate. The reaction was conducted for 24 h. Column chromatography on silica gel (eluent–EtOAc/Hex 1/4 *v/v*). Yellow solid; yield 44% (205 mg); m.p. = 190–191°C; R_f = 0.23 (EtOAc/Hex 1/4 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.14 (d, *J* = 8.9 Hz, 2H, C-Ph 2,6-H), 7.78 (s, 1H, Pyr 5-H), 7.61–7.55 (m, 3H, Ind 7-H, N-Ph 2,6-H), 7.45–7.41 (m, 2H, Ind 4,6-H), 7.16 (d, *J* = 16.3 Hz, 1H, Pyr–CH=CH–Ind), 7.14–7.09 (m, 2H, N-Ph 3,5-H), 7.00 (d, *J* = 8.9 Hz, 2H, C-Ph 3,5-H), 6.93 (d, *J* = 16.3 Hz, 1H, Pyr–CH=CH–Ind), 4.11 (s, 3H, Pyr 3-OCH$_3$), 3.88 (s, 3H, C-Ph 4-OCH$_3$), 1.61 (s, 6H, Ind 3-(CH$_3$)$_2$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 182.4 (Ind C-2), 162.5 (Pyr C-3), 161.5 (C-Ph C-4), 160.4 (d, $^1J_{CF}$ = 244.5 Hz, N-Ph C-4), 152.7 (Ind C-7a), 148.1 (Ind C-3a), 136.3 (d, $^4J_{CF}$ = 2.7 Hz, N-Ph C-1), 135.5 (Ind C-5), 130.0 (C-Ph C-2,6), 128.1 (Pyr–CH=CH–Ind), 126.1 (Ind C-6), 126.0 (C-Ph C-1), 125.0 (Pyr C-5), 120.4 (Ind C-7), 119.2 (d, $^3J_{CF}$ = 8.0 Hz, N-Ph C-2,6), 118.1 (Ind C-4), 116.1 (d, $^2J_{CF}$ = 23.0 Hz, N-Ph C-3,5), 116.09 (Pyr–CH=CH–Ind), 114.0 (C-Ph C-3,5), 108.6 (Pyr C-4), 56.3 (Pyr 3-OCH$_3$), 55.4 (C-Ph 4-OCH$_3$), 53.1 (Ind C-3), 25.1 (Ind 3-(CH$_3$)$_2$. ^{15}N NMR (71 MHz, $CDCl_3$): δ −77.2 (Ind N-1), −119.7 (Pyr N-2), −190.9 (Pyr N-1). ^{19}F NMR (376 MHz, $CDCl_3$): δ −117.6. IR (ν_{max}, cm^{-1}): 2986, 2966, 2953, 2928, 2838 (CH_{arom}, CH_{aliph}), 1633, 1599, 1567, 1506, 1456, 1415, 1395, 1304, 1245, 1182, 1161, 1029, 1007, 969, 937, 834, 825, 765 (C=C, CH_3 bending, C–O, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{29}H_{27}FN_3O_2$, 468.2082; found, 468.2085.

5-{(*E*)-2-[3-Methoxy-1-(4-methoxyphenyl)-1*H*-pyrazol-4-yl]ethenyl}-2-(4-methoxyphenyl)-3,3-dimethyl-3*H*-indole (**8l**): Synthesized according to the General procedure V from 4-ethenyl-3-methoxy-1-(4-methoxyphenyl)-1*H*-pyrazole (**7d**) (230 mg, 1 mmol) and 5-bromo-2-(4-methoxyphenyl)-3,3-dimethyl-3*H*-indole (**3c**) (413 mg, 1.25 mmol) using TBAC (416 mg) and 10 mol% of palladium(II) acetate. The reaction was conducted for 24 h. Column chromatography on silica gel (eluent–EtOAc/Hex 1/4 to 1/2 *v/v*). Yellow solid; yield 47% (225 mg); m.p. = 207–209°C; R_f = 0.15 (EtOAc/Hex 1/4 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.14 (d, *J* = 8.9 Hz, 2H, C-Ph 2,6-H), 7.75 (s, 1H, Pyr 5-H), 7.59 (d, *J* = 7.9 Hz, 1H, Ind 7-H), 7.54–7.50 (m, 2H, N-Ph 2,6-H), 7.46–7.40 (m, 2H, Ind 4,6-H), 7.13 (d, *J* = 16.3 Hz, 1H, Pyr–CH=CH–Ind), 7.03–6.91 (m, 5H, Pyr–CH=CH–Ind, C-Ph 3,5-H, N-Ph 3,5-H), 4.11 (s, 3H, Pyr 3-OCH$_3$), 3.88 (s, 3H, C-Ph 4-OCH$_3$), 3.83 (s, 3H, N-Ph 4-OCH$_3$), 1.61 (s, 6H, Ind 3-(CH$_3$)$_2$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 182.3 (Ind C-2), 162.3 (Pyr C-3), 161.5 (C-Ph C-4), 157.5 (N-Ph C-4), 152.6 (Ind C-7a), 148.1 (Ind C-3a), 135.7 (Ind C-5), 133.8 (C-Ph C-1, N-Ph C-1), 130.0 (C-Ph C-2,6), 127.5 (Pyr–CH=CH–Ind), 126.0 (Ind C-6), 125.1 (Pyr C-5), 120.4 (Ind C-7), 119.4 (N-Ph C-2,6), 118.0 (Ind C-4), 116.4 (Pyr–CH=CH–Ind), 114.5 (N-Ph C-3,5), 114.0 (C-Ph C-3,5), 107.8 (Pyr C-4), 56.3 (Pyr 3-OCH$_3$), 55.6 (N-Ph 4-OCH$_3$), 55.4 (C-Ph 4-OCH$_3$), 53.1 (Ind C-3), 25.1 (Ind 3-(CH$_3$)$_2$). ^{15}N NMR (71 MHz, $CDCl_3$): δ −76.9 (Ind N-1), −119.3 (Pyr N-2), −189.5 (Pyr N-1). IR (ν_{max}, cm^{-1}): 3000, 2971, 2931, 2837 (CH_{arom}, CH_{aliph}), 1633, 1599, 1566, 1509, 1456, 1440, 1416, 1398, 1302, 1242, 1178,

1160, 1029, 972, 940, 839, 830, 822, 711 (C=C, CH_3 bending, C–O, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{30}H_{30}N_3O_3$, 480.2282; found, 480.2280.

2-(4-Fluorophenyl)-5-{(*E*)-2-[3-methoxy-1-(4-methoxyphenyl)-1*H*-pyrazol-4-yl]ethenyl}-3,3-dimethyl-3*H*-indole (**8m**): Synthesized according to the General procedure V from 4-ethenyl-3-methoxy-1-(4-methoxyphenyl)-1*H*-pyrazole (**7d**) (230 mg, 1 mmol) and 5-bromo-2-(4-fluorophenyl)-3,3-dimethyl-3*H*-indole (**3b**) (398 mg, 1.25 mmol) using TBAC (416 mg) and 10 mol% of palladium(II) acetate. The reaction was conducted for 24 h. Column chromatography on silica gel (eluent–EtOAc/Hex 1/4 *v/v*). Yellow amorphous material; yield 61% (282 mg); R_f = 0.36 (EtOAc/Hex 1/4 *v/v*). ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.18–8.13 (m, 2H, C-Ph 2,6-H), 7.76 (s, 1H, Pyr 5-H), 7.61 (d, *J* = 7.9 Hz, 1H, Ind 7-H), 7.55–7.51 (m, 2H, N-Ph 2,6-H), 7.46–7.42 (m, 2H, Ind 4,6-H), 7.20–7.14 (m, 2H, C-Ph 3,5-H), 7.14 (d, *J* = 16.3 Hz, 1H, Pyr–CH=CH–Ind), 6.98–6.93 (m, 3H, Pyr–CH=CH–Ind, N-Ph 3,5-H), 4.11 (s, 3H, Pyr 3-OCH_3), 3.84 (s, 3H, N-Ph 4-OCH_3), 1.60 (s, 6H, Ind 3-(CH_3)$_2$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 181.6 (Ind C-2), 164.1 (d, $^1J_{CF}$ = 252.0 Hz, C-Ph C-4), 162.3 (Pyr C-3), 157.5 (N-Ph C-4), 152.3 (Ind C-7a), 148.0 (Ind C-3a), 136.2 (Ind C-5), 133.8 (N-Ph C-1), 130.3 (d, $^3J_{CF}$ = 8.4 Hz, C-Ph C-2,6), 129.6 (d, $^4J_{CF}$ = 3.3 Hz, C-Ph C-1), 127.3 (Pyr–CH=CH–Ind), 126.0 (Ind C-6), 125.1 (Pyr C-5), 120.8 (Ind C-7), 119.4 (N-Ph C-2,6), 118.1 (Ind C-4), 116.8 (Pyr–CH=CH–Ind), 115.7 (d, $^2J_{CF}$ = 21.8 Hz, C-Ph C-3,5), 114.5 (N-Ph C-3,5), 107.7 (Pyr C-4), 56.3 (Pyr 3-OCH_3), 55.6 (N-Ph 4-OCH_3), 53.3 (Ind C-3), 24.9 (Ind 3-(CH_3)$_2$). ^{15}N NMR (71 MHz, $CDCl_3$): δ −71.0 (Ind N-1), −119.2 (Pyr N-2), −189.6 (Pyr N-1). ^{19}F NMR (376 MHz, $CDCl_3$): δ −109.4. IR (ν_{max}, cm^{-1}): 2961, 2932, 2868, 2835 (CH_{arom}, CH_{aliph}), 1736, 1637, 1601, 1565, 1505. 1460, 1444, 1413, 1301, 1240, 1154, 1013. 964. 943. 824. 712 (C=C, CH_3 bending, C–O, C–N, CH_{arom} oop bending). HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{29}H_{27}FN_3O_2$, 468.2082; found, 468.2083.

4.2 | Biological assays

4.2.1 | Cell cultures

G361 (human skin melanoma) and MCF-7 (human breast adenocarcinoma) were obtained from the European Collection of Authenticated Cell Cultures. MCF-7-SSBP1-GFP-HSF1-mCHERRY cell line was kindly gifted by Petr Müller (Masaryk Memorial Cancer Institute, Brno). All cell lines were cultivated in Dulbecco's modified eagle medium without phenol red supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 µg/mL) at 37°C in 5% CO_2 atmosphere.

4.2.2 | Photodynamic treatment

An in-house constructed LED-based light source specifically designed for the irradiation of 96-well microplates and Petri dishes[54] was used; a maximal wavelength emission of 414 nm, light intensity set to 20 mW/cm^2. The total dose of irradiation did not affect the temperature during irradiation. For photodynamic treatments, cells were seeded and the next day treated with test compounds, after 4 h incubation, cells were irradiated and further cultivated.

4.2.3 | Cell viability assay

Cell viability was determined using the MTT (Sigma-Aldrich) assay in 96-well microplates. The test compounds were added 24 h post plating, the cells were then incubated for additional 4 h and irradiated (a maximal wavelength emission of 414 nm and total dose of 10 J/cm^2). After irradiation, the cells were incubated for further 20 or 72 h and then the MTT solution was added. Cells were incubated for another 4 h at 37°C and in 5% CO_2 and then the 10% SDS was added to the wells to solubilize the violet formazan crystals. The measurement of absorbance was carried out on reader Tecan Infinite M200Pro at 570 nm. Dark viability was measured in parallel under the same conditions without irradiation.

4.2.4 | Reactive oxygen species (ROS) production

The ROS production measurement using CM-H_2DCFDA (Invitrogen) fluorescent probe was performed according to the manufacturer's guidelines. Briefly, the MCF-7 cells were plated and the next day labeled with the 5 µM probe (30 min loading time), loading buffer was removed and replaced with prewarmed phosphate-buffered saline (PBS) with 5 mM glucose and for 4 h treated with test compound. After irradiation (2, 6, and 10 J/cm^2), the ROS levels were measured immediately on Tecan Infinite M200Pro at 492/530 nm (ex/em). Negative control samples were established by 10 min pretreatment with *N*-acetylcysteine (5 mM), inhibitor of ROS.

Similarly, the DHR123 (Enzo), SOSG (Molecular Probes), and HPF (Enzo) probes were used. Briefly, the MCF-7 cells were plated and treated with the test compound the following day. After a 4 h incubation, the medium with the test compound was replaced with PBS containing 5 µM probe for 30 min. Then, the labeling solution was discarded and the cells in PBS were irradiated (2, 6, and 10 J/cm^2). ROS levels were measured immediately on Tecan Infinite M200Pro at 490/525 nm (ex/em).

To measure ROS production by **8d** in plain water, solutions of **8d** were mixed with DHR123, SOSG, or HPF probes at a final concentration of 5 µM and measured immediately after irradiation with blue light (10 J/cm^2) on Tecan Infinite M200Pro at 490/525 nm (ex/em). ROS production in samples kept in the dark was measured in parallel under the same conditions without irradiation.

4.2.5 | Immunoblotting

Cell lysates were prepared in radio-immunoprecipitation assay buffer. Proteins were separated on sodium dodecyl sulfate

(SDS)-polyacrylamide gels, electroblotted onto nitrocellulose membranes, and after blocking, overnight incubation with specific primary antibodies and incubation with peroxidase-conjugated secondary antibodies, the peroxidase activity was detected with SuperSignal West Pico reagents (Thermo Scientific) using a CCD camera LAS-4000 (Fujifilm). All primary antibodies were diluted in tris buffered saline containing 4% bovine serum albumin and 0.1% Tween 20. The specific antibodies were purchased from Cell Signaling (anti-PARP-1, clone 46D11; anti-BAX, clone D2E11; anti-HO-1, clone E7U4W; anti-cytochrome C, clone 136F3; anti-HSP60, clone D307; anti-HSP40, clone C64B4; anti-HSP90, clone C45G5; anti-HSF1), Sigma Aldrich (anti-Bcl-2), Millipore (anti-phospho-histone H2A.X, Ser139, clone JBW301) and Santa Cruz Biotechnology (anti-GAPDH, clone 0411).

4.2.6 | Phase contrast and fluorescence microscopy

MCF-7 or MCF-7-SSBP1-GFP-HSF1-mCHERRY cells were seeded into 8-well µSlides (Ibidi), next day treated with a test compound and irradiated with 10 J/cm^2 blue light (414 nm). Samples were analyzed after the indicated incubation period using an inverted fluorescent microscope IX51 (Olympus).

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CONFLICTS OF INTEREST STATEMENT

Patents CZ 309070 and EP 4107145B1 describing indole-pyrazole hybrids as photodynamic agents (with inventors G.V., N.K., A.B., A.Š., V.K. and A.Ž.) are assigned to Palacký University and Kaunas University of Technology.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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