

This is the accepted version of the following article:

*Beatričė Razmienė, Veronika Vojáčková, Eva Řezníčková, Lukáš Malina, Vaida Dambrauskienė, Martin Kubala, Robert Bajgar, Hana Kolářová, Asta Žukauskaitė, Eglė Arbačiauskienė, Algirdas Šačkus, Vladimír Kryštof. Synthesis of N-aryl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-amines and their photodynamic properties in the human skin melanoma cell line G361 // Bioorganic chemistry. ISSN 0045-2068. 2022, vol. 119, art. no. 105570, p. 1-10. DOI: 10.1016/j.bioorg.2021.105570 which has been published in final form at <https://www.sciencedirect.com/science/article/pii/S0045206821009482>.*

1 **Synthesis of N-aryl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-amines and their**  
2 **photodynamic properties in the human skin melanoma cell line G361**

3  
4 Beatričė Razmienė<sup>1,2</sup>, Veronika Vojáčková<sup>3</sup>, Eva Řezníčková<sup>3</sup>, Lukáš Malina<sup>4</sup>, Vaida  
5 Dambrauskienė<sup>1</sup>, Martin Kubala<sup>5</sup>, Robert Bajgar<sup>4</sup>, Hana Kolářová<sup>4</sup>, Asta Žukauskaitė<sup>6\*</sup>, Eglė  
6 Arbačiauskienė<sup>1\*\*</sup>, Algirdas Šačkus<sup>1,2</sup>, Vladimír Kryštof<sup>3</sup>

7  
8 <sup>1</sup> Department of Organic Chemistry, Kaunas University of Technology, Radvilėnų pl. 19, LT-  
9 50254 Kaunas, Lithuania

10 <sup>2</sup> Institute of Synthetic Chemistry, Kaunas University of Technology, K. Baršausko g. 59, LT-  
11 51423, Kaunas, Lithuania

12 <sup>3</sup> Department of Experimental Biology, Faculty of Science, Palacký University, Šlechtitelů 27, CZ-  
13 78371 Olomouc, Czech Republic

14 <sup>4</sup> Department of Medical Biophysics, Faculty of Medicine and Dentistry, Palacký University,  
15 Hněvotínská 3, Olomouc, CZ-77515, Czech Republic

16 <sup>5</sup> Department of Experimental Physics, Faculty of Science, Palacký University, 17. listopadu 12,  
17 CZ-77146 Olomouc, Czech Republic

18 <sup>6</sup> Department of Chemical Biology, Faculty of Science, Palacký University, Šlechtitelů 27, CZ-  
19 78371 Olomouc, Czech Republic

20

21 \* Corresponding author

22 \*\* Corresponding author

23

24 *E-mail address:* asta.zukauskaite@upol.cz (A. Žukauskaitė), egle.arbačiauskiene@ktu.lt (E.  
25 Arbačiauskienė)

26

## 27 **Abstract**

28 A small series of *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines was synthesized from  
29 easily accessible 1-phenyl-1*H*-pyrazol-3-ol *via* 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine  
30 and 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine intermediates and their subsequent  
31 use in palladium catalyzed Buchwald-Hartwig cross-coupling reaction with various anilines.  
32 Majority of the compounds were not significantly cytotoxic to melanoma G361 cells in the dark  
33 up to 10  $\mu\text{M}$  concentration, but their activity could be increased by irradiation with visible blue  
34 light (414 nm). The most active compound **10** possessed  $\text{EC}_{50}$  values of 3.5, 1.6 and 0.9  $\mu\text{M}$  in  
35 cells irradiated with 1, 5 and 10  $\text{J}/\text{cm}^2$ , respectively. The treatment caused generation of reactive  
36 oxygen species in cells and extensive DNA damage, documented by the comet assay and by  
37 detection of phosphorylated histone H2A.X, followed by apoptotic cell death. Our results suggest  
38 that *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines could serve as a potential source of  
39 photosensitizing compounds with anticancer activities.

40

## 41 **Keywords**

42 apoptosis; Buchwald–Hartwig amination; cytotoxicity; DNA damage; photodynamic effect;  
43 pyrazole; reactive oxygen species

44

## 45 **1. Introduction**

46 Photodynamic therapy (PDT) uses by itself non-toxic medication – photosensitizer (PS), which  
47 upon activation by specific light source within target cells produces reactive oxygen species and  
48 causes cell destruction [1]. The main advantages of PDT over traditional treatments include low  
49 systemic toxicity, spatiotemporal selectivity and the possibility of undergoing repeated treatments  
50 without the development of resistance [2,3]. It is thus a promising treatment for various, especially  
51 oncological, diseases [4], including but not limited to skin basal cell carcinoma [5,6], oral [7], lung  
52 [8,9], cervical [10], breast [11], prostate [12], head and neck [13] cancers. Furthermore, it can be  
53 used for treating acne [14], macular degeneration [15], Barrett's esophagus [16], atherosclerosis  
54 [17] or inactivating pathogens [18–22].

55 Despite huge potential of PDT over classical treatments, up to date, only five photosensitizers have  
56 been commercialized and around fifteen more are in clinical trials [4,23]. The most researched  
57 photosensitizers are by far derivatives of porphyrins [24]. Besides them, other types of elongated

58 conjugated systems possessing compounds have also been reported to possess photodynamic  
59 properties, such as cyanines [25–27], BODIPYs [28–30], aza-BODIPYs [31], rhodamines [32,33],  
60 coumarines [34], squaraines [35], etc. While pyrazoles are acknowledged for their diverse variety  
61 of biological activities [36–38], only several studies address their potential use in photodynamic  
62 therapy. Pyrazole-fused porphyrins were prepared by Yang *et al.*, unfortunately, as of now their  
63 properties were not reported [39]. Several pyrazolo[3,4-*h*]quinolones were revealed to possess sub-  
64 micromolar phototoxicity and to photoinduce cell death without DNA damage in cancer cells [40].  
65 Pereira *et al.* developed dihydroxymethyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridine-fused *meso*-  
66 tetraphenylchlorin which showed nanomolar activity against melanoma cells which are typically  
67 resistant to photodynamic therapy [41]. We recently reported strong photodynamic effect in  
68 melanoma G361 cells possessing pyrazole–indole hybrid which induces cell death through the  
69 production of ROS and extensive DNA damage [42].

70 In our previous works, we also investigated synthesis and biological activities of various annulated  
71 pyrazole ring systems such as benzopyrano[2,3-*c*]pyrazol-4(2*H*)-ones [43], 2*H*-furo[2,3-  
72 *c*]pyrazoles [44], 2*H*-pyrazolo[4,3-*c*]pyridines [45,46], pyrazolo[4,3-*f*][1,2,3]triazolo[5,1-  
73 *c*][1,4]oxazepines [47], pyrazolo[4',3':3,4]pyrido[1,2-*a*]benzimidazoles [48], dipyrazolo[1,5-  
74 *a*:4',3'-*c*]pyridines [49], thieno[2,3-*c*]pyrazoles [50], 2,6-dihydropyrano[2,3-*c*]pyrazoles [51],  
75 pyrazolo[4',3':5,6]pyrano[4,3-*c*][1,2]oxazoles [52] and others. In continuation on our research on  
76 pyrazole derivatives, in this work we present synthesis of *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-  
77 *c*]pyridin-7-amines from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines by Buchwald-Hartwig  
78 reaction and investigation of their photodynamic properties in the human skin melanoma cell line  
79 G361.

80

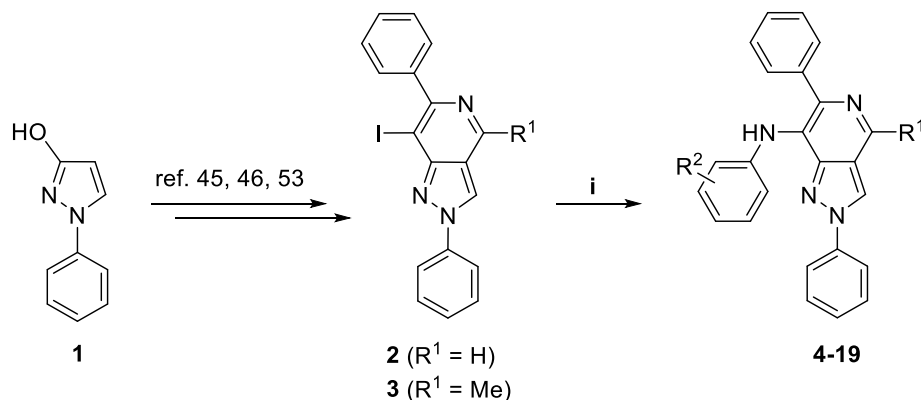
## 81 **2. Results and discussion**

### 82 **2.1. Chemistry**

83 To access a library of various *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines,  
84 intermediate 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines **2-3** were prepared starting from 1-  
85 phenyl-1*H*-pyrazol-3-ol **1** *via* a multi-step synthetic sequence in accordance to our previously  
86 published procedures (Scheme S1, Supplementary File) [45,46,53]. In short, hydroxy group of 1-  
87 phenyl-1*H*-pyrazol-3-ol **1** was protected as a benzyl ether and a formyl group was introduced to  
88 4- position by Vilsmeier–Haack reaction. After debenzylation with TFA, hydroxy group was

89 activated as a triflate and subsequently used in Sonogashira cross-coupling reaction with  
 90 phenylacetylene to form 1-phenyl-3-(2-phenylethynyl)-1*H*-pyrazole-4-carbaldehyde. The  
 91 obtained carbaldehyde was reduced to primary or secondary alcohols using either sodium  
 92 borohydride as a reducing agent or methylmagnesium bromide as a Grignard reagent. The obtained  
 93 alcohols were further converted into azides using TMSN<sub>3</sub> and catalytic amount of boron trifluoride  
 94 diethyl etherate. Lastly, azides were treated with iodine and a proper base to form the pyrazolo[4,3-  
 95 *c*]pyridine core with iodine in 7-position *via* electrophilic substitution reaction.  
 96 Prepared 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **2** and 7-iodo-4-methyl-2,6-diphenyl-  
 97 2*H*-pyrazolo[4,3-*c*]pyridine **3** were subsequently used in palladium catalysed Buchwald-Hartwig  
 98 cross-coupling reaction adopting previously described reaction conditions [54]. 7-  
 99 Iodopyrazolo[4,3-*c*]pyridines **2** and **3** were treated with an appropriate aniline, palladium acetate  
 100 as a catalyst, 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl as a ligand and sodium *tert*-  
 101 butoxide as a base in dioxane. To enhance the rate of the cross-coupling reactions, they were  
 102 performed under microwave irradiation. The compounds **4-19** were obtained from iodo derivatives  
 103 **2-3** in moderate to excellent yields (48-95%) (Table 1). Noteworthy, reactions were carried out  
 104 under argon atmosphere and dry solvent due to the sensitivity of ligand and catalyst to air and  
 105 moisture.

106



107

108 **Scheme 1.** Synthesis of compounds (**4-19**). *Reagents and conditions:* (i) R<sup>2</sup>PhNH<sub>2</sub>, Pd(OAc)<sub>2</sub>, SPhos, NaOtBu,  
 109 dioxane, MW, 120 °C, 280 W, 1 h.

110

111 **Table 1** Yields of new compounds **4-19** prepared from iodo derivatives **2-3**.

compound	R <sup>1</sup>	R <sup>2</sup>	Yield, %
----------	----------------	----------------	----------

<b>4</b>	H	H	92
<b>5</b>	H	4-MeO	48
<b>6</b>	H	3,4-diMeO	86
<b>7</b>	H	3,5-diMeO	60
<b>8</b>	H	2,4-diMeO	63
<b>9</b>	H	3,4,5-triMeO	95
<b>10</b>	H	2-CF <sub>3</sub> O	56
<b>11</b>	H	3-CF <sub>3</sub> O	56
<b>12</b>	Me	H	65
<b>13</b>	Me	4-MeO	77
<b>14</b>	Me	3,4-diMeO	60
<b>15</b>	Me	3,5-diMeO	49
<b>16</b>	Me	2,4-diMeO	73
<b>17</b>	Me	3,4,5-triMeO	63
<b>18</b>	Me	2-CF <sub>3</sub> O	62
<b>19</b>	Me	3-CF <sub>3</sub> O	82

112

113 The structures of compounds **4-19** were confirmed by NMR and IR spectroscopy, MS and HRMS  
 114 spectrometry. The formation of amine can be quickly confirmed by IR spectrum in which peaks  
 115 of amine group can be found at 3204-3404 cm<sup>-1</sup>. Also, in comparison to starting materials **2** and **3**,  
 116 the <sup>1</sup>H NMR spectra of all new derivatives have a broad NH singlet at 5.89-6.46 ppm, appropriate  
 117 signals of the *N*-aryl protons at 5.97-7.21 ppm, while compounds **5-9** and **13-17** additionally have  
 118 singlets of methoxy groups protons in the area of 3.65-3.83 ppm.

119

## 120 **2.2. Optical properties**

121 The fluorescence properties of final compounds **4-19** were briefly assessed in pH 7 Britton–  
 122 Robinson buffer (Figure S1, Supplementary File). The excitation wavelength  $\lambda_{\text{ex}}$  was set to 380  
 123 nm. With an exception of 2,4-dimethoxyphenyl and 3,4,5-trimethoxyphenyl substituents at 7-  
 124 position possessing compounds **8**, **9**, **16** and **17**, which had extremely low fluorescence intensity,  
 125 the emission maxima  $\lambda_{\text{em}}$  of the remaining compounds were located in the 460-540 nm range,

126 which corresponds to the blue part of the visible light spectrum. Even though compounds absorbed  
127 both UV and visible blue light, to minimize negative effects of UV and near-UV light to the cells  
128 [55], a 414 nm emitting LED source was used for photodynamic experiments [56].

129

### 130 2.3. Biology

131 To assess photodynamic potency of the prepared compounds, G361 cells were treated with  
132 compounds for 4 h and then exposed to blue light (LED source, 414 nm), which was selected based  
133 on optical properties of the compounds (Figure S1, Supplementary File), at a total irradiation dose  
134 of 10 J/cm<sup>2</sup>. The viability of the cells was quantified 72 h after irradiation by an MTT assay,  
135 revealing that most of the compounds possess photodynamic properties, with 4-unsubstituted  
136 derivatives **4-11** being typically more active than their 4-methyl counterparts **12-19**. However,  
137 there were few exceptions from this trend; 4-methyl substituted phenylamino and (3,5-  
138 dimethoxyphenyl)amino derivatives **12** and **15** were slightly more active than 4-unsubstituted  
139 homologs **4** and **7**. Compounds **5** and **10**, both of which lack methyl at position **4** and have either  
140 (4-methoxyphenyl)amino or [2-(trifluoromethoxy)phenyl]amino substituents at position **7**,  
141 respectively, were revealed to be the most potent photosensitizers with the lowest EC<sub>50</sub> (1.1 μM).  
142 Noteworthy, with an exception of compound **8**, no dark toxicity was observed up to tested  
143 concentration (10 μM) (Table 1).

144

145 **Table 2** EC<sub>50</sub> values of compounds **4-19** in dark kept and light irradiated (414 nm, 10 J/cm<sup>2</sup>) G361 cells.

compound	EC <sub>50</sub> ± SD (μM)	
	dark	light energy (10 J/cm <sup>2</sup> )
<b>4</b>	>10	2.7 ± 0.5
<b>5</b>	>10	1.1 ± 0.5
<b>6</b>	>10	1.3 ± 0.3
<b>7</b>	>10	8.0 ± 1.7
<b>8</b>	9.6 ± 0.2	3.1 ± 0.1
<b>9</b>	>10	>10
<b>10</b>	>10	1.1 ± 0.0
<b>11</b>	>10	1.8 ± 0.8

<b>12</b>	>10	2.1 ± 0.7	146
<b>13</b>	>10	8.8 ± 1.7	
<b>14</b>	>10	7.1 ± 0.5	
<b>15</b>	>10	4.5 ± 1.8	
<b>16</b>	>10	9.9 ± 0.2	
<b>17</b>	>10	>10	
<b>18</b>	>10	5.3 ± 2.3	
<b>19</b>	>10	2.0 ± 0.5	

147

148 Subsequently, the viability of G361 cells upon treatment with one of the most potent compounds  
 149 **10** was evaluated in a range of irradiation doses (1, 5, and 10 J/cm<sup>2</sup>). As anticipated, the  
 150 photoactivation proved to be light exposure-dependent. Namely, decreasing irradiation dose to 5  
 151 and 1 J/cm<sup>2</sup> increased the EC<sub>50</sub> value to 1.6 and 3.5 μM, respectively. Conducting the experiment  
 152 on human keratinocyte HaCaT cells revealed that these non-cancerous cells are approximately 2-  
 153 fold less sensitive to the treatment, confirming the potential of compound **10** as a photodynamic  
 154 agent (Table 3).

155

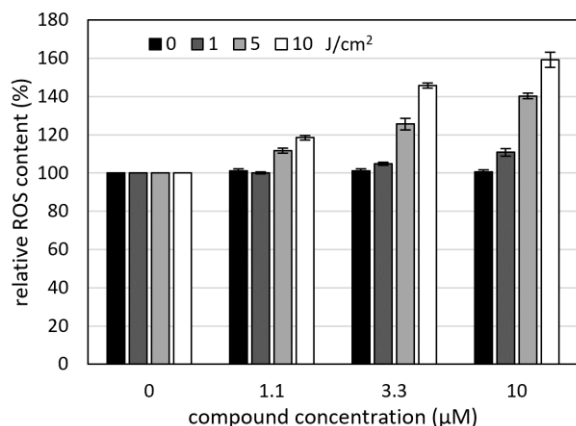
156 **Table 3** Sensitivity of G361 and HaCaT cells to compound **10** in the dark and after exposure to various light doses  
 157 (414 nm).

light energy (J/cm <sup>2</sup> )	EC <sub>50</sub> ± SD (μM)	
	G361	HaCaT
0	>10	>10
1	3.5 ± 0.3	5.7 ± 2.2
5	1.6 ± 0.4	1.9 ± 0.1
10	0.9 ± 0.3	2.0 ± 0.1

158

159 Generation of reactive oxygen species (ROS) is the key mechanism by which PDT causes localized  
 160 cell death [57]. Therefore, to further assess the phototoxic activity of compound **10**, the production  
 161 of ROS in treated G361 cells was quantified by using 2',7'-dichlorodihydrofluorescein diacetate.  
 162 As expected, no ROS production was observed in untreated and/or only irradiated cells, while

163 combination of compound **10** in a range of tested concentrations (1.1, 3.3 and 10  $\mu\text{M}$ ) and  
 164 irradiation doses (1, 5 and 10  $\text{J}/\text{cm}^2$ ) caused ROS generation (Figure 1).



165  
 166 **Figure 1** Production of reactive oxygen species in the G361 cells treated with compound **10** (1.1, 3.3 or 10  $\mu\text{M}$ ) in  
 167 combination with light irradiation (414 nm).

168  
 169 Next, the analysis of cellular DNA content of compound **10** treated and irradiated G361 cells by  
 170 flow cytometry revealed an increase of subdiploid populations in both concentration and light  
 171 irradiation dependent manner (Table 4). This result demonstrates that apoptosis is a probable cause  
 172 of cell death. Notably, at higher irradiation doses, a mild increase of subdiploid populations was  
 173 also detected in compound-untreated cells, which is to be anticipated as high fluences irradiation  
 174 with blue light (412–426 nm) is known to exert toxic effects in human skin cells [58]. Moreover,  
 175 slightly increased subdiploid populations in cell cultures kept in the dark indicated that compound  
 176 **10** has some toxic effect by itself, however this effect is low as documented by  $\text{EC}_{50}$  values  $>10$   
 177  $\mu\text{M}$ .

178  
 179 **Table 4** Quantification (%) of subdiploid population of G361 cells treated with compound **10** in combination with  
 180 light irradiation (414 nm).

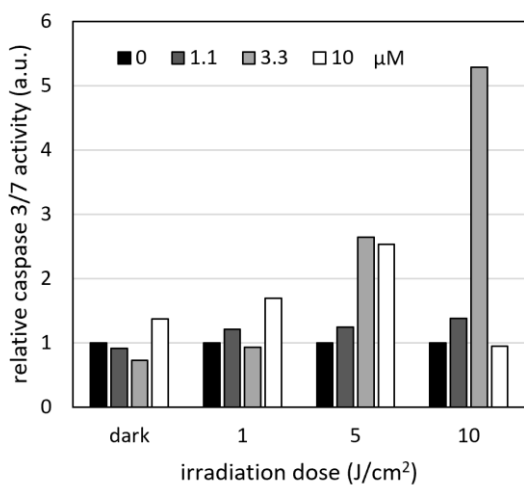
concentration ( $\mu\text{M}$ )	light energy ( $\text{J}/\text{cm}^2$ )			
	0	1	5	10
0	3.4	5.0	4.4	6.1
1.1	4.6	5.4	4.5	9.7
3.3	5.0	5.0	15.0	70.0
10	5.9	5.3	63.3	88.3

181



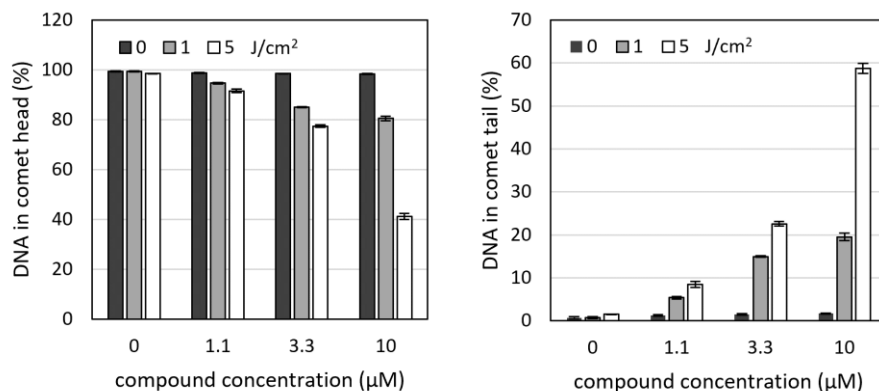
182 To confirm apoptosis as a mechanism of cell death induced by irradiation in cells treated by  
183 compound **10**, activity of pro-apoptotic caspases 3 and 7, which are involved in mediating cell  
184 death signaling transduction, was quantified (Figure 2). The obtained results further confirmed  
185 initially observed capacity of compound **10** to induce apoptosis under light irradiation. Combining  
186 10  $\mu\text{M}$  concentration and 10  $\text{J}/\text{cm}^2$  irradiation, however, resulted in a severe cell death and no  
187 caspase 3/7 activity could be detected.

188



189 **Figure 2** Relative caspase 3/7 activity in G361 cells treated with compound **10** (1.1, 3.3 or 10  $\mu\text{M}$ ) in combination  
190 with light irradiation (414 nm). The cells were treated with compound **10** for 4 h, irradiated (414 nm) and cultivated  
191 for further 24 h. After this period, the cells were harvested and the lysates were assayed for caspase 3/7 activity.  
192

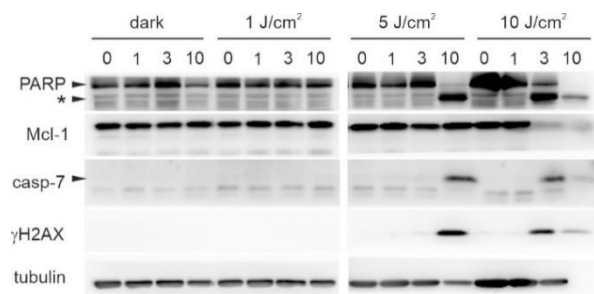
193  
194 The compounds can kill cancer cells by inducing apoptotic cell death, leading to extensive DNA  
195 damage. In order to determine the extent of DNA fragmentation in G361 cells exposed to a  
196 combination of compound **10** and irradiation, a comet assay was performed. Percentual DNA  
197 content in the comet heads (nuclei) and tails were quantified. Expectedly, and in line with other  
198 performed experiments, compound **10** caused significant DNA fragmentation, which was  
199 dependent both on compound dose and light energy (Figure 3). The degree of DNA fragmentation  
200 in individual treatment combinations is available from Table S1, Supplementary File. Importantly,  
201 control cells (i.e. cells treated by **10** and kept in the dark or irradiated cells untreated with the  
202 compound) exhibited no marks of DNA damage.



203  
 204 **Figure 3.** Percentual DNA content in the comet heads (nuclei) and tails of G361 cells treated with compound **10** (1.1,  
 205 3.3 or 10 μM) in combination with light irradiation (414 nm).

206  
 207 Finally, DNA damage and apoptotic cell death were independently confirmed by the means of  
 208 immunoblotting analysis. G361 cells were treated with compound **10** at 1, 3 and 10 μM  
 209 concentrations for 4 h, then either kept in the dark or exposed to light with a total irradiation dose  
 210 of 1, 5 or 10 J/cm<sup>2</sup> followed by subsequent cultivation for 24 h. Increased levels of phosphorylated  
 211 histone H2A.X at Ser-139, a marker of DNA damage, were observed when combining the higher  
 212 concentrations of compound **10** and irradiation doses; the effect was the most apparent at 10 μM  
 213 concentration and 5 J/cm<sup>2</sup> irradiation or at 3 μM concentration and 10 J/cm<sup>2</sup> irradiation (Figure 4).  
 214 Analogously, the cleavage of PARP, an enzyme involved in DNA repair, which gets cut and  
 215 inactivated by caspases during apoptotic cell death, was also apparent when combining said  
 216 concentrations and irradiation doses. Finally, cleavage activation of caspase 7 further confirmed  
 217 apoptosis. Absence of tubulin in the last lane (despite equal protein loading) could be attributed to  
 218 severe cell death. Notably, the aforementioned changes could not be observed when the cells were  
 219 kept in the dark or without exposure to compound **10**. It confirms that the above observation is a  
 220 consequence of a photodynamic effect.

221



222

223 **Figure 4** Light irradiation (414 nm) increases sensitivity of G361 cells to compound **10**. The cells were treated with  
 224 1, 3 or 10  $\mu\text{M}$  compound for 4 h, irradiated and harvested 24 h after irradiation. Asterisk indicates 89 kDa cleavage  
 225 fragment of PARP.

226

### 227 3. Conclusion

228 Synthesis of *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines was accomplished from  
 229 easily accessible 1-phenyl-1*H*-pyrazol-3-ol *via* 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine  
 230 and 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine intermediates *via* palladium  
 231 catalysed Buchwald-Hartwig cross-coupling reaction with various anilines. Biological evaluation  
 232 revealed that the prepared compounds exhibit very low dark cytotoxicity ( $>10 \mu\text{M}$  in most cases)  
 233 but high photocytotoxicity to melanoma G361 cells under blue light (414 nm) irradiation. The  
 234 most active compound **10** displays  $\text{EC}_{50}$  values of 3.5, 1.6 and 0.9  $\mu\text{M}$  in cells irradiated with 1, 5  
 235 and 10  $\text{J}/\text{cm}^2$ , respectively. Further experiments revealed that compound **10** causes cell death by  
 236 producing ROS and damaging DNA. *N*-Aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines  
 237 could thus serve as a potential source of photosensitizing compounds with anticancer activities.

238

### 239 4. Materials and methods

#### 240 4.1. Chemistry

##### 241 4.1.1. General

242 All chemicals and solvents were purchased from commercial suppliers and used without further  
 243 purification unless otherwise specified. The  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$  NMR spectra were recorded in  $\text{CDCl}_3$   
 244 or  $\text{DMSO}-d_6$  solutions at 25  $^\circ\text{C}$  on either a Bruker Avance III 700 (700 MHz for  $^1\text{H}$ , 176 MHz for  
 245  $^{13}\text{C}$ , 71 MHz for  $^{15}\text{N}$ ) spectrometer equipped with a 5 mm TCI  $^1\text{H}-^{13}\text{C}/^{15}\text{N}/\text{D}$  z-gradient cryoprobe  
 246 or on Jeol ECA-500 (500 MHz for  $^1\text{H}$ , 126 MHz for  $^{13}\text{C}$ ) spectrometer equipped with a 5 mm  
 247 Royal probe. The chemical shifts, expressed in ppm, were relative to tetramethylsilane (TMS). The

248 <sup>15</sup>N NMR spectra were referenced to neat, external nitromethane (coaxial capillary). FT-IR spectra  
249 were collected using the ATR method on a Bruker Vertex 70v spectrometer with an integrated  
250 Platinum ATR accessory or on a Bruker Tensor 27 spectrometer in KBr pellets. The melting points  
251 of crystalline compounds were determined in open capillary tubes with a Buchi M – 565 apparatus  
252 (temperature gradient – 2 °C/min) and are uncorrected. Mass spectra were recorded on Q-TOF  
253 MICRO spectrometer (Waters), analyses were performed in positive (ES<sup>+</sup>) mode and molecular  
254 ions were recorded in [M+H]<sup>+</sup> forms. High-resolution mass spectrometry (HRMS) spectra were  
255 obtained in ESI mode on a Bruker MicrOTOF-Q III spectrometer. All reactions were performed  
256 in oven-dried flasks under an argon atmosphere with magnetic stirring. Reaction progress was  
257 monitored by TLC analysis on Macherey-Nagel<sup>TM</sup> ALUGRAM® Xtra SIL G/UV254 plates. TLC  
258 plates were visualized with UV light (wavelengths 254 and 365 nm) or iodine vapour. Compounds  
259 were purified by flash chromatography in a glass column (stationary phase – silica gel, high-purity  
260 grade 9385, pore size 60 Å, particle size - 230–400 mesh, supplier Sigma-Aldrich). <sup>1</sup>H, <sup>13</sup>C NMR  
261 spectra, as well as the HRMS data of new compounds, are provided in the Supplementary File.

262

## 263 **4.1.2. Synthesis**

### 264 **4.1.2.1 General procedure for the synthesis of 7-substituted pyrazolo[4,3-*c*]pyridine** 265 **derivatives 4-19 by Buchwald-Hartwig cross-coupling reaction with anilines**

266 Appropriate 7-iodo-2*H*-pyrazolo[4,3-*c*]pyridine **2** or **3** (1 eq) was dissolved in dry dioxane. Then  
267 appropriate aniline (1.1 eq), NaO*t*Bu (1.6 eq), SPhos (0.3 eq) and Pd(OAc)<sub>2</sub> (0.1 eq) were added  
268 to the solution under argon atmosphere. The mixture was stirred at 120 °C under microwave  
269 irradiation (280 W, 300 Pa) for 1 h. Upon completion (monitored by TLC), the reaction mixture  
270 was cooled to room temperature, filtered through a pad of Celite and the filter cake was washed  
271 with EtOAc (20 mL). Filtrate was diluted with water (20 mL) and extracted with EtOAc (3 × 25  
272 mL). The combined organic layers were washed with brine (10 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and  
273 evaporated under reduced pressure. The residue was purified by column chromatography.

274

#### 275 **4.1.2.1.1. *N*,2,6-Triphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine **4****

276 Prepared in accordance to general procedure from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-  
277 *c*]pyridine **2** (60 mg, 0.15 mmol), aniline (0.015 mL, 0.16 mmol), NaO*t*Bu (23 mg, 0.24 mmol),

278 SPhos (18.6 mg, 0.045 mmol) and Pd(OAc)<sub>2</sub> (3.4 mg, 0.015mmol) and dioxane (4.5 mL). The  
279 residue was purified by column chromatography (EtOAc/Hex, 1:3 to 1:2, v/v).

280 Yield: 50 mg (92%), orange crystals, mp = 234–235 °C, R<sub>f</sub> = 0.11 (EtOAc/Hex, 1:3).

281 <sup>1</sup>H NMR (700 MHz, CDCl<sub>3</sub>): δ 6.17 (1H, s, NH), 6.80-6.89 (3H, m, NH-Ph 2,4,6-H), 7.10-7.17  
282 (2H, m, NH-Ph 3,5-H), 7.24-7.29 (1H, m, C-Ph 4-H), 7.33-7.38 (2H, m, C-Ph 3,5-H), 7.41-7.45  
283 (1H, m, N-Ph 4-H), 7.48-7.54 (2H, m, N-Ph 3,5-H), 7.69-7.77 (2H, m, C-Ph 2,6-H), 7.82-7.90  
284 (2H, m, N-Ph 2,6-H), 8.59 (1H, s, 3-H), 9.07 (1H, s, 4-H). <sup>13</sup>C NMR (176 MHz, CDCl<sub>3</sub>): δ 117.4  
285 (NH-Ph C-2,6), 120.5 (NH-Ph C-4), 121.1 (C-3a), 121.2 (N-Ph C-2,6), 121.6 (C-3), 124.3 (C-7),  
286 127.6 (C-Ph C-4), 128.4 (C-Ph C-3,5), 128.5 (NH-Ph C-3,5), 128.7 (N-Ph C-4), 128.8 (C-Ph C-  
287 2,6), 129.7 (N-Ph C-3,5), 139.2 (C-6), 139.9 (C-Ph C-1), 140.8 (N-Ph C-1), 141.1 (C-4), 143.5  
288 (NH-Ph C-1), 147.5 (C-7a). <sup>15</sup>N NMR (71 MHz, CDCl<sub>3</sub>): δ -304.0 (NH), -147.3 (N-2), -100.5  
289 (N-1), -78.1 (N-5). IR (ν, cm<sup>-1</sup>): 3299 (NH), 3059, 3020 (CH<sub>arom</sub>), 1601, 1590, 1497, 1426, 1346,  
290 1202 (C=C, C=N, C-N), 751, 692, 684 (CH=CH of monosubstituted benzenes). MS (ES<sup>+</sup>): m/z  
291 (%): 363 ([M+H]<sup>+</sup>, 100). HRMS (ESI) for C<sub>24</sub>H<sub>19</sub>N<sub>4</sub> ([M+H]<sup>+</sup>): calcd 363.1604, found 363.1606.

292

#### 293 4.1.2.1.2. *N*-(4-Methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine 5

294 Prepared in accordance to general procedure from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-  
295 *c*]pyridine **2** (60 mg, 0.15 mmol), 4-methoxyaniline (20 mg, 0.16 mmol), NaOtBu (23 mg, 0.24  
296 mmol), SPhos (18.6 mg, 0.045 mmol) and Pd(OAc)<sub>2</sub> (3.4 mg, 0.015 mmol) and dioxane (4.5 mL).  
297 The residue was purified by column chromatography (EtOAc/Hex, 1:3 to 1:2, v/v).

298 Yield: 29 mg (48%), red crystals, mp = 135–136 °C, R<sub>f</sub> = 0.08 (EtOAc/Hex, 1:3). <sup>1</sup>H NMR (500  
299 MHz, CDCl<sub>3</sub>): δ 3.74 (3H, s, OCH<sub>3</sub>), 6.19 (1H, s, NH), 6.68-6.72 (2H, m, NH-Ph 3,5-H), 6.81-  
300 6.85 (2H, m, NH-Ph 2,6-H), 7.24-7.29 (1H, m, C-Ph 4-H), 7.33-7.37 (2H, m, C-Ph 3,5-H), 7.42-  
301 7.45 (1H, m, N-Ph 4-H), 7.50-7.54 (2H, m, N-Ph 3,5-H), 7.67-7.71 (2H, m, C-Ph 2,6-H), 7.83-  
302 7.87 (2H, m, N-Ph 2,6-H), 8.63 (1H, s, 3-H), 9.05 (1H, s, 4-H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ  
303 55.7 (OCH<sub>3</sub>), 113.9 (NH-Ph C-3,5), 120.3 (NH-Ph C-2,6), 120.8 (C-3a), 121.2 (N-Ph C-2,6), 122.2  
304 (C-3), 126.2 (C-7), 127.8 (C-Ph C-4), 128.6 (C-Ph C-3,5), 128.8 (C-Ph C-2,6), 128.9 (N-Ph C-4),  
305 129.8 (N-Ph C-3,5), 136.5 (NH-Ph C-1), 137.7 (C-6), 138.4 (C-Ph C-1), 139.3 (C-4), 139.9 (N-Ph  
306 C-1), 146.9 (C-7a), 154.8 (NH-Ph C-4). IR (ν, cm<sup>-1</sup>): 3376 (NH), 3056, 3025 (CH<sub>arom</sub>), 2923, 2852  
307 (CH<sub>aliph</sub>), 1595, 1505, 1464, 1234, 1212 (C=C, C=N, C-N), 1033 (C-O-C), 755, 699, 688 (CH=CH

308 of mono- and disubstituted benzenes).MS (ES<sup>+</sup>): *m/z* (%): 393 ([M+H]<sup>+</sup>, 100). HRMS (ESI) for  
309 C<sub>25</sub>H<sub>21</sub>N<sub>4</sub>O ([M+H]<sup>+</sup>) calcd 393.1710, found 393.1710.

310

#### 311 **4.1.2.1.3. *N*-(3,4-Dimethoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine 6**

312 Prepared in accordance to general procedure from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-  
313 *c*]pyridine **2** (60 mg, 0.15 mmol), 4-methoxyaniline (20 mg, 0.16 mmol), NaOtBu (23 mg, 0.24  
314 mmol), SPhos (18.6 mg, 0.045 mmol) and Pd(OAc)<sub>2</sub> (3.4 mg, 0.015 mmol) and dioxane (4.5 mL).

315 The residue was purified by column chromatography (EtOAc/Hex, 1:3 to 1:2, v/v).

316 Yield: 55 mg (86%), orange crystals, mp = 93–94 °C, R<sub>f</sub> = 0.20 (EtOAc/Hex, 1:1). <sup>1</sup>H NMR (700  
317 MHz, CDCl<sub>3</sub>): δ 3.66 (3H, s, 3-OCH<sub>3</sub>), 3.80 (3H, s, 4-OCH<sub>3</sub>), 6.21 (1H, s, NH), 6.41-6.48 (2H, m,  
318 NH-Ph 2,6-H), 6.65-6.70 (1H, m, NH-Ph 3-H), 7.22-7.28 (1H, m, C-Ph 4-H), 7.31-7.36 (2H, m,  
319 C-Ph 3,5-H), 7.41-7.45 (1H, m, N-Ph 4-H), 7.49-7.54 (2H, m, N-Ph 3,5-H), 7.71-7.77 (2H, m, C-  
320 Ph 2,6-H), 7.83-7.90 (2H, m, N-Ph 2,6-H), 8.57 (1H, s, 3-H), 9.02 (1H, s, 4-H). <sup>13</sup>C NMR (176  
321 MHz, CDCl<sub>3</sub>): δ 55.8 (3-OCH<sub>3</sub>), 56.4 (4-OCH<sub>3</sub>), 103.4 (NH-Ph C-2), 110.2 (NH-Ph C-6), 111.8  
322 (NH-Ph C-5), 121.0 (C-3a), 121.2 (N-Ph C-2,6), 121.7 (C-3), 125.3 (C-7), 127.6 (C-Ph C-4), 128.5  
323 (C-Ph C-3,5), 128.7 (N-Ph C-4), 128.8 (N-Ph C-2,6), 129.8 (N-Ph C-3,5), 137.3 (NH-Ph C-1),  
324 139.0 (C-6), 139.6 (N-Ph C-1), 140.0 (N-Ph C-1), 140.1 (C-4), 143.8 (NH-Ph C-4), 147.3 (C-7a),  
325 149.0 (NH-Ph C-3). <sup>15</sup>N NMR (71 MHz, CDCl<sub>3</sub>): δ -306.4 (NH), -148.2 (N-2), -101.4 (N-1),  
326 -77.3 (N-5). IR (v, cm<sup>-1</sup>): 3363 (NH), 3055, 2995 (CH<sub>arom</sub>), 2929, 2831 (CH<sub>aliph</sub>), 1596, 1509, 1439,  
327 1228 (C=C, C=N, C-N), 1024 (C-O-C), 756, 696 (CH=CH of mono- and disubstituted benzenes).  
328 MS (ES<sup>+</sup>): *m/z* (%): 423 ([M+H]<sup>+</sup>, 100). HRMS (ESI) for C<sub>26</sub>H<sub>23</sub>N<sub>4</sub>O<sub>2</sub> ([M+H]<sup>+</sup>) calcd 423.1816,  
329 found 423.1816.

330

#### 331 **4.1.2.1.4. *N*-(3,5-Dimethoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine 7**

332 Prepared in accordance to general procedure from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-  
333 *c*]pyridine **2** (60 mg, 0.15 mmol), 3,5-dimethoxyaniline (25 mg, 0.16 mmol), NaOtBu (23 mg, 0.24  
334 mmol), SPhos (18.6 mg, 0.045 mmol) and Pd(OAc)<sub>2</sub> (3.4 mg, 0.015 mmol) and dioxane (4.5 mL).

335 The residue was purified by column chromatography (EtOAc/Hex, 1:3 to 1:2, v/v).

336 Yield: 38 mg (60%), brown crystals, mp = 140–141 °C, R<sub>f</sub> = 0.28 (Hex:EtOAc 1:1). <sup>1</sup>H NMR (700  
337 MHz, CDCl<sub>3</sub>): δ 3.64 (3H, s, OCH<sub>3</sub>), 5.98-6.01 (1H, m, NH-Ph 4-H), 6.01-6.05 (2H, m, NH-Ph  
338 2,6-H), 6.17 (1H, s, NH), 7.27-7.30 (1H, m, C-Ph 4-H), 7.35-7.39 (2H, m, C-Ph 3,5-H), 7.42-7.45

339 (1H, m, N-Ph 4-H), 7.50-7.54 (2H, m, N-Ph 3,5-H), 7.74-7.78 (2H, m, C-Ph 2,6-H), 7.85-7.91  
340 (2H, m, N-Ph 2,6-H), 8.58 (1H, s, 3-H), 9.08 (1H, s, 4-H). <sup>13</sup>C NMR (176 MHz, CDCl<sub>3</sub>): δ 55.2  
341 (OCH<sub>3</sub>), 93.2 (NH-Ph C-4), 95.7 (NH-Ph C-2,6), 121.0 (C-3a), 121.2 (N-Ph C-2,6), 121.8 (C-4),  
342 124.0 (C-7), 127.7 (C-Ph C-4), 128.4 (C-Ph C-3,5), 128.70 (N-Ph C-4), 128.74 (C-Ph C-2,6), 129.7  
343 (N-Ph C-3,5), 139.2 (C-Ph C-1), 139.9 (N-Ph C-1), 141.3 (C-6), 141.5 (C-4), 145.6 (NH-Ph C-1),  
344 147.6 (C-7a), 161.1 (NH-Ph C-3,5). <sup>15</sup>N NMR (71 MHz, CDCl<sub>3</sub>): -302.6 (NH), -146.9 (N-2),  
345 -101.7 (N-1), -79.3 (N-5). IR (ν, cm<sup>-1</sup>): 3376 (NH), 3057, 2998 (CH<sub>arom</sub>), 2933, 2837 (CH<sub>aliph</sub>),  
346 159, 1480, 1461, (C=C, C=N, C-N), 1199, 1148, 1054 (C-O-C), 756, 696, 686 (CH=CH of mono-  
347 and disubstituted benzenes). MS (ES<sup>+</sup>): *m/z* (%): 423 ([M+H]<sup>+</sup>, 99.7). HRMS (ESI) C<sub>26</sub>H<sub>23</sub>N<sub>4</sub>O<sub>2</sub>  
348 ([M+H]<sup>+</sup>) calcd 423.1816, found 423.1816.

349

#### 350 **4.1.2.1.5. *N*-(2,4-Dimethoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine 8**

351 Prepared in accordance to general procedure from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-  
352 *c*]pyridine **2** (60 mg, 0.15 mmol), 2,4-dimethoxyaniline (25 mg, 0.16 mmol), NaOtBu (23 mg, 0.24  
353 mmol), SPhos (18.6 mg, 0.045 mmol) and Pd(OAc)<sub>2</sub> (3.4 mg, 0.015 mmol) and dioxane (4.5 mL).  
354 The residue was purified by column chromatography (EtOAc/Hex, 1:3 to 1:2, v/v).

355 Yield: 40 mg (63%), brown crystals, mp = 108–110 °C, R<sub>f</sub> = 0.28 (Hex:EtOAc 1:1). <sup>1</sup>H NMR (700  
356 MHz, CDCl<sub>3</sub>): δ 3.74 (3H, s, OCH<sub>3</sub>), 3.83 (3H, s, OCH<sub>3</sub>), 6.18-6.24 (1H, m, NH-Ph 4-H), 6.32  
357 (1H, s, NH), 6.44-6.51 (1H, m, NH-Ph 3-H), 6.65-6.73 (1H, m, NH-Ph 6-H), 7.24-7.28 (1H, m, C-  
358 Ph 4-H), 7.32-7.37 (2H, m, C-Ph 3,5-H), 7.38-7.43 (1H, m, N-Ph 4-H), 7.47-7.53 (2H, m, N-Ph  
359 3,5-H), 7.68-7.76 (2H, m, C-Ph 2,6-H), 7.83-7.91 (2H, m, N-Ph 2,6-H), 8.55 (1H, s, 3-H), 9.00  
360 (1H, s, 4-H). <sup>13</sup>C NMR (176 MHz, CDCl<sub>3</sub>): δ 55.7 (2,4-OCH<sub>3</sub>), 98.9 (NH-Ph C-3), 103.3 (NH-Ph  
361 C-5), 117.3 (NH-Ph C-6), 121.1 (C-3a), 121.2 (N-Ph C-2,6), 121.5 (C-3), 125.4 (C-7), 126.7 (NH-  
362 Ph C-1), 127.5 (C-Ph C-4), 128.4 (C-Ph C-3,5), 128.6 (N-Ph C-4), 128.9 (C-Ph C-2,6), 129.7 (N-  
363 Ph C-3,5), 139.6 (C-Ph C-1), 139.8 (C-6), 140.1 (C-4 and N-Ph C-1), 147.5 (C-7a), 150.1 (NH-Ph  
364 C-2), 154.4 (NH-Ph C-4). IR (ν, cm<sup>-1</sup>): 3381 (NH), 3056, 2999 (CH<sub>arom</sub>), 2932, 2832 (CH<sub>aliph</sub>),  
365 1595, 1512, 1463, 1283, 1203 (C=C, C=N, C-N), 1155, 1030 (C-O-C), 755, 699, 689 (CH=CH of  
366 mono- and disubstituted benzenes). MS (ES<sup>+</sup>): *m/z* (%): 423 ([M+H]<sup>+</sup>, 100). HRMS (ESI)  
367 C<sub>26</sub>H<sub>23</sub>N<sub>4</sub>O<sub>2</sub> ([M+H]<sup>+</sup>) calcd 423.1816, found 423.1816.

368

#### 369 **4.1.2.1.6. *N*-(3,4,5-Trimethoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine 9**

370 Prepared in accordance to general procedure from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-  
371 *c*]pyridine **2** (60 mg, 0.15 mmol), 3,4,5-trimethoxyaniline (29 mg, 0.16 mmol), NaOtBu (23 mg,  
372 0.24 mmol), SPhos (18.6 mg, 0.045 mmol) and Pd(OAc)<sub>2</sub> (3.4 mg, 0.015 mmol) and dioxane (4.5  
373 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:3 to 1:2, v/v).  
374 Yield: 65 mg (95%), orange crystals, mp = 90–91 °C, R<sub>f</sub> = 0.21 (Hex:EtOAc 1:1). <sup>1</sup>H NMR (700  
375 MHz, CDCl<sub>3</sub>): δ 3.65 (6H, s, 3,5-OCH<sub>3</sub>), 3.75 (3H s, 4-OCH<sub>3</sub>), 6.02-6.12 (2H, m, NH-Ph 2,6-H),  
376 6.27 (1H, s, NH), 7.23-7.27 (1H, m, C-Ph 4-H), 7.32-7.37 (2H, m, C-Ph 3,5-H), 7.42-7.45 (1H, m,  
377 N-Ph 4-H), 7.50-7.55 (2H, m, N-Ph C-3,5), 7.72-7.79 (2H, m, C-Ph 2,6-H), 7.83-7.91 (2H, m, N-  
378 Ph 2,6-H), 8.57 (1H, s, 3-H), 9.04 (1H, s, 4-H). <sup>13</sup>C NMR (176 MHz, CDCl<sub>3</sub>): δ 56.0 (3,5-OCH<sub>3</sub>),  
379 61.1 (OCH<sub>3</sub>), 95.6 (NH-Ph C-2,6) 121.0 (C-3a), 121.2 (N-Ph C-2,6), 121.9 (C-3), 124.6 (C-7),  
380 127.7 (C-Ph C-4), 128.5 (C-Ph C-3,5), 128.8 (C-Ph C-2,6), 128.9 (N-Ph C-4), 129.9 (N-Ph C-3,5),  
381 132.4 (NH-Ph C-4), 139.4 (NH-Ph C-1), 139.6 (C-6 and C-Ph C-1), 140.0 (N-Ph C-1), 140.7 (C-  
382 4), 147.6 (C-7a), 153.2 (NH-Ph C-3,5). <sup>15</sup>N NMR (71 MHz, CDCl<sub>3</sub>): δ -304.0 (NH), -147.3 (N-  
383 2), -101.7 (N-1), -76.9 (N-5). IR (ν, cm<sup>-1</sup>): 3345 (NH), 3120, 3057 (CH<sub>arom</sub>), 2930, 2838 (CH<sub>aliph</sub>),  
384 1595, 1501, 1463, 1409, 1231 (C=C, C=N, C-N), 1122, 1006 (C-O-C), 804, 756, 697, 689  
385 (CH=CH of mono- and trisubstituted benzenes). MS (ES<sup>+</sup>): *m/z* (%): 453 ([M+H]<sup>+</sup>, 98). HRMS  
386 (ESI) C<sub>27</sub>H<sub>25</sub>N<sub>4</sub>O<sub>3</sub> ([M+H]<sup>+</sup>) calcd 453.1921, found 453.1921.

387  
388 **4.1.2.1.7. 2,6-Diphenyl-*N*-[2-(trifluoromethoxy)phenyl]-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine**  
389 **10**

390 Prepared in accordance to general procedure from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-  
391 *c*]pyridine **2** (60 mg, 0.15 mmol), 2-(trifluoromethoxy)aniline (28 mg, 0.16 mmol), NaOtBu (23  
392 mg, 0.24 mmol), SPhos (18.6 mg, 0.045 mmol) and Pd(OAc)<sub>2</sub> (3.4 mg, 0.015 mmol) and dioxane  
393 (4.5 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:3 to 1:2, v/v).  
394 Yield: 38 mg (56%), yellow crystals, mp = 66–68 °C, R<sub>f</sub> = 0.38 (Hex:EtOAc 1:1). <sup>1</sup>H NMR (500  
395 MHz, CDCl<sub>3</sub>): δ 6.46 (1H, s, NH), 6.67-6.73 (1H, m, NH-Ph 6-H), 6.75-6.79 (1H, m, NH-Ph 4-  
396 H), 6.86-6.91 (1H, m, NH-Ph 5-H), 7.17-7.21 (1H, m, NH-Ph 3-H), 7.24-7.28 (1H, m, C-Ph 4-H),  
397 7.31-7.35 (2H, m, C-Ph 3,5-H), 7.44-7.48 (1H, m, N-Ph 4-H), 7.52-7.57 (2H, m, N-Ph 3,5-H),  
398 7.72-7.76 (2H, m, C-Ph 2,6-H), 7.87-7.92 (2H, m, N-Ph 2,6-H), 8.68 (1H, s, 3-H), 9.18 (1H, s, 4-  
399 H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ 117.7 (NH-Ph C-6), 119.9, 120.9, 122.0 (CF<sub>3</sub>, *J* = 126 Hz),  
400 120.2 (NH-Ph C-4), 120.8 (NH-Ph C-3), 121.3 (N-Ph C-2,6), 122.5 (C-3), 123.5 (C-7), 126.6 (NH-



401 Ph C-5), 128.2 (C-Ph C-4), 128.6 (C-Ph C-3,5), 128.7 (C-Ph C-2,6), 129.1 (N-Ph C-4), 129.9 (N-  
402 Ph C-3,5), 135.7 (NH-Ph C-1), 138.3 (C-Ph C-1 and NH-Ph C-2), 139.9 (N-Ph C-1), 140.9 (C-6),  
403 141.7 (C-4), 147.8 (C-7a). IR ( $\nu$ ,  $\text{cm}^{-1}$ ): 3404 (NH), 3060, 3031 ( $\text{CH}_{\text{arom}}$ ), 1606, 1501, 1484, 1244,  
404 1213, 1165 (C=C, C=N, C-N, C-F), 751, 697, 687 (CH=CH of mono- and disubstituted benzenes).  
405 MS ( $\text{ES}^+$ ):  $m/z$  (%): 447 ( $[\text{M}+\text{H}]^+$ , 97.8). HRMS (ESI)  $\text{C}_{25}\text{H}_{18}\text{F}_3\text{N}_4\text{O}$  ( $[\text{M}+\text{H}]^+$ ) calcd 447.1427,  
406 found 447.1427.

407  
408 **4.1.2.1.8. 2,6-Diphenyl-N-[3-(trifluoromethoxy)phenyl]-2H-pyrazolo[4,3-c]pyridin-7-amine**  
409 **11**

410 Prepared in accordance to general procedure from 7-iodo-2,6-diphenyl-2H-pyrazolo[4,3-  
411 c]pyridine **2** (60 mg, 0.15 mmol), 3-(trifluoromethoxy)aniline (28 mg, 0.16 mmol), NaOtBu (23  
412 mg, 0.24 mmol), SPhos (18.6 mg, 0.045 mmol) and Pd(OAc)<sub>2</sub> (3.4 mg, 0.015 mmol) and dioxane  
413 (4.5 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:3 to 1:2, v/v).

414 Yield: 38 mg (56%) orange crystals, mp = 77–78 °C,  $R_f$  = 0.38 (Hex:EtOAc 1:1). <sup>1</sup>H NMR (500  
415 MHz, CDCl<sub>3</sub>):  $\delta$  6.22 (1H, s, NH), 6.65-6.71 (2H, m, NH-Ph 4-H, 2-H or 6-H), 6.72-6.76 (1H, m,  
416 NH-Ph 2-H or 6-H), 7.09-7.16 (1H, m, NH-Ph 5-H), 7.28-7.32 (1H, m, C-Ph 4-H), 7.36-7.40 (2H,  
417 m, C-Ph 3,5-H), 7.43-7.48 (1H, m, N-Ph 4-H), 7.51-7.56 (2H, m, N-Ph 3,5-H), 7.68-7.75 (2H, m,  
418 C-Ph 2,6-H), 7.84-7.90 (2H, m, N-Ph 2,6-H), 8.64 (1H, s, 3-H), 9.14 (1H, s, 4-H). <sup>13</sup>C NMR (126  
419 MHz, CDCl<sub>3</sub>):  $\delta$  109.7 (NH-Ph C-2 or C-6), 112.5 (NH-Ph C-5), 115.5 (NH-Ph C-2 or C-6), 119.5  
420 (CF<sub>3</sub>), 121.1 (C-3a), 121.4 (N-Ph C-2,6), 122.4 (C-3), 123.5 (C-7), 128.1 (C-Ph C-4), 128.7 (C-Ph  
421 C-3,5), 128.9 (C-Ph C-2,6), 129.0 (N-Ph C-4), 129.6 (NH-Ph C-5), 129.8 (N-Ph C-3,5), 138.6 (C-  
422 Ph C-1), 139.9 (N-Ph C-1), 141.6 (C-6), 142.0 (C-4), 145.2 (NH-Ph C-1), 147.5 (C-7a), 149.8  
423 (NH-Ph C-3). IR ( $\nu$ ,  $\text{cm}^{-1}$ ): 3204 (NH), 3061, 3048 ( $\text{CH}_{\text{arom}}$ ), 1610, 1596, 1490, 1486, 1249, 1213,  
424 1153 (C=C, C=N, C-N, C-F), 756, 695 (CH=CH of mono- and disubstituted benzenes). MS ( $\text{ES}^+$ ):  
425  $m/z$  (%): 447 ( $[\text{M}+\text{H}]^+$ , 100). HRMS (ESI)  $\text{C}_{25}\text{H}_{18}\text{F}_3\text{N}_4\text{O}$  ( $[\text{M}+\text{H}]^+$ ) calcd 447.1427, found  
426 447.1427.

427  
428 **4.1.2.1.9 4-Methyl-N-2,6-triphenyl-2H-pyrazolo[4,3-c]pyridin-7-amine 12**

429 Prepared in accordance to general procedure from 7-iodo-4-methyl-2,6-diphenyl-2H-  
430 pyrazolo[4,3-c]pyridine **3** (50 mg, 0.12 mmol), aniline (0.012 mL, 0.13 mmol), NaOtBu (18.7 mg,

431 0.19 mmol), SPhos (15 mg, 0.036 mmol) and Pd(OAc)<sub>2</sub> (1.7 mg, 0.012 mmol) and dioxane (4 mL).  
432 The residue was purified by column chromatography (EtOAc/PE, 1:4 to 1:2, v/v).  
433 Yield: 31 mg (65 %), mp = 165–169 °C. <sup>1</sup>H NMR (700 MHz, CDCl<sub>3</sub>): δ 2.87 (3H, s, CH<sub>3</sub>), 5.94  
434 (1H, s, NH), 6.81–6.83 (3H, m, NH-Ph 2,4,6-H), 7.13–7.15 (2H, m, NH-Ph 3,5-H), 7.26–7.29  
435 (1H, m, C-Ph 4-H), 7.35–7.37 (2H, m, C-Ph 3,5-H), 7.40–7.42 (1H, m, N-Ph 4-H), 7.48–7.51 (2H,  
436 m, N-Ph 3,5-H), 7.72–7.73 (2H, m, C-Ph 2,6-H), 7.85–7.87 (2H, m, N-Ph 2,6-H), 8.57 (s, 1H, 3-  
437 H). <sup>13</sup>C NMR (176 MHz, CDCl<sub>3</sub>): δ 22.8 (CH<sub>3</sub>), 117.0 (NH-Ph C-2,6), 120.1 (NH-Ph C-4), 121.20  
438 (C-3a), 121.23 (N-Ph C-2,6), 122.0 (C-3), 122.4 (C-7), 127.8 (C-Ph C-4), 128.6 (C-Ph C-3,5),  
439 128.65 (N-Ph C-4), 128.7 (NH-Ph C-3,5), 129.1 (C-Ph C-2,6), 129.7 (N-Ph C-3,5), 139.1 (C-6),  
440 140.0 (N-Ph C-1), 141.6 (C-Ph C-1), 144.5 (NH-Ph C-1), 148.0 (C-7a), 150.4 (C-4). <sup>15</sup>N NMR (71  
441 MHz, CDCl<sub>3</sub>): δ –285.3 (NH), –160.2 (N-1), –148.3 (N-2), –82.2 (N-5). IR (KBr, v, cm<sup>-1</sup>): 3387  
442 (NH), 3031, 3051 (CH<sub>arom</sub>), 2985, 2914, 2850 (CH<sub>aliph</sub>), 1598, 1511, 1499, 1310 (C=C, C=N,  
443 C–N), 760, 747, 696 (CH=CH of mono- and disubstituted benzenes). MS m/z (%): 377 ([M+H]<sup>+</sup>,  
444 100). HRMS (ESI) C<sub>25</sub>H<sub>21</sub>N<sub>4</sub> ([M+H]<sup>+</sup>) calcd 377.1761, found 377.1763.

#### 445 446 **4.1.2.1.10 N-4-Methoxyphenyl-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-amine 13**

447 Prepared in accordance to general procedure from 7-iodo-4-methyl-2,6-diphenyl-2H-  
448 pyrazolo[4,3-c]pyridine **3** (50 mg, 0.12 mmol), 4-methoxyaniline (16 mg, 0.13 mmol), NaOtBu  
449 (18.7 mg, 0.19 mmol), SPhos (15 mg, 0.036 mmol) and Pd(OAc)<sub>2</sub> (1.7 mg, 0.012 mmol) and  
450 dioxane (4 mL). The residue was purified by column chromatography (EtOAc/PE, 1:4 to 1:2, v/v).  
451 Yield: 38 mg (77%), light yellow crystals, mp = 89–90 °C, R<sub>f</sub> = 0.11 (EtOAc/Hex, 1:3). <sup>1</sup>H NMR  
452 (500 MHz, CDCl<sub>3</sub>): δ 2.86 (3H, s, CH<sub>3</sub>), 3.74 (3H, s, OCH<sub>3</sub>), 5.89 (1H, s, NH), 6.67–6.75 (2H, m,  
453 NH-Ph 3,5-H), 6.76–6.85 (2H, m, NH-Ph 2,6-H), 7.26–7.29 (1H, m, C-Ph 4-H), 7.32–7.38 (2H, m,  
454 C-Ph 3,5-H), 7.39–7.43 (1H, m, N-Ph 4-H), 7.48–7.53 (2H, m, N-Ph 3,5-H), 7.66–7.73 (2H, m, C-  
455 Ph 2,6-H), 7.81–7.88 (2H, m, N-Ph 2,6-H), 8.55 (1H, s, 3-H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ 22.8  
456 (CH<sub>3</sub>), 55.7 (OCH<sub>3</sub>), 114.0 (NH-Ph C-2,6), 119.1 (NH-Ph C-3,5), 121.1 (N-Ph C-2,6), 121.2 (C-  
457 3a), 121.7 (C-3), 123.4 (C-7), 127.6 (C-Ph C-4), 128.5 (C-Ph C-3,5), 128.6 (N-Ph C-4), 129.0 (C-  
458 Ph C-2,6), 129.7 (N-Ph C-3,5), 138.0 (NH-Ph C-1), 139.3 (C-Ph C-1), 140.0 (N-Ph C-1), 140.3  
459 (C-6), 147.6 (C-7a), 149.3 (C-4), 154.1 (NH-Ph C-4). IR (v, cm<sup>-1</sup>): 3375 (NH), 3053, 2994  
460 (CH<sub>arom</sub>), 2947, 2929, 2831 (CH<sub>aliph</sub>), 1596, 1506, 1488, 1410, 1234, 1212 (C=C, C=N, C–N), 1029

461 (C-O-C), 756, 696, 689 (CH=CH of mono- and disubstituted benzenes). MS (ES<sup>+</sup>): *m/z* (%): 407  
462 ([M+H]<sup>+</sup>, 100). HRMS (ESI) for C<sub>26</sub>H<sub>23</sub>N<sub>4</sub>O ([M+H]<sup>+</sup>): calcd 407.1866, found 407.1866.

463  
464 **4.1.2.1.11 N-(3,4-Dimethoxyphenyl)-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-**  
465 **amine 14**

466 Prepared in accordance to general procedure from 7-iodo-4-methyl-2,6-diphenyl-2H-  
467 pyrazolo[4,3-c]pyridine **3** (50 mg, 0.12 mmol), 3,4-dimethoxyaniline (20 mg, 0.13 mmol),  
468 NaOtBu (18.7 mg, 0.19 mmol), SPhos (15 mg, 0.036 mmol) and Pd(OAc)<sub>2</sub> (1.7 mg, 0.012 mmol)  
469 and dioxane (4 mL). The residue was purified by column chromatography (EtOAc/PE, 1:4 to 1:2,  
470 v/v).

471 Yield: 32 mg (60%), brown crystals, mp = 202–203 °C, R<sub>f</sub> = 0.08 (EtOAc/Hex, 1:3). <sup>1</sup>H NMR  
472 (500 MHz, CDCl<sub>3</sub>): δ 2.88 (3H, s, CH<sub>3</sub>), 3.67 (3H, s, 3-OCH<sub>3</sub>), 3.81 (3H, s, 4-OCH<sub>3</sub>), 5.98 (1H, s,  
473 NH), 6.38-6.43 (1H, m, NH-Ph 6-H), 6.43-6.46 (1H, m, NH-Ph 2-H), 6.65-6.72 (1H, m, NH-Ph  
474 5-H), 7.23-7.29 (1H, m, C-Ph 4-H), 7.32-7.37 (2H, m, C-Ph 3,5-H), 7.41-7.45 (1H, m, N-Ph 4-H),  
475 7.49-7.54 (2H, m N-Ph, 3,5-H), 7.70-7.75 (2H, m, C-Ph 2,6-H), 7.84-7.90 (2H, m, N-Ph 2,6-H),  
476 8.57 (1H, s, 3-H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ 22.5 (CH<sub>3</sub>), 55.8 (3-OCH<sub>3</sub>), 56.4 (4-OCH<sub>3</sub>),  
477 103.0 (NH-Ph C-2), 109.6 (NH-Ph C-6), 111.8 (NH-Ph C-5), 121.0 (C-3a), 121.1 (N-Ph C-2,6),  
478 122.0 (C-3), 123.5 (C-7), 127.8 (C-Ph C-4), 128.6 (C-Ph C-3,5), 128.7 (N-Ph C-4), 129.1 (C-Ph  
479 C-2,6), 129.8 (N-Ph C-3,5), 138.0 (NH-Ph C-1), 138.8 (N-Ph C-1), 139.4 (C-6), 139.9 (N-Ph C-  
480 1), 143.6 (NH-Ph C-4), 147.6 (C-7a), 149.1 (NH-Ph C-3), 149.3 (C-4). IR (ν, cm<sup>-1</sup>): 3335 (NH),  
481 3124, 3000 (CH<sub>arom</sub>), 2951, 2931, 2830 (CH<sub>aliph</sub>), 1595, 1526, 1507 1464, 1405, 1220, 1207 (C=C,  
482 C=N, C-N), 1153, 1027 (C-O-C), 759, 691 (CH=CH of mono- and trisubstituted benzenes). MS  
483 (ES<sup>+</sup>): *m/z* (%): 437 ([M+H]<sup>+</sup>, 100). HRMS (ESI) for C<sub>27</sub>H<sub>25</sub>N<sub>4</sub>O<sub>2</sub> ([M+H]<sup>+</sup>): calcd 437.1972,  
484 found 437.1972.

485  
486 **4.1.2.1.12 N-(3,5-Dimethoxyphenyl)-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-**  
487 **amine 15**

488 Prepared in accordance to general procedure from 7-iodo-4-methyl-2,6-diphenyl-2H-  
489 pyrazolo[4,3-c]pyridine **3** (50 mg, 0.12 mmol), 3,5-dimethoxyaniline (20 mg, 0.13 mmol),  
490 NaOtBu (18.7 mg, 0.19 mmol), SPhos (15 mg, 0.036 mmol) and Pd(OAc)<sub>2</sub> (1.7 mg, 0.012 mmol)

491 and dioxane (4 mL). The residue was purified by column chromatography (EtOAc/PE, 1:4 to 1:2,  
492 v/v).

493 Yield: 31 mg (49%), brown crystals, mp = 157–159 °C,  $R_f$  = 0.49 (Hex:EtOAc 1:1).  $^1\text{H}$  NMR (500  
494 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.89 (3H, s,  $\text{CH}_3$ ), 3.65 (6H, s,  $\text{OCH}_3$ ), 5.95 (1H, s, NH), 5.97-6.02 (3H, m, NH-  
495 Ph 2,5,6-H), 7.27-7.32 (1H, m, C-Ph 4-H), 7.35-7.40 (2H, m, C-Ph 3,5-H), 7.40-7.45 (1H, m, N-  
496 Ph 4-H), 7.49-7.54 (2H, m, N-Ph 3,5-H), 7.72-7.78 (2H, m, C-Ph 2,6-H), 7.85-7.91 (2H, m, N-Ph  
497 2,6-H), 8.58 (1H, s, 3-H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ ):  $\delta$  22.7 ( $\text{CH}_3$ ), 55.3 ( $\text{OCH}_3$ ), 92.9 (NH-Ph  
498 C-4), 95.4 (NH-Ph C-2,6), 121.1 (C-3a), 121.3 (N-Ph C-2,6), 122.3 (C-3a and C-7), 128.0 (C-Ph  
499 C-4), 128.6 (C-Ph C-3,5), 128.8 (N-Ph C-4), 129.1 (C-Ph C-2,6), 129.8 (N-Ph C-3,5), 138.8 (C-  
500 Ph C-1), 140.0 (N-Ph C-1), 141.9 (C-6), 146.5 (NH-Ph C-1), 148.3 (C-7a), 151.0 (C-3), 161.2  
501 (NH-Ph C-3,5). IR (v,  $\text{cm}^{-1}$ ): 3394, 3310 (NH), 3058, 3000 ( $\text{CH}_{\text{arom}}$ ), 2920, 2851 ( $\text{CH}_{\text{aliph}}$ ), 1595,  
502 1516, 1459, 1416, 1378 (C=C, C=N, C-N), 1197, 1144, 1068, 1050 (C-O-C), 818, 763, 753, 688  
503 (CH=CH of mono- and trisubstituted benzenes). MS ( $\text{ES}^+$ ):  $m/z$  (%): 437 ( $[\text{M}+\text{H}]^+$ , 97.3). HRMS  
504 (ESI)  $\text{C}_{27}\text{H}_{25}\text{N}_4\text{O}_2$  ( $[\text{M}+\text{H}]^+$ ) calcd 437.1972, found 437.1972.

505  
506 **4.1.2.1.13 *N*-(2,4-Dimethoxyphenyl)-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-**  
507 **amine 16**

508 Prepared in accordance to general procedure from 7-iodo-4-methyl-2,6-diphenyl-2*H*-  
509 pyrazolo[4,3-*c*]pyridine **3** (50 mg, 0.12 mmol), 3,5-dimethoxyaniline (20 mg, 0.13 mmol),  
510  $\text{NaOtBu}$  (18.7 mg, 0.19 mmol), SPhos (15 mg, 0.036 mmol) and  $\text{Pd}(\text{OAc})_2$  (1.7 mg, 0.012 mmol)  
511 and dioxane (4 mL). The residue was purified by column chromatography (EtOAc/PE, 1:4 to 1:2,  
512 v/v).

513 Yield: 39 mg (73%), orange crystals, mp = 92–95 °C,  $R_f$  = 0.44 (Hex:EtOAc 1:1).  $^1\text{H}$  NMR (500  
514 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.89 (3H, s,  $\text{CH}_3$ ), 3.74 (3H, s, 4- $\text{OCH}_3$ ), 3.80 (3H, s, 3- $\text{OCH}_3$ ), 6.14 (1H, s, NH),  
515 6.18-6.26 (1H, m, NH-Ph 5-H), 6.42-6.51 (1H, m, NH-Ph 3-H), 6.61-6.73 (1H, m, NH-Ph 6-H),  
516 7.24-7.29 (1H, m, C-Ph 4-H), 7.31-7.37 (2H, m, C-Ph 3,5-H), 7.39-7.44 (1H, m, N-Ph 4-H), 7.48-  
517 7.54 (2H, m, N-Ph 3,5-H), 7.64-7.74 (2H, m, C-Ph 2,6-H), 7.83-7.94 (2H, m, N-Ph 2,6-H), 8.57  
518 (1H, s, 3-H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ ):  $\delta$  22.8 (Me), 55.66 (4-MeO), 55.69 (2-MeO), 98.9  
519 (NH-Ph C-3), 103.4 (NH-Ph C-5), 116.1 (NH-Ph C-6), 121.1 (C-3a), 121.2 (N-Ph C-2,6), 121.6  
520 (C-3), 123.4 (C-7), 127.6 (C-Ph C-4), 127.8 (NH-Ph C-1), 128.4 (C-Ph C-3,5), 128.5 (N-Ph C-4),  
521 129.0 (C-Ph C-2,6), 129.7 (N-Ph C-3,5), 139.4 (C-Ph C-1), 140.2 (N-Ph C-1), 140.9 (C-6), 148.1

522 (C-7a), 149.4 (C-4), 149.6 (NH-Ph C-2), 154.0 (NH-Ph C-4). <sup>15</sup>N NMR (51 MHz, CDCl<sub>3</sub>): δ  
523 -316.2 (NH), -149.2 (N-2), -98.8 (N-5), -83.4 (N-1). IR (ν, cm<sup>-1</sup>): 3382 (NH), 3053, 2997  
524 (CH<sub>arom</sub>), 2933, 2832 (CH<sub>aliph</sub>), 1595, 1511, 1463, 1283, 1204 (C=C, C=N, C-N), 1155, 1029 (C-  
525 O-C), 829, 756, 696, 689 (CH=CH of mono- and trisubstituted benzenes). MS (ES<sup>+</sup>): *m/z* (%): 437  
526 ([M+H]<sup>+</sup>, 98.7). HRMS (ESI) C<sub>27</sub>H<sub>25</sub>N<sub>4</sub>O<sub>2</sub> ([M+H]<sup>+</sup>) calcd 437.1972, found 437.1972.

527

#### 528 **4.1.2.1.14 N-(3,4,5-Trimethoxyphenyl)-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-** 529 **amine 17**

530 Prepared in accordance to general procedure from 7-iodo-4-methyl-2,6-diphenyl-2H-  
531 pyrazolo[4,3-c]pyridine **3** (50 mg, 0.12 mmol), 3,4,5-trimethoxyaniline (24 mg, 0.13 mmol),  
532 NaOtBu (18.7 mg, 0.19 mmol), SPhos (15 mg, 0.036 mmol) and Pd(OAc)<sub>2</sub> (1.7 mg, 0.012 mmol)  
533 and dioxane (4 mL). The residue was purified by column chromatography (EtOAc/PE, 1:4 to 1:2,  
534 v/v).

535 Yield: 36 mg (63%), reddish brown crystals, mp = 107–108 °C, R<sub>f</sub> = 0.31 (Hex:EtOAc 1:1). <sup>1</sup>H  
536 NMR (500 MHz, CDCl<sub>3</sub>): δ 2.93 (3H, s, CH<sub>3</sub>), 3.66 (6H, s, 3,5-OCH<sub>3</sub>), 3.75 (3H, s, 4-OCH<sub>3</sub>), 6.06  
537 (2H, s, NH-Ph 2,6-H), 6.07-6.09 (1H, br s, NH), 7.26-7.30 (1H, m, C-Ph 4-H), 7.34-7.38 (2H, m,  
538 C-Ph 3,5-H), 7.43-7.47 (1H, m, N-Ph 4-H), 7.21-7.56 (2H, m, N-Ph 3,5-H), 7.73-7.77 (2H, m, C-  
539 Ph 2,6-H), 7.87-7.90 (2H, m, N-Ph 2,6-H), 8.62 (1H, s, 3-H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ 22.8  
540 (CH<sub>3</sub>), 56.0 (3,5-OCH<sub>3</sub>), 61.1 (4-OCH<sub>3</sub>), 95.0 (NH-Ph C-2,6), 121.1 (C-3a), 121.2 (N-Ph C-2,6),  
541 122.0 (C-7), 122.6 (C-3), 127.8 (C-Ph C-4), 128.5 (C-Ph C-3.5), 128.7 (N-Ph C-4), 129.0 (C-Ph  
542 C-2.6), 129.8 (N-Ph C-3.5), 132.1 (NH-Ph C-4), 139.4 (NH-Ph C-1), 140.0, 140.3 and 140.5 (C-  
543 6, C-Ph C-1 or N-Ph C-1), 148.0 (C-7a), 150.0 (C-4), 153.3 (NH-Ph C-4,5). IR (ν, cm<sup>-1</sup>): 3346,  
544 3314 (NH), 3112, 3057 (CH<sub>arom</sub>), 2923, 2852 (CH<sub>aliph</sub>), 1596, 1502, 1463, 1412, 1232 (C=C, C=N,  
545 C-N), 1125, 1006 (C-O-C), 804, 757, 690 (CH=CH of mono- and tetrasubstituted benzenes). MS  
546 (ES<sup>+</sup>): *m/z* (%): 467 ([M+H]<sup>+</sup>, 100). HRMS (ESI) C<sub>28</sub>H<sub>27</sub>N<sub>4</sub>O<sub>3</sub> ([M+H]<sup>+</sup>) calcd 467.2078, found  
547 467.2078.

548

#### 549 **4.1.2.1.15 4-Methyl-2,6-diphenyl-N-[2-(trifluoromethoxy)phenyl]-2H-pyrazolo[4,3-** 550 **c]pyridin-7-amine 18**

551 Prepared in accordance to general procedure from 7-iodo-4-methyl-2,6-diphenyl-2H-  
552 pyrazolo[4,3-c]pyridine **3** (50 mg, 0.12 mmol), 2-(trifluoromethoxy)aniline (23 mg, 0.13 mmol),

553 NaOtBu (18.7 mg, 0.19 mmol), SPhos (15 mg, 0.036 mmol) and Pd(OAc)<sub>2</sub> (1.7 mg, 0.012 mmol)  
554 and dioxane (4 mL). The residue was purified by column chromatography (EtOAc/PE, 1:4 to 1:2,  
555 v/v).

556 Yield: 46 mg (82%), yellowish crystals, mp = 113–114 °C, R<sub>f</sub> = 0.62 (Hex:EtOAc 1:1). <sup>1</sup>H NMR  
557 (500 MHz, CDCl<sub>3</sub>): δ 2.87 (3H, s, CH<sub>3</sub>), 6.28 (1H, s, NH), 6.68–6.77 (2H, m, NH-Ph 4,6-H), 6.87–  
558 6.93 (1H, m, NH-Ph 3-H or 5-H), 7.16–7.21 (1H, m, NH-P, 3-H or 5-H), 7.24–7.29 (1H, m, C-P,  
559 4-H), 7.32–7.36 (2H, m, C-Ph 3,5-H), 7.40–7.44 (1H, m, N-Ph 4-H), 7.49–7.53 (2H, m, N-Ph 3,5-  
560 H), 7.73–7.79 (2H, m, C-Ph 2,6-H), 7.85–7.89 (2H, m, N-Ph 2,6-H), 8.56 (1H, s, 3-H). <sup>13</sup>C NMR  
561 (126 MHz, CDCl<sub>3</sub>): δ 23.0 (CH<sub>3</sub>) 116.7 (NH-Ph C-6), 117.9, 119.2, 124.0 (CF<sub>3</sub> J = 252 Hz), 120.7  
562 (NH-Ph C-3 or C-5), 120.8 (C-7), 121.1 (N-Ph C-2,6), 121.2 (C-3a), 122.0 (C-3), 126.7 (NH-Ph  
563 C-3 or C-5), 127.9 (C-Ph C-4), 128.5 (C-Ph C-3,5), 128.7 (N-Ph C-4), 128.8 (C-Ph C-2,6), 129.7  
564 (N-Ph C-3,5), 136.8 (NH-Ph C-1), 137.7 (NH-Ph C-2), 139.1 (C-Ph C-1), 140.0 (N-Ph C-1), 143.1  
565 (C-6), 148.3 (C-7a), 151.5 (C-4). IR (ν, cm<sup>-1</sup>): 3412, 3396 (NH), 3133, 3056 (CH<sub>arom</sub>), 2920, 2852  
566 (CH<sub>aliph</sub>), 1600, 1511, 1485, 1434, 1248, 1213, 1167 (C=C, C=N, C–N, C-F), 755, 697, 688  
567 (CH=CH of mono- and disubstituted benzenes). MS (ES<sup>+</sup>): m/z (%): 461 ([M+H]<sup>+</sup>, 100). HRMS  
568 (ESI) C<sub>26</sub>H<sub>20</sub>F<sub>3</sub>N<sub>4</sub>O ([M+H]<sup>+</sup>) calcd 461.1584, found 461.1584.

569

#### 570 **4.1.2.1.16**            **4-Methyl-2,6-diphenyl-N-[3-(trifluoromethoxy)phenyl]-2H-pyrazolo[4,3-** 571 **c]pyridin-7-amine 19**

572 Prepared in accordance to general procedure from 7-iodo-4-methyl-2,6-diphenyl-2H-  
573 pyrazolo[4,3-c]pyridine **3** (50 mg, 0.12 mmol), 3-(trifluoromethoxy)aniline (23 mg, 0.13 mmol),  
574 NaOtBu (18.7 mg, 0.19 mmol), SPhos (15 mg, 0.036 mmol) and Pd(OAc)<sub>2</sub> (1.7 mg, 0.012 mmol)  
575 and dioxane (4 mL). The residue was purified by column chromatography (EtOAc/PE, 1:4 to 1:2,  
576 v/v).

577 Yield: 35mg (62%), yellowish crystals, mp = 76–78 °C, R<sub>f</sub> = 0.59 (Hex:EtOAc 1:1). <sup>1</sup>H NMR (700  
578 MHz, CDCl<sub>3</sub>): δ 2.87 (3H, s, CH<sub>3</sub>), 5.99 (1H, s, NH), 6.63–6.65 (1H, m, NH-Ph 2-H), 6.65–6.67  
579 (1H, m, NH-Ph 4-H), 6.68–6.72 (1H, m, NH-Ph 6-H), 7.10–7.14 (1H, m, NH-Ph 5-H), 7.28–7.31  
580 (1H, m, C-Ph 4-H), 7.36–7.39 (2H, m, C-Ph 3,5-H), 7.42–7.45 (1H, m, N-Ph 4-H), 7.50–7.53 (2H,  
581 m, N-Ph 3,5-H), 7.69–7.72 (2H, m, C-Ph 2,6-H), 7.84–7.88 (2H, m, N-Ph 2,6-H), 8.57 (1H, s, 3-  
582 H). <sup>13</sup>C NMR (176 MHz, CDCl<sub>3</sub>): δ 22.9 (CH<sub>3</sub>), 108.8 (NH-Ph C-2), 111.7 (NH-Ph C-4), 114.7  
583 (NH-Ph C-6), 119.7 (OCF<sub>3</sub>), 121.0 (C-7), 121.1 (C-3a), 121.2 (N-Ph C-2,6), 122.0 (C-3), 127.8

584 (C-Ph C-4), 128.5 (C-Ph C-3,5), 128.6 (N-Ph C-4), 128.9 (C-Ph C-2,6), 129.5 (NH-Ph C-5), 129.6  
585 (N-Ph C-3,5), 138.8 (C-Ph C-1), 139.9 (N-Ph C-1), 142.9 (C-6), 146.1 (NH-Ph C-1 or C-3), 147.9  
586 (C-7a), 149.8 (NH-Ph C-1 or C-3), 151.4 (C-4). <sup>15</sup>N NMR (71 MHz, CDCl<sub>3</sub>): δ -304.5 (NH),  
587 -148.3 (N-2), -82.2 (N-5). IR (ν, cm<sup>-1</sup>): 3384 (NH), 3059 (CH<sub>arom</sub>), 2923, 2853 (CH<sub>aliph</sub>), 1610,  
588 1598, 1519, 1487, 1249, 1213, 1178, 1151 (C=C, C=N, C-N, C-F), 756, 697, 689 (CH=CH of  
589 mono- and disubstituted benzenes). MS (ES<sup>+</sup>): *m/z* (%): 461 ([M+H]<sup>+</sup>, 100). HRMS (ESI)  
590 C<sub>26</sub>H<sub>20</sub>F<sub>3</sub>N<sub>4</sub>O ([M+H]<sup>+</sup>) calcd 461.1584, found 461.1583.

591

## 592 **4.2. Optical measurements**

593 Stock solutions (4 mM) of the compounds were prepared in DMSO, which were further diluted to  
594 a final concentration of 4 μM in pH 7 Britton–Robinson buffer, a solution consisting of 0.04 M  
595 H<sub>3</sub>PO<sub>4</sub>, 0.04 M CH<sub>3</sub>COOH and 0.04 M H<sub>3</sub>BO<sub>3</sub>, which was adjusted to pH 7 by 0.2 M NaOH.  
596 Absorption spectra were measured using a Specord 250 Plus spectrophotometer in a 250-500 nm  
597 range with the step of 1 nm, 1 nm bandpass and 2 nm/s scan-speed. Fluorescence emission spectra  
598 were measured in a 400-700 nm range with excitation at 380 nm using Fluorolog-3 (Horiba)  
599 spectrofluorometer. Bandpasses in both the excitation and emission were set to 5 nm, the spectra  
600 were scanned with the 1 nm step and integration time 0.2 s per data point. Signal of pure solvent  
601 was subtracted as a background.

602

## 603 **4.3. Biology**

### 604 **4.3.1. Cell cultures**

605 G361 cell line (human skin melanoma) was cultivated in DMEM medium and HaCaT cell line  
606 (immortalized keratinocytes) was cultivated in DMEM with high glucose (4.5 g/L) medium.  
607 Medium was supplemented with 10% fetal bovine serum, penicillin (100 U/mL) and streptomycin  
608 (100 μg/mL) and cells were cultivated at 37 °C in 5% CO<sub>2</sub> atmosphere.

609

### 610 **4.3.2. Photodynamic treatment**

611 An in-house constructed LED based light source specifically designed for the irradiation of 96-  
612 well microplates and Petri dishes [56] was used; a maximal wavelength emission of 414 nm, light  
613 intensity set to 20 mW/cm<sup>2</sup>. The total dose of irradiation used was 1, 5 or 10 J/cm<sup>2</sup> and there were  
614 no significant changes of temperature during irradiation. For photodynamic treatments, cells were

615 seeded and next day treated with test compounds; after 4 h incubation, cells were irradiated and  
616 cultivated for further 24 h or 72 h.

617

#### 618 **4.3.3. Cell viability assay**

619 Cell viability was determined using the MTT (Sigma-Aldrich) assay in 96-well microplates (5000  
620 cells per well). The test compounds were added 24 h post plating, the cells were then incubated  
621 for additional 4 h and irradiated (the maximal wavelength emission of 414 nm and total dose of 1,  
622 5 or 10 J/cm<sup>2</sup>). After irradiation, the cells were incubated for further 72 h and then the MTT  
623 solution was added, cells were incubated for another 4 h at 37 °C and then the 0.1% SDS was  
624 added to the wells to solubilize the violet formazan crystals. The measurement of absorbance was  
625 carried out on reader Tecan Infinite M200Pro at 570 nm. Dark viability was measured in parallel  
626 under the same conditions without irradiation.

627

#### 628 **4.3.4. Measurement of reactive oxygen species (ROS) production**

629 The cells were treated for 4 h with compound **10** and then ROS was quantified using a CM-  
630 H<sub>2</sub>DCFDA (Invitrogen), which is converted to the highly fluorescent 2',7'-dichlorofluorescein  
631 (DCF) in the presence of ROS. After adding 50 µl of H<sub>2</sub>DCFDA solution (10 µl 10 µM DCF + 1  
632 ml PBS).and 25 min incubation, the cells were irradiated and the ROS production was determined  
633 by measuring fluorescence using Tecan Infinite M200Pro at 480/540 (excitation/emission) as  
634 described earlier [42].

635

#### 636 **4.3.5. Flow cytometry**

637 Asynchronous cells were seeded and, after a preincubation period, treated with test compound for  
638 4 h, irradiated and after further 24h of incubation, samples were fixed and stained with propidium  
639 iodide. DNA content was analyzed by flow cytometry using a 488 nm laser (BD FACS Verse with  
640 software BD FACSuite™, version 1.0.6.) and cell cycle distribution was analyzed using ModFit  
641 LT (Verity Software House).

642

#### 643 **4.3.6. Caspase-3/7 assay**



644 The cell lysates were incubated for 4 h with 100  $\mu$ M Ac-DEVD-AMC (the substrate of caspases 3  
645 and 7) in the assay buffer (25 mM PIPES, 2 mM EGTA, 2 mM MgCl<sub>2</sub>, 5 mM DTT, pH 7.3). The  
646 fluorescence of the product was measured using a Fluoroskan Ascent microplate reader  
647 (Labsystems) at 355/460 nm (excitation/emission).

648

#### 649 **4.3.7. Comet assay**

650 Treated G361 cells were trypsinized, rinsed by DMEM and then the cell suspension in 1% LMP  
651 agarose was pipetted to the microscope slides with agarose gel. The microscope slides were  
652 immersed in a lysis buffer with 1% Triton X for 1 h and then placed in an electrophoretic tank and  
653 dipped into a cool electrophoresis solution for 40 min. After the electrophoresis (20 V, 350 mA,  
654 20 min), the microscopic slides were immersed in a neutralisation buffer (10 min twice). The  
655 samples were then stained by SYBR Green (Invitrogen) for 15 min and scored by the SW Comet  
656 Score (TriTek Corp.).

657

#### 658 **4.3.8. Immunoblotting**

659 Cell lysates were prepared in RIPA buffer. Equal protein amounts (17  $\mu$ g/well) were loaded and  
660 separated on SDS-polyacrylamide gels, electroblotted onto nitrocellulose membranes and after  
661 blocking, overnight incubation with specific primary antibodies and incubation with peroxidase-  
662 conjugated secondary antibodies, the peroxidase activity was detected with SuperSignal West Pico  
663 reagents (Thermo Scientific) using a CCD camera LAS-4000 (Fujifilm). All primary antibodies  
664 were diluted in TBS containing 4% BSA and 0.1% Tween 20. The specific antibodies were  
665 purchased from Cell Signaling (anti-PARP, clone 46D11; anti-cleaved caspase 7; anti-Mcl-1,  
666 clone D35A5; HRP-linked secondary antibodies), Sigma Aldrich (anti- $\alpha$ -tubulin, clone DM1A),  
667 Millipore (anti-phospho-histone H2A.X, Ser139, clone JBW301).

668

#### 669 **CRedit authorship contribution statement**

670 **Beatričė Razmienė:** Investigation, Formal analysis, Writing - Original Draft. **Veronika**  
671 **Vojáčková:** Investigation, Formal analysis. **Eva Řezníčková:** Investigation, Formal analysis,  
672 Visualization. **Lukáš Malina:** Investigation, Formal analysis. **Vaida Dambrauskienė:**  
673 Investigation. **Martin Kubala:** Investigation, Formal analysis. **Robert Bajgar:** Resources,  
674 Methodology. **Hana Kolářová:** Supervision, Resources, Funding acquisition. **Asta Žukauskaitė:**

675 Supervision, Visualization, Writing - Original Draft, Writing - Review & Editing. **Eglė**  
676 **Arbačiauskienė**: Methodology, Supervision, Funding acquisition, Writing - Original Draft,  
677 Writing - Review & Editing. **Algirdas Šačkus**: Conceptualization, Resources. **Vladimír Kryštof**:  
678 Conceptualization, Supervision, Resources, Writing - Original Draft, Writing - Review & Editing.

679

#### 680 **Declaration of competing interest**

681 The authors declare that they have no known competing financial interests or personal  
682 relationships that could have appeared to influence the work reported in this paper.

683

#### 684 **Acknowledgments**

685 This work was supported by the Research Council of Lithuania (LMTLT, Agreement No. [S-MIP-  
686 20-60]) and by the Ministry of Health of the Czech Republic (No. NU21-09-00357).

687

#### 688 **References**

- 689 [1] B.W. Henderson, T.J. Dougherty, How does photodynamic therapy work?, *Photochem.*  
690 *Photobiol.* 55 (1992) 145–157. <https://doi.org/10.1111/j.1751-1097.1992.tb04222.x>.
- 691 [2] R.R. Allison, V.S. Bagnato, R. Cuenca, G.H. Downie, C.H. Sibata, The future of  
692 photodynamic therapy in oncology, *Futur. Oncol.* 2 (2006) 53–71.  
693 <https://doi.org/10.2217/14796694.2.1.53>.
- 694 [3] A.-G. Niculescu, A.M. Grumezescu, Photodynamic Therapy—An Up-to-Date Review,  
695 *Appl. Sci.* 11 (2021) 3626. <https://doi.org/10.3390/app11083626>.
- 696 [4] X. Zhao, J. Liu, J. Fan, H. Chao, X. Peng, Recent progress in photosensitizers for  
697 overcoming the challenges of photodynamic therapy: from molecular design to  
698 application, *Chem. Soc. Rev.* (2021). <https://doi.org/10.1039/d0cs00173b>.
- 699 [5] I.O. de Albuquerque, J. Nunes, J.P. Figueiró Longo, L.A. Muehlmann, R.B. Azevedo,  
700 Photodynamic therapy in superficial basal cell carcinoma treatment, *Photodiagnosis*  
701 *Photodyn. Ther.* 27 (2019) 428–432. <https://doi.org/10.1016/j.pdpdt.2019.07.017>.
- 702 [6] D.M. Ozog, A.M. Rkein, S.G. Fabi, M.H. Gold, M.P. Goldman, N.J. Lowe, G.M. Martin,  
703 G.S. Munavalli, Photodynamic Therapy: A Clinical Consensus Guide, *Dermatologic Surg.*  
704 42 (2016) 804–827. <https://doi.org/10.1097/DSS.0000000000000800>.
- 705 [7] M. Olek, J. Kasperski, D. Skaba, R. Wiench, G. Cieślak, A. Kawczyk-Krupka,

- 706 Photodynamic therapy for the treatment of oral squamous carcinoma—Clinical  
707 implications resulting from in vitro research, *Photodiagnosis Photodyn. Ther.* 27 (2019)  
708 255–267. <https://doi.org/10.1016/j.pdpdt.2019.06.012>.
- 709 [8] G. Shafirstein, A. Battoo, K. Harris, H. Baumann, S.O. Gollnick, J. Lindenmann, C.E.  
710 Nwogu, Photodynamic therapy of non-small cell lung cancer narrative review and future  
711 directions, *Ann. Am. Thorac. Soc.* 13 (2016) 265–275.  
712 <https://doi.org/10.1513/AnnalsATS.201509-650FR>.
- 713 [9] C. Nwogu, P. Pera, W. Bshara, K. Attwood, R. Pandey, Photodynamic therapy of human  
714 lung cancer xenografts in mice, *J. Surg. Res.* 200 (2015) 8–12.  
715 <https://doi.org/10.1016/j.jss.2015.07.024>.
- 716 [10] D.T. Mlynarczyk, J. Piskorz, L. Popenda, M. Stolarska, W. Szczolko, K. Konopka, S.  
717 Jurga, L. Sobotta, J. Mielcarek, N. Düzgüneş, T. Goslinski, S-seco-porphyrzine as a new  
718 member of the seco-porphyrzine family – Synthesis, characterization and  
719 photocytotoxicity against cancer cells, *Bioorg. Chem.* 96 (2020) 103634.  
720 <https://doi.org/10.1016/j.bioorg.2020.103634>.
- 721 [11] E. Ostańska, D. Aebisher, D. Bartusik-Aebisher, The potential of photodynamic therapy in  
722 current breast cancer treatment methodologies, *Biomed. Pharmacother.* 137 (2021)  
723 111302. <https://doi.org/10.1016/j.biopha.2021.111302>.
- 724 [12] T. Gheewala, T. Skwor, G. Munirathinam, Photosensitizers in prostate cancer therapy,  
725 *Oncotarget.* 8 (2017) 30524–30538. <https://doi.org/10.18632/oncotarget.15496>.
- 726 [13] F.J. Civantos, B. Karakullukcu, M. Biel, C.E. Silver, A. Rinaldo, N.F. Saba, R.P. Takes,  
727 V. Vander Poorten, A. Ferlito, A Review of Photodynamic Therapy for Neoplasms of the  
728 Head and Neck, *Adv. Ther.* 35 (2018) 324–340. [https://doi.org/10.1007/s12325-018-0659-](https://doi.org/10.1007/s12325-018-0659-3)  
729 3.
- 730 [14] M. Boen, J. Brownell, P. Patel, M.M. Tsoukas, The Role of Photodynamic Therapy in  
731 Acne: An Evidence-Based Review, *Am. J. Clin. Dermatol.* 18 (2017) 311–321.  
732 <https://doi.org/10.1007/s40257-017-0255-3>.
- 733 [15] R. Wormald, J. Evans, L. Smeeth, K. Henshaw, Photodynamic therapy for neovascular  
734 age-related macular degeneration, in: *Cochrane Database Syst. Rev.*, John Wiley & Sons,  
735 Ltd, Chichester, UK, 2003. <https://doi.org/10.1002/14651858.CD002030>.
- 736 [16] B.J. Qumseya, W. David, H.C. Wolfsen, Photodynamic Therapy for Barrett’s Esophagus

- 737 and Esophageal Carcinoma, *Clin. Endosc.* 46 (2013) 30.  
738 <https://doi.org/10.5946/ce.2013.46.1.30>.
- 739 [17] S. Houthoofd, M. Vuylsteke, S. Mordon, I. Fourneau, Photodynamic therapy for  
740 atherosclerosis. The potential of indocyanine green, *Photodiagnosis Photodyn. Ther.* 29  
741 (2020) 101568. <https://doi.org/10.1016/j.pdpdt.2019.10.003>.
- 742 [18] M. Glueck, C. Hamminger, M. Fefer, J. Liu, K. Plaetzer, Save the crop: Photodynamic  
743 Inactivation of plant pathogens I: bacteria, *Photochem. Photobiol. Sci.* 18 (2019) 1700–  
744 1708. <https://doi.org/10.1039/C9PP00128J>.
- 745 [19] E. Polat, K. Kang, Natural Photosensitizers in Antimicrobial Photodynamic Therapy,  
746 *Biomedicines.* 9 (2021) 584. <https://doi.org/10.3390/biomedicines9060584>.
- 747 [20] D. de C.R. Picco, L.L.R. Cavalcante, R.L.B. Trevisan, A.E. Souza-Gabriel, M.C. Borsatto,  
748 S.A.M. Corona, Effect of curcumin-mediated photodynamic therapy on *Streptococcus*  
749 *mutans* and *Candida albicans*: A systematic review of in vitro studies, *Photodiagnosis*  
750 *Photodyn. Ther.* 27 (2019) 455–461. <https://doi.org/10.1016/j.pdpdt.2019.07.010>.
- 751 [21] L. Polo, A. Segalla, G. Bertoloni, G. Jori, K. Schaffner, E. Reddi, Polylysine–porphycene  
752 conjugates as efficient photosensitizers for the inactivation of microbial pathogens, *J.*  
753 *Photochem. Photobiol. B Biol.* 59 (2000) 152–158. [https://doi.org/10.1016/S1011-](https://doi.org/10.1016/S1011-1344(01)00114-2)  
754 [1344\(01\)00114-2](https://doi.org/10.1016/S1011-1344(01)00114-2).
- 755 [22] Z. Malá, L. Žárská, L. Malina, K. Langová, R. Večeřová, M. Kolář, P. Henke, J.  
756 Mosinger, H. Kolářová, Photodynamic effect of TPP encapsulated in polystyrene  
757 nanoparticles toward multi-resistant pathogenic bacterial strains: AFM evaluation, *Sci.*  
758 *Rep.* 11 (2021) 6786. <https://doi.org/10.1038/s41598-021-85828-9>.
- 759 [23] P. Agostinis, K. Berg, K.A. Cengel, T.H. Foster, A.W. Girotti, S.O. Gollnick, S.M. Hahn,  
760 M.R. Hamblin, A. Juzeniene, D. Kessel, M. Korbelik, J. Moan, P. Mroz, D. Nowis, J.  
761 Piette, B.C. Wilson, J. Golab, Photodynamic therapy of cancer: An update, *CA. Cancer J.*  
762 *Clin.* 61 (2011) 250–281. <https://doi.org/10.3322/caac.20114>.
- 763 [24] M. Dichiara, O. Prezzavento, A. Marrazzo, V. Pittalà, L. Salerno, A. Rescifina, E. Amata,  
764 Recent advances in drug discovery of phototherapeutic non-porphyrinic anticancer agents,  
765 *Eur. J. Med. Chem.* 142 (2017) 459–485. <https://doi.org/10.1016/J.EJMECH.2017.08.070>.
- 766 [25] S.M. Usama, S. Thavornpradit, K. Burgess, Optimized Heptamethine Cyanines for  
767 Photodynamic Therapy, *ACS Appl. Bio Mater.* 1 (2018) 1195–1205.

- 768 <https://doi.org/10.1021/acsabm.8b00414>.
- 769 [26] J. Atchison, S. Kamila, H. Nesbitt, K.A. Logan, D.M. Nicholas, C. Fowley, J. Davis, B.  
770 Callan, A.P. McHale, J.F. Callan, Iodinated cyanine dyes: a new class of sensitizers for  
771 use in NIR activated photodynamic therapy (PDT), *Chem. Commun.* 53 (2017) 2009–  
772 2012. <https://doi.org/10.1039/C6CC09624G>.
- 773 [27] C. Shirata, J. Kaneko, Y. Inagaki, T. Kokudo, M. Sato, S. Kiritani, N. Akamatsu, J. Arita,  
774 Y. Sakamoto, K. Hasegawa, N. Kokudo, Near-infrared photothermal/photodynamic  
775 therapy with indocyanine green induces apoptosis of hepatocellular carcinoma cells  
776 through oxidative stress, *Sci. Rep.* 7 (2017) 13958. <https://doi.org/10.1038/s41598-017-14401-0>.
- 778 [28] J. Zhang, C. Jiang, J.P. Figueiró Longo, R.B. Azevedo, H. Zhang, L.A. Muehlmann, An  
779 updated overview on the development of new photosensitizers for anticancer  
780 photodynamic therapy, *Acta Pharm. Sin. B.* 8 (2018) 137–146.  
781 <https://doi.org/10.1016/J.APSB.2017.09.003>.
- 782 [29] A. Kamkaew, S.H. Lim, H.B. Lee, L.V. Kiew, L.Y. Chung, K. Burgess, BODIPY dyes in  
783 photodynamic therapy, *Chem. Soc. Rev.* 42 (2013) 77–88.  
784 <https://doi.org/10.1039/C2CS35216H>.
- 785 [30] J.-Y. Liu, P.-Z. Zhou, J.-L. Ma, X. Jia, J.-Y. Liu, P.-Z. Zhou, J.-L. Ma, X. Jia,  
786 Trifluoromethyl Boron Dipyrromethene Derivatives as Potential Photosensitizers for  
787 Photodynamic Therapy, *Molecules.* 23 (2018) 458.  
788 <https://doi.org/10.3390/molecules23020458>.
- 789 [31] M. Zhao, Y. Xu, M. Xie, L. Zou, Z. Wang, S. Liu, Q. Zhao, Halogenated Aza-BODIPY  
790 for Imaging-Guided Synergistic Photodynamic and Photothermal Tumor Therapy, *Adv.*  
791 *Healthc. Mater.* 7 (2018) 1800606. <https://doi.org/10.1002/adhm.201800606>.
- 792 [32] W. Lv, S. Chi, W. Feng, T. Liang, D. Song, Z. Liu, Development of a red absorbing Se-  
793 rhodamine photosensitizer and its application for bio-orthogonally activatable  
794 photodynamic therapy, *Chem. Commun.* 55 (2019) 7037–7040.  
795 <https://doi.org/10.1039/C9CC03018B>.
- 796 [33] K.S. Davies, M.K. Linder, M.W. Kryman, M.R. Detty, Extended rhodamine  
797 photosensitizers for photodynamic therapy of cancer cells, *Bioorg. Med. Chem.* 24 (2016)  
798 3908–3917. <https://doi.org/10.1016/j.bmc.2016.05.033>.

- 799 [34] Q. Zou, Y. Fang, Y. Zhao, H. Zhao, Y. Wang, Y. Gu, F. Wu, Synthesis and in Vitro  
800 Photocytotoxicity of Coumarin Derivatives for One- and Two-Photon Excited  
801 Photodynamic Therapy, *J. Med. Chem.* 56 (2013) 5288–5294.  
802 <https://doi.org/10.1021/jm400025g>.
- 803 [35] P.S. Saneesh Babu, P.M. Manu, T.J. Dhanya, P. Tapas, R.N. Meera, A. Surendran, K.A.  
804 Aneesh, S.J. Vadakkancheril, D. Ramaiah, S.A. Nair, M.R. Pillai, Bis(3,5-diiodo-2,4,6-  
805 trihydroxyphenyl)squaraine photodynamic therapy disrupts redox homeostasis and induce  
806 mitochondria-mediated apoptosis in human breast cancer cells, *Sci. Rep.* 7 (2017) 42126.  
807 <https://doi.org/10.1038/srep42126>.
- 808 [36] S.G. Kucukguzel, S. Senkardes, Recent advances in bioactive pyrazoles, *Eur. J. Med.*  
809 *Chem.* 97 (2015) 786–815. <https://doi.org/10.1016/j.ejmech.2014.11.059>.
- 810 [37] J.V. Faria, P.F. Vegi, A.G.C. Migueta, M.S. dos Santos, N. Boechat, A.M.R. Bernardino,  
811 Recently reported biological activities of pyrazole compounds, *Bioorg. Med. Chem.* 25  
812 (2017) 5891–5903. <https://doi.org/10.1016/J.BMC.2017.09.035>.
- 813 [38] M.F. Khan, M.M. Alam, G. Verma, W. Akhtar, M. Akhter, M. Shaquiquzzaman, The  
814 therapeutic voyage of pyrazole and its analogs: A review, *Eur. J. Med. Chem.* 120 (2016)  
815 170–201. <https://doi.org/10.1016/j.ejmech.2016.04.077>.
- 816 [39] L.L. Yang, X.F. Li, X.L. Hu, X.Y. Yu, Simple and efficient synthesis of pyrazole-fused  
817 porphyrins, *Tetrahedron Lett.* 57 (2016) 1265–1267.  
818 <https://doi.org/10.1016/j.tetlet.2016.02.020>.
- 819 [40] V. Spanò, B. Parrino, A. Carbone, A. Montalbano, A. Salvador, P. Brun, D. Vedaldi, P.  
820 Diana, G. Cirrincione, P. Barraja, Pyrazolo[3,4-h]quinolines promising photosensitizing  
821 agents in the treatment of cancer, *Eur. J. Med. Chem.* 102 (2015) 334–351.  
822 <https://doi.org/10.1016/j.ejmech.2015.08.003>.
- 823 [41] N.A.M. Pereira, M. Laranjo, M. Pineiro, A.C. Serra, K. Santos, R. Teixeira, A.M. Abrantes,  
824 A.C. Gonçalves, A.B. Sarmiento Ribeiro, J. Casalta-Lopes, M.F. Botelho, T.M.V.D. Pinho  
825 E Melo, Novel 4,5,6,7-tetrahydropyrazolo[1,5-a]pyridine fused chlorins as very active  
826 photodynamic agents for melanoma cells, *Eur. J. Med. Chem.* 103 (2015) 374–380.  
827 <https://doi.org/10.1016/j.ejmech.2015.08.059>.
- 828 [42] G. Varvuolytė, L. Malina, A. Bieliauskas, B. Hošíková, H. Simerská, H. Kolářová, N.  
829 Kleizienė, V. Kryštof, A. Šačkus, A. Žukauskaitė, Synthesis and photodynamic properties

- 830 of pyrazole-indole hybrids in the human skin melanoma cell line G361, *Dye. Pigment.* 183  
831 (2020) 108666. <https://doi.org/10.1016/j.dyepig.2020.108666>.
- 832 [43] V. Milišiūnaitė, A. Kadlecová, A. Žukauskaitė, K. Doležal, M. Strnad, J. Voller, E.  
833 Arbačiauskienė, W. Holzer, A. Šačkus, Synthesis and anthelmintic activity of  
834 benzopyrano[2,3-*c*]pyrazol-4(2H)-one derivatives, *Mol. Divers.* 24 (2020) 1025–1042.  
835 <https://doi.org/10.1007/s11030-019-10010-3>.
- 836 [44] V. Milišiūnaitė, R. Paulavičiūtė, E. Arbačiauskienė, V. Martynaitis, W. Holzer, A. Šačkus,  
837 Synthesis of 2 *H* -furo[2,3- *c*]pyrazole ring systems through silver(I) ion-mediated ring-  
838 closure reaction, *Beilstein J. Org. Chem.* 15 (2019) 679–684.  
839 <https://doi.org/10.3762/bjoc.15.62>.
- 840 [45] V. Milišiūnaitė, E. Arbačiauskienė, E. Řezníčková, R. Jorda, V. Malínková, A.  
841 Žukauskaitė, W. Holzer, A. Šačkus, V. Kryštof, Synthesis and anti-mitotic activity of 2,4-  
842 or 2,6-disubstituted- and 2,4,6-trisubstituted-2H-pyrazolo[4,3-*c*]pyridines, *Eur. J. Med.*  
843 *Chem.* 150 (2018) 908–919. <https://doi.org/https://doi.org/10.1016/j.ejmech.2018.03.037>.
- 844 [46] B. Razmienė, E. Řezníčková, V. Dambrauskienė, R. Ostruszka, M. Kubala, A.  
845 Žukauskaitė, V. Kryštof, A. Šačkus, E. Arbačiauskienė, Synthesis and Antiproliferative  
846 Activity of 2,4,6,7-Tetrasubstituted-2H-pyrazolo[4,3-*c*]pyridines, *Molecules.* 26 (2021)  
847 6747. <https://doi.org/10.3390/molecules26216747>.
- 848 [47] E. Arbačiauskienė, V. Laukaitytė, W. Holzer, A. Šačkus, Metal-Free Intramolecular  
849 Alkyne-Azide Cycloaddition To Construct the Pyrazolo[4,3-*f*][1,2,3]triazolo[5,1-*c*]  
850 [[1,4]oxazepine Ring System, *European J. Org. Chem.* 2015 (2015) 5663–5670.  
851 <https://doi.org/10.1002/ejoc.201500541>.
- 852 [48] V. Milišiūnaitė, E. Arbačiauskienė, A. Bieliauskas, G. Vilkauskaitė, A. Šačkus, W.  
853 Holzer, Synthesis of pyrazolo[4',3':3,4]pyrido[1,2-*a*]benzimidazoles and related new ring  
854 systems by tandem cyclisation of vic-alkynylpyrazole-4-carbaldehydes with (het)aryl-1,2-  
855 diamines and investigation of their optical properties, *Tetrahedron.* 71 (2015) 3385–3395.  
856 <https://doi.org/10.1016/J.TET.2015.03.092>.
- 857 [49] W. Holzer, G. Vilkauskaitė, E. Arbačiauskienė, A. Šačkus, Dipyrazolo[1,5-*a*:4',3'-  
858 *c*]pyridines - a new heterocyclic system accessed via multicomponent reaction, *Beilstein J*  
859 *Org Chem.* 8 (2012) 2223–2229. <https://doi.org/10.3762/bjoc.8.251>.
- 860 [50] G.A. Eller, G. Vilkauskaitė, E. Arbačiauskienė, A. Šačkus, W. Holzer, Sonogashira

861 Coupling Offers a New Synthetic Route to Thieno[2,3- c ]pyrazoles, *Synth. Commun.* 41  
862 (2011) 541–547. <https://doi.org/10.1080/00397911003629382>.

863 [51] A. Bieliauskas, S. Krikštolaitytė, W. Holzer, A. Šačkus, Ring-closing metathesis as a key  
864 step to construct 2,6-dihydropyrano[2,3-c]pyrazole ring system, *Arkivoc.* 2018 (2018)  
865 296–307. <https://doi.org/10.24820/ark.5550190.p010.407>.

866 [52] V. Milišiūnaitė, E. Plytninkienė, R. Bakšienė, A. Bieliauskas, S. Krikštolaitytė, G.  
867 Račkauskienė, E. Arbačiauskienė, A. Šačkus, Convenient Synthesis of  
868 Pyrazolo[4',3':5,6]pyrano[4,3-c][1,2]oxazoles via Intramolecular Nitrile Oxide  
869 Cycloaddition, *Molecules.* 26 (2021) 5604. <https://doi.org/10.3390/molecules26185604>.

870 [53] E. Arbačiauskienė, V. Martynaitis, S. Krikštolaitytė, W. Holzer, A. Šačkus, Synthesis of  
871 3-substituted 1-phenyl-1H-pyrazole-4-carbaldehydes and the corresponding ethanones by  
872 Pd-catalysed cross-coupling reactions, *Arkivoc.* (2011) 1–21. [internal-pdf://11-6643zp-4-  
873 2269486342/11-6643ZP-4.pdf](https://doi.org/10.1080/15226948634211-6643ZP-4).

874 [54] N. Proisy, S. Taylor, A. Nelson, I. Collins, Rapid Synthesis of 3-Aminoisoquinoline-5-  
875 sulfonamides Using the Buchwald-Hartwig Reaction, *Synthesis (Stuttg).* 2009 (2009)  
876 561–566. <https://doi.org/10.1055/s-0028-1083336>.

877 [55] S. Kwiatkowski, B. Knap, D. Przystupski, J. Saczko, E. Kędzierska, K. Knap-Czop, J.  
878 Kotlińska, O. Michel, K. Kotowski, J. Kulbacka, Photodynamic therapy – mechanisms,  
879 photosensitizers and combinations, *Biomed. Pharmacother.* 106 (2018) 1098–1107.  
880 <https://doi.org/10.1016/j.biopha.2018.07.049>.

881 [56] R. Bajgar, H. Kolarova, P. Kolar, K. Pizova, A. Hanakova, Light source intended  
882 particularly for in vitro creating and monitoring photodynamic phenomena, *CZ28377U1,*  
883 2015.

884 [57] D.L. Sai, J. Lee, D.L. Nguyen, Y.-P. Kim, Tailoring photosensitive ROS for advanced  
885 photodynamic therapy, *Exp. Mol. Med.* 53 (2021) 495–504.  
886 <https://doi.org/10.1038/s12276-021-00599-7>.

887 [58] J. Liebmann, M. Born, V. Kolb-Bachofen, Blue-Light Irradiation Regulates Proliferation  
888 and Differentiation in Human Skin Cells, *J. Invest. Dermatol.* 130 (2010) 259–269.  
889 <https://doi.org/10.1038/jid.2009.194>.

890