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Synthesis of *N*-alkyl substituted bioactive indolocarbazoles related to Gö6976

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Abstract—The syntheses of new nitrile and amide analogues of 7-keto Gö6976 are described. The amide analogue **22** was formed via the condensation with a new functionalized indoleacetic acid derivative **25** to overcome the solubility problem during the coupling reaction.

1. Introduction

Cell cycle checkpoints are activated in response to DNA damage thereby delaying cell cycle progression in order to provide more time for DNA repair. Cell cycle arrest in G1 or S phase prevents replication of damaged DNA, while arrest in G2 prevents damaged chromosomes from being segregated in mitosis; thus preventing the propagation of genetic abnormalities. Inhibition of the G2 checkpoint has attracted widespread interest because most cancer cells have an inoperative G1 checkpoint. The activity of the G1 checkpoint is dependent on the p53 tumor suppressor protein, which is deleted or mutated in more than 50% of all cancers. Although cells with defective p53 are unable to activate the G1 checkpoint in response to DNA damage, they retain the ability to arrest in S and G2. This provides the cells with an opportunity to repair their DNA and thereby survive and grow. The S and G2 checkpoints are regulated by various kinases among which checkpoint kinase 1 (Chk1) plays a major role. Inhibitors of Chk1 preferentially abrogate cell cycle arrest in p53-defective cells and selectively sensitize cancer cells with mutated p53 to killing by DNA-damaging agents. Therefore, combining a Chk1 inhibitor with a DNA-damaging agent should selectively drive p53-defective cells into a premature and lethal mitosis.[1]

Several small molecules that inhibit checkpoint proteins have been described in the literature, the first of which were the purine alkaloids caffeine (**1**) and pentoxifylline (**2**), which inhibit two upstream kinases, ATM and ATR (Fig. 1).[2]

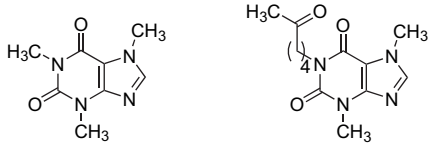

Figure 1.

However, caffeine cannot be used to inhibit this pathway in human beings as the doses required cause central nervous system and cardiac toxicities.

UCN-01 (**3**), one member of the indolocarbazole family,[3] generated considerable interest in our laboratory when it was found to be a potent inhibitor of DNA damage-induced S and G2 cell cycle checkpoints, which led to increased killing of tumor cells (Fig. 2).[4] Although UCN-01 is well recognized as a protein kinase C inhibitor,[5] this checkpoint inhibition was attributed to its ability to inhibit Chk1.[6] Unfortunately, UCN-01 binds avidly to human serum proteins thereby compromising its potential therapeutic activity.[7] Subsequent research identified other Chk1 inhibitors such as staurosporine (**4**),[2] granulatimide (**5**), and isogranulatimide (**6**),[8] but their nonselectivity and/or their weak inhibitory activity justifies the search for new selective Chk1 inhibitors. Accordingly, we initiated a synthetic program to develop novel analogues rationally designed to overcome the obstacles observed with the other analogues and with improved therapeutic potential.

Initially, a K252a (**7**) analogue, ICP-1 (**8**), was synthesized and tested, and was found to overcome the problem of protein binding but it has considerably reduced potency (Fig. 3).[9]

Keywords: Indolocarbazoles; Bisindolylmaleimide; Gö6976; UCN-01; Checkpoint inhibitors.
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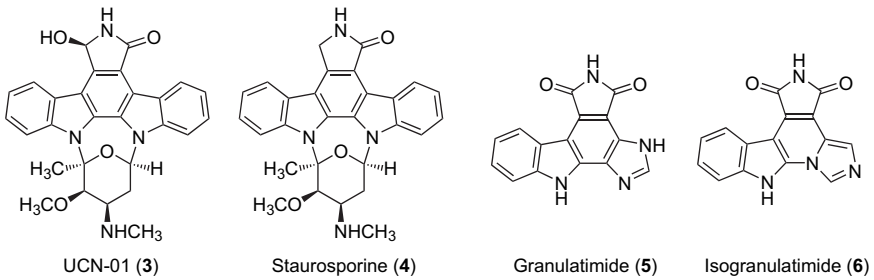


Figure 2.

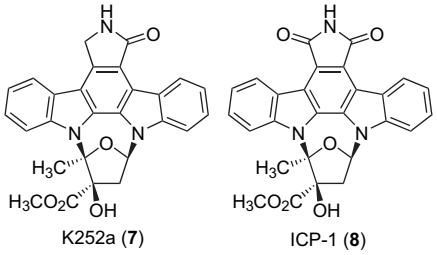


Figure 3.

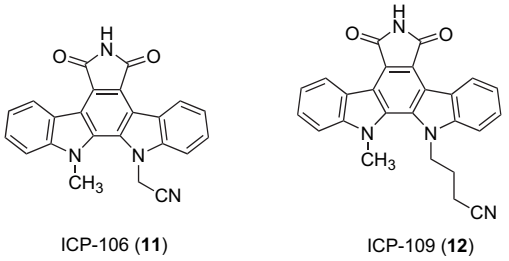


Figure 5.

More recently, we found that Gö6976 (**9**) is a very potent checkpoint inhibitor even in the presence of human serum,[10] and this has also been attributed to the inhibition of Chk1 (Fig. 4).[11] Additionally, Gö6976 abrogated S and G2 arrest at a concentration substantially lower than that required to inhibit PKC. Interestingly, UCN-01 (**3**) did not demonstrate this selectivity for checkpoint inhibition. As a consequence, we have begun a structure–activity study of analogues of Gö6976 (**9**). During our screening to identify novel inhibitors of Chk1 related to Gö6976, we found that ICP-103 (**10**) is also a potent checkpoint inhibitor.[12] Therefore, we have focused our investigation on this class of molecules as potential inhibitors of Chk1. Our initial objective was to investigate the effect of the nitrile chain-length on Chk1 activity, and we now report our results.

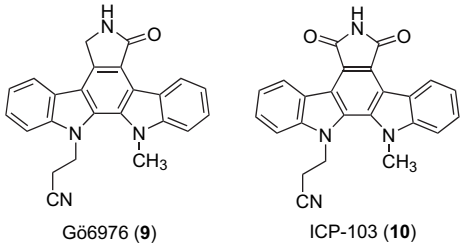


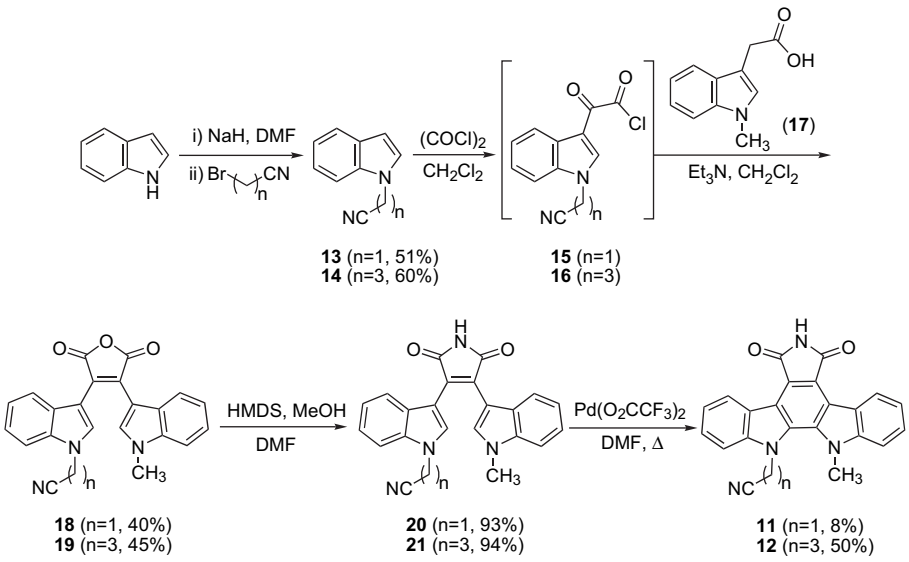
Figure 4.

2. Results and discussion

In the course of our structure–activity relationship studies on ICP-103 analogues, we have synthesized and tested two new nitrile homologues, ICP-106 (**11**) and ICP-109 (**12**) (Fig. 5).

Thus, for the synthesis of **11** and **12**, we alkylated the indole nitrogen with bromoacetonitrile and 4-bromobutyronitrile in the presence of NaH to furnish indole-1-acetonitrile (**13**) and indole-1-butanenitrile (**14**), respectively. Compound **13** was obtained in 51% yield and compound **14** was obtained in 60% yield (Scheme 1). Similar N-alkylation of indole-3-acetic acid using methyl iodide in the presence of excess NaH gave 1-methylindole-3-acetic acid (**17**) in 94% yield. Indole-1-acetonitrile (**13**) was then treated with oxalyl chloride in dichloromethane to furnish the glyoxylyl chloride **15**, which was immediately treated with 1-methylindole-3-acetic acid (**17**) in the presence of triethylamine to produce anhydride **18** in 40% yield in two steps from **13**.[13] Similarly, indole-1-butanenitrile (**14**) furnished **19** in 45% yield via the intermediate glyoxylyl chloride **16**. Due to the potentially labile nitrile functionality, ammonia was generated in situ from 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and methanol and these conditions were used to convert anhydrides **18** and **19** to imides **20** and **21**, respectively, at rt.[14] During our synthesis of ICP-103 (**10**), we found that the final oxidative cyclization was quite challenging for the bisindolylmaleimide with substituents present on both *N*-12 and *N*-13.[12,15,16] However, heating bisindolylmaleimide **20** and **21** in DMF in the presence of palladium(II) trifluoroacetate gave the target compounds **11** and **12** in 8 and 50% yields, respectively. The lower yield of **11** may be a consequence of the strong inductive electron withdrawing effect of the cyano group that retards the (electrophilic) oxidative addition of Pd(II) to bisindolylmaleimide **20**.

In work to be reported separately, we find that ICP-106 (**11**) and ICP-109 (**12**) are less potent than ICP-103 (**10**) at abrogating DNA damage-induced cell cycle arrest. In an assay using flow cytometry analysis,[10] ICP-106 and ICP-109 were found to abrogate S phase arrest at 3 μM and 10 μM, respectively, whereas ICP-103 (**10**) was effective at 100 nM. These values can be compared to the efficacy of Gö6976 (**9**) of 30 nM in the same assay.[10] For drugs to be used in patients, it is also essential that they do not elicit undesirable toxicities. In this regard, the breast cancer MDA-MB-231 cell line was used in an assay of growth inhibition. Cells were



Scheme 1.

incubated with the compounds for 24 h, and the cell number assessed after 7 days. The concentration that inhibited 50% of growth was: Gö6976, 6 μM; ICP-103, 2.5 μM; ICP-106, >10 μM; ICP-109, 2 μM. Accordingly, the concentrations that inhibit growth do not correlate with those that overcome cell cycle arrest, and Gö6976 and ICP-103 are clearly active as checkpoint inhibitors at noncytotoxic concentrations.

From this activity data for the nitrile analogues **10**, **11**, and **12**, it was found that two methylene groups provide maximum activity. Therefore, we attempted to synthesize a novel amide analogue of ICP-103, ICP-112 (**22**), bearing the same number of carbons in the amide arm (Fig. 6). We wanted to further explore the SAR by replacing the nitrile with an amide.

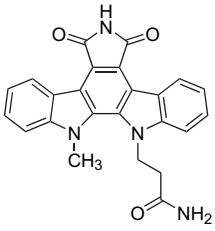
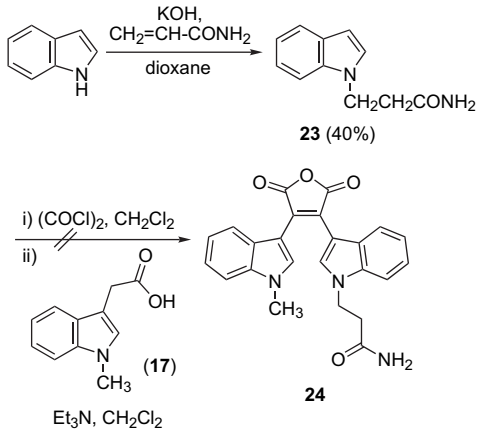


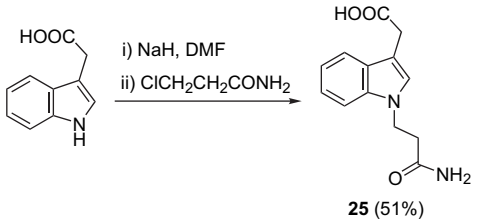
Figure 6.

For the synthesis of ICP-112 (**22**), we initially proceeded through the same route that we used for the nitrile compounds. For this purpose, indole-1-propionamide (**23**) was synthesized from indole and acrylamide in the presence of KOH in 40% yield (Scheme 2). We then treated **23** with oxalyl chloride to prepare the corresponding glyoxylyl chloride that was to be coupled with 1-methylindole-3-acetic acid (**17**) in the presence of triethylamine. To our surprise, the desired anhydride **24** was not formed. The coupling reaction failed probably due to the highly insoluble nature of the glyoxylyl chloride intermediate bearing the polar amide functionality. Use of more polar solvents, such as 1,2-dichloroethane, THF, and DMF, also failed to furnish the desired coupling product **24**.

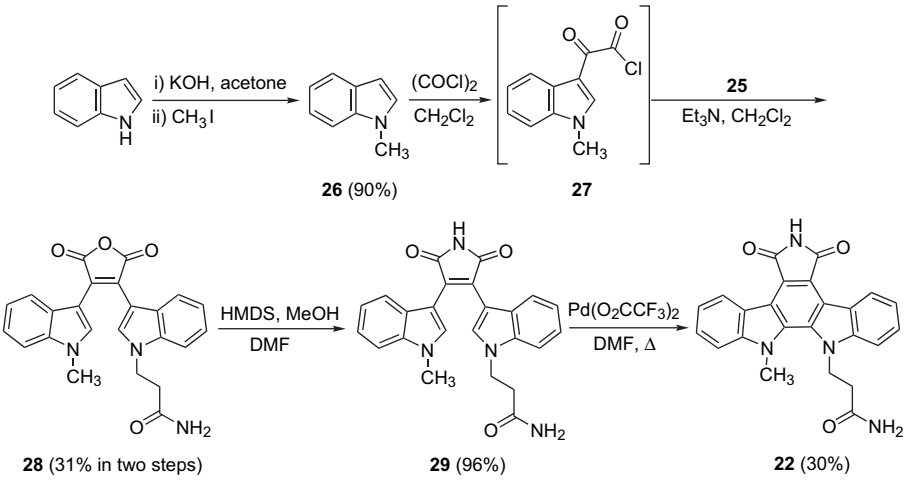


Scheme 2.

To possibly circumvent this solubility issue in the coupling reaction, we decided to attach the amide-chain on the indole-3-acetic acid fragment. Although there does not appear to be literature precedent for these compounds, we were able to synthesize 1-(2-carbamoyl-ethyl)indole-3-acetic acid (**25**) from indole-3-acetic acid in one step, using 3-chloropropionamide as an alkylating agent in the presence of excess NaH (51% yield) (Scheme 3).



Scheme 3.



Scheme 4.

Compound **25** was treated with 1-methylindole-3-glyoxylyl chloride (**27**), derived from the reaction between 1-methylindole (**26**) and oxalyl chloride, to form the desired anhydride **28** in 31% yield over the two steps (Scheme 4). Anhydride **28** was treated with HMDS and methanol to give imide **29** in 96% yield. Heating **29** in DMF with palladium(II) trifluoroacetate afforded the target compound ICP-112 (**22**) in 30% yield. An alternative oxidative cyclization of **29** using ultraviolet light in the presence of a catalytic amount of iodine in THF:acetonitrile (1:1) yielded **22** only in 5% yield.[17]

The new indolocarbazole ICP-112 (**22**) abrogated S phase arrest at 1–3 μM. This value should be compared to the 100 nM efficacy observed with the nitrile analogue, ICP-103 (**10**). Accordingly, it appears that the nitrile is the preferred structure for further study. Our detailed biological activity data will be reported separately.

3. Conclusion

In summary, we have explored one aspect of the structure–activity relationships (SARs) of Gö6976 and discovered that a three-carbon nitrile chain seems essential for optimal activity. Also, we find that a nitrile is the more desirable functionality than an amide for activity. Work is in progress in our laboratory with other nitrogen-bearing functionalities and these will be reported in due course.

4. Experimental

4.1. General experimental procedures

Melting points were determined with a Mel-Temp Laboratory Device apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 600 series FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on either a Varian XL-300 or 500 Fourier-transform NMR spectrometer. Both low- and high-resolution mass spectra were carried out at the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois at Urbana Champaign. Anhydrous THF and CH_2Cl_2 were prepared by a solvent purification system; anhydrous DMF and Et_3N were purchased from Aldrich.

4.1.1. 1*H*-Indole-1-acetonitrile (13). To a stirred suspension of NaH (600 mg, 15 mmol, 60% dispersion in mineral oil) in DMF (35 mL) at 0 °C was added dropwise a solution of indole (1.17 g, 10 mmol) in DMF (15 mL). After stirring the mixture for 30 min at 0 °C, a solution of bromoacetonitrile (1.1 mL, 15 mmol) in DMF (15 mL) was added dropwise. The mixture was allowed to slowly reach rt and continued to stir overnight. The mixture was poured into cold water (100 mL) and extracted with ether (3×50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (3:1 hexane:ethyl acetate) to give the desired product (796 mg, 51%) as a colorless oil, which solidified after prolonged drying: mp 75–77 °C (lit.[18] 74–75 °C); IR (thin film) 3097, 2978, 2941, 2252, 1611, 1462, 1317 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.75–7.71 (m, 1H), 7.44–7.35 (m, 2H), 7.31–7.25 (m, 1H), 7.13 (d, 1H, J=3.4 Hz), 6.67 (dd, 1H, J=3.2 Hz, 0.7 Hz), 4.99 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 129.1, 127.3, 123.1, 121.7, 121.0, 114.6, 109.0, 104.3, 34.4.

4.1.2. 1*H*-Indole-1-butanenitrile (14). To a stirred suspension of NaH (600 mg, 15 mmol, 60% dispersion in mineral oil) in DMF (35 mL) at 0 °C was added dropwise a solution of indole (1.17 g, 10 mmol) in DMF (15 mL). After stirring the mixture for 30 min at 0 °C, a solution of bromobutyronitrile (1.6 mL, 15 mmol) in DMF (15 mL) was added dropwise. The mixture was allowed to slowly reach rt and continued to stir overnight. The mixture was poured into cold water (100 mL) and extracted with ethyl acetate (3×50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (2:1 hexane: ethyl acetate) to give the desired product (1.1 g, 60%) as a colorless oil:[19] IR (thin film) 3051, 2939, 2246, 1610, 1512, 1463, 1314 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.77–7.74 (m, 1H), 7.45–7.42 (m, 1H), 7.37–7.31 (m, 1H), 7.27–7.22 (m, 1H), 7.17 (d, 1H, J=3.2 Hz), 6.63 (dd, 1H, J=3.2 Hz, 0.7 Hz), 4.35–4.31 (m, 2H), 2.27–2.18 (m, 4H); ^{13}C NMR ($CDCl_3$): δ 135.8, 128.8, 127.8, 122.0, 121.3, 119.8, 118.9, 109.2, 102.1, 44.4, 26.0, 14.6.

4.1.3. 1-Methyl-1*H*-indole-3-acetic acid (17). To a stirred suspension of NaH (6.0 g, 150 mmol, 60% dispersion in mineral oil) in THF (125 mL) at 0 °C was added a solution of indole-3-acetic acid (5.25 g, 30 mmol) in THF (50 mL). After stirring the mixture for 30 min at 0 °C, a solution of methyl iodide (14.2 g, 100 mmol) in THF (50 mL) was added dropwise. The mixture was allowed to slowly reach rt and continued to stir for 16 h. The reaction mixture was then cooled to 0 °C and excess hydride was carefully destroyed by slow addition of MeOH (5 mL) with vigorous stirring followed by cold water until a clear yellow solution resulted. Ether (100 mL) was added. The aqueous phase was separated, acidified with 6 N HCl, and extracted with dichloromethane (3×100 mL). The combined dichloromethane extracts were dried (Na_2SO_4) and concentrated to about 40–50 mL. Pet ether was then added slowly until a brownish colored solid completely precipitated out. The crude solid was recrystallized from ethanol to give the desired product (5.33 g, 94%) as a pale brown solid: mp 127–128 °C (lit.[20] 127–129 °C); IR (thin film) 3059, 2933, 1699, 1617, 1474 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.64–7.62 (m, 1H), 7.34–7.26 (m, 2H), 7.19–7.15 (m, 1H), 7.07 (s, 1H), 3.83 (s, 2H), 3.78 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 178.8, 137.0, 128.1, 127.7, 122.0, 119.5, 119.1, 109.5, 106.2, 32.9, 31.2.

4.1.4. 3-[2,5-Dihydro-4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-3-furanyl]-1*H*-indole-1-acetonitrile (18). To a stirred solution of indole-1-acetonitrile (683 mg, 4.37 mmol) in dichloromethane (45 mL) at 0 °C was added dropwise oxalyl chloride (594 mg, 4.68 mmol). The mixture was stirred at 0 °C for 2 h and again oxalyl chloride (0.5 mL) was added. The mixture was further stirred at 0 °C for 3 h and allowed to slowly reach rt and continued to stir for 8 h. Oxalyl chloride (1 mL) was again added and stirred for 1 h at rt. The solvent was removed in vacuo. The residue was redissolved in dichloromethane (60 mL) and added dropwise to a stirred solution of 1-methylindole-3-acetic acid (827 mg, 4.37 mmol) and triethylamine (885 mg, 8.74 mmol) in dichloromethane (15 mL). The mixture was stirred overnight and concentrated in vacuo. The residue was purified by column chromatography on silica gel (97:3 dichloromethane:methanol) to give the desired product (666 mg, 40%) as an orange-red solid: mp 231–233 °C; IR (thin film) 3119, 1816, 1750, 1629, 1531, 1255 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.04 (s, 1H), 7.92 (s, 1H), 7.63 (d, 1H, J=8.3 Hz), 7.49 (d, 1H, J=8.3 Hz), 7.24–7.19 (m, 1H), 7.13–7.08 (m, 1H), 7.01 (d, 1H, J=7.8 Hz), 6.89–6.84 (m, 1H), 6.74–6.66 (m, 2H), 5.66 (s, 2H), 3.89 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 166.4, 166.3, 136.9, 135.6, 135.0, 132.3, 130.1, 126.0, 125.7, 125.1, 123.2, 122.4, 121.7, 121.4, 121.0, 120.4, 116.1, 110.7, 110.4, 106.2, 103.9, 34.2, 33.2; LRMS (EI): m/z 381 (M^+), 223, 203, 168, 156, 144, 141, 116, 91, 77, 62 (100%); HRMS (EI) calcd for $C_{23}H_{15}N_3O_3$: 381.1113, found: 381.1108.

4.1.5. 3-[2,5-Dihydro-4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-3-furanyl]-1*H*-indole-1 butanenitrile (19). To a stirred solution of indole-1-butyronitrile (829 mg, 4.5 mmol) in dichloromethane (45 mL) at 0 °C was added dropwise oxalyl chloride (600 mg, 4.73 mmol). After stirring the mixture for 1 h at 0 °C, the solvent was removed in vacuo. The residue was redissolved in dichloromethane (45 mL) and added dropwise to a stirred solution of 1-methylindole-3-acetic acid (851 mg, 4.5 mmol) and triethylamine (911 mg, 9 mmol) in dichloromethane (15 mL). The mixture was stirred overnight at rt and the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel (97:3 dichloromethane:methanol) to give the desired product (829 mg, 45%) as a red solid: mp 89–91 °C (dec); IR (thin film) 3052, 2941, 2247, 1816, 1750, 1628, 1529, 1255 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.98 (s, 1H), 7.80 (s, 1H), 7.56 (d, 1H, J=8.3 Hz), 7.49 (d, 1H, J=8.1 Hz), 7.15–7.09 (m, 2H), 7.05 (d, 1H, J=7.8 Hz), 6.83 (t, 1H, J=7.6 Hz), 6.74–6.70 (m, 2H), 4.30 (t, 2H, J=6.8 Hz), 3.87 (s, 3H), 2.41 (t, 2H, J=7.3 Hz), 2.02 (qn, 2H, J=7.1 Hz); ^{13}C NMR (DMSO-d_6): δ 166.5, 166.3, 136.9, 135.9, 134.6, 132.8, 128.5, 126.8, 125.7, 124.9, 122.5, 122.3, 121.7, 121.5, 120.3, 120.2, 120.0, 110.7, 110.5, 104.7, 103.9, 44.7, 33.1, 25.6, 13.8; LRMS (EI): m/z 409 (M^+), 380, 355, 203, 144 (100%), 62; HRMS (EI) calcd for $C_{25}H_{19}N_3O_3$: 409.1426, found: 409.1424.

4.1.6. 3-[2,5-Dihydro-4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-1*H*-pyrrol-3-yl]-1*H*-indole-1-acetonitrile (20). To a stirred solution of anhydride **18** (381 mg, 1 mmol) in DMF (4 mL) was added 1,1,1,3,3,3-hexamethyldisilazane (1.61 g, 10 mmol) followed by methanol (0.16 g, 5 mmol). The tightly closed reaction mixture was stirred for 24 h at rt. The mixture was then poured into water (25 mL) and extracted with ethyl acetate (3×50 mL). The combined organic extracts were washed with water (50 mL). The separated organic phase was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (93:7 dichloromethane:methanol) to give the desired product (354 mg, 93%) as an orange-red solid: mp 278–280 °C; IR (thin film) 3311, 1692, 1628, 1532, 1329 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.02 (s, 1H), 7.91 (s, 1H), 7.83 (s, 1H), 7.58 (d, 1H, J=8.4 Hz), 7.42 (d, 1H, J=8.4 Hz), 7.15 (t, 1H, J=7.7 Hz), 7.07–7.01 (m, 1H), 6.92 (d, 1H, J=7.7 Hz), 6.78 (t, 1H, J=7.5 Hz), 6.68–6.60 (m, 2H), 5.63 (s, 2H), 3.86 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 172.8, 172.7, 136.6, 135.4, 133.6, 131.3, 129.2, 126.5, 125.6, 125.4, 122.6, 121.8, 121.4, 121.0, 120.5, 119.7, 116.2, 110.2, 110.0, 107.0, 104.4, 34.1, 32.9; LRMS (EI): m/z 380 (M^+, 100%), 340, 297, 269, 176, 135, 121, 62; HRMS (EI) calcd for $C_{23}H_{16}N_4O_2$: 380.1273, found: 380.1272.

4.1.7. 3-[2,5-Dihydro-4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-1*H*-pyrrol-3-yl]-1*H*-indole-1-butanenitrile (21). To a stirred solution of anhydride **19** (409 mg, 1 mmol) in DMF (4 mL) was added 1,1,1,3,3,3-hexamethyldisilazane (1.61 g, 10 mmol) followed by methanol (0.16 g, 5 mmol). The tightly closed reaction mixture was stirred for 24 h at rt. The mixture was then poured into water (25 mL) and extracted with ethyl acetate (3×50 mL). The combined organic extracts were washed with water (50 mL). The separated organic phase was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (93:7 dichloromethane:methanol) to give the desired product (384 mg, 94%) as a red solid: mp 249–251 °C; IR (thin film) 3281, 2246, 1756, 1704, 1610, 1532, 1334 cm^{-1}; ^1H NMR (DMSO-d_6): δ 10.95 (s, 1H), 7.86 (s, 1H), 7.68 (s, 1H), 7.50 (d, 1H, J=8.4 Hz), 7.43 (d, 1H, J=8.4 Hz), 7.10–6.98 (m, 3H), 6.75 (t, 1H, J=7.7 Hz), 6.68–6.61 (m, 2H), 4.27 (t, 2H, J=6.8 Hz), 3.85 (s, 3H), 2.38 (t, 2H, J=7.3 Hz), 2.01 (qn, 2H, J=7.0 Hz); ^{13}C NMR

(DMSO-d_6): δ 172.9, 172.8, 136.6, 135.7, 133.3, 131.6, 128.0, 126.6, 126.1, 125.4, 121.9, 121.7, 121.4, 121.1, 119.9, 119.7, 119.5, 110.2, 110.0, 105.5, 104.4, 44.4, 32.9, 25.6, 13.7; LRMS (EI): *m/z* 408 (M^+, 100%), 380, 354, 297, 283, 269, 204, 142, 121, 62; HRMS (EI) calcd for $C_{25}H_{20}N_4O_2$: 408.1586, found: 408.1595.

4.1.8. 5,6,7,13-Tetrahydro-13-methyl-5,7-dioxo-12*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-12-acetonitrile (11). A mixture of maleimide **20** (19 mg, 0.05 mmol) and palladium(II) trifluoroacetate (83 mg, 0.25 mmol) in DMF (3 mL) was heated at 90 °C for 9 h. The reaction mixture was then cooled, diluted with ethyl acetate (25 mL), and washed with 0.5 N HCl (50 mL). The organic phase was dried (Na_2SO_4) and filtered through Hyflo. The filtrate was concentrated in vacuo and the residue was purified by column chromatography on silica gel (97:3 dichloromethane:methanol) to give the desired product (1.5 mg, 8%) as a fluorescent yellow solid: mp >250 °C (dec); IR (thin film) 3201, 2919, 2615, 2282, 1696 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.26 (s, 1H), 9.14–9.11 (m, 2H), 7.95 (d, 1H, *J*=8.1 Hz), 7.82 (d, 1H, *J*=8.6 Hz), 7.75–7.68 (m, 2H), 7.53 (t, 1H, *J*=7.4 Hz), 7.45 (t, 1H, *J*=7.7 Hz), 5.87 (s, 2H), 4.29 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 171.2, 170.5, 144.4, 144.3, 132.8, 128.3, 123.4, 122.3, 121.4, 120.3, 119.7, 118.9, 116.3, 112.7, 111.4, 38.5, 35.6; LRMS (EI): *m/z* 378 (M^+), 272, 239, 229, 213, 161, 149 (100%), 133, 119, 109, 104, 95, 91, 83, 78, 69, 62; HRMS (EI) calcd for $C_{23}H_{14}N_4O_2$: 378.1117, found: 378.1119.

4.1.9. 5,6,7,13-Tetrahydro-13-methyl-5,7-dioxo-12*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-12-butanenitrile (12). A mixture of maleimide **21** (20 mg, 0.05 mmol) and palladium(II) trifluoroacetate (83 mg, 0.25 mmol) in DMF (3 mL) was heated at 90 °C for 2 h. The reaction mixture was then cooled, diluted with ethyl acetate (25 mL), and washed with 0.5 N HCl (50 mL). The organic phase was dried (Na_2SO_4) and filtered through Hyflo. The filtrate was concentrated in vacuo and the residue was purified by column chromatography on silica gel (97:3 dichloromethane:methanol) to give the desired product (10 mg, 50%) as a fluorescent yellow solid: mp >200 °C (dec); IR (thin film) 3201, 2245, 1748, 1710, 1695, 1317 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.14 (s, 1H), 9.14 (d, 1H, *J*=7.8 Hz), 9.11 (d, 1H, *J*=7.8 Hz), 7.89 (d, 1H, *J*=8.3 Hz), 7.77 (d, 1H, *J*=8.3 Hz), 7.67–7.63 (m, 2H), 7.44–7.40 (m, 2H), 4.86 (t, 2H, *J*=7.6 Hz), 4.24 (s, 3H), 2.26 (t, 2H, *J*=7.1 Hz), 1.85 (qn, 2H, *J*=7.3 Hz); ^{13}C NMR (DMSO-d_6): δ 171.5, 171.4, 145.5, 144.1, 133.8, 132.5, 128.3, 128.1, 125.5, 125.2, 123.6, 122.5, 122.1, 121.8, 121.7, 120.8, 120.5, 120.1, 119.4, 112.9, 112.0, 47.6, 37.4, 24.6, 14.3; LRMS (EI): *m/z* 406 (M^+), 378, 338, 239, 211, 161, 149, 133, 130, 119, 109, 104, 97, 91, 83, 77, 71, 69, 62 (100%); HRMS (EI) calcd for $C_{25}H_{18}N_4O_2$: 406.1430, found: 406.1436.

4.1.10. 1*H*-Indole-1-propanamide (23). To a stirred solution of indole (2.93 g, 25 mmol) in dioxane (75 ml) at 0 °C was added acrylamide (2.67 g, 37.5 mmol) and freshly powdered KOH (1.4 g, 25 mmol). The reaction mixture was slowly allowed to come to rt and stirred for 24 h. The solution was filtered to remove the insoluble materials and the solvent was removed in vacuo. The residue was purified with column chromatography on silica gel (15:1 chloroform:methanol) to give the desired product (1.88 g, 40%) as a white solid: mp 101–102 °C (lit.[21] 90–92 °C); IR (thin film) 3446, 3358, 3190, 1668, 1611, 1462, 1402, 1314 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.54–7.53 (m, 1H), 7.49–7.48 (m, 1H), 7.39 (s, 1H), 7.31 (d, 1H, *J*=3.2 Hz), 7.15–7.11 (m, 1H), 7.03–7.00 (m, 1H), 6.92 (s, 1H), 6.41–6.40 (m, 1H), 4.39 (t, 2H, *J*=6.9 Hz), 2.57 (t, 2H, *J*=6.8 Hz); ^{13}C NMR (DMSO-d_6): δ 171.9, 135.5, 128.6, 128.1, 121.0, 120.4, 119.0, 109.8, 100.5, 41.8, 35.8.

4.1.11. (2-Carbamoyl-ethyl)-1*H*-indole-3-acetic acid (25). To a stirred suspension of NaH (1 g, 25 mmol, 60% dispersion in mineral oil) in DMF (10 mL) at 0 °C was added dropwise a solution of indole-3-acetic acid (1.7 g, 10 mmol) in DMF (10 mL). After the addition, the solution was allowed to warm to rt and stirred for 1 h. It was then cooled to 0 °C and a solution of 3-chloropropionamide (1.2 g, 11 mmol) was added dropwise. The solution was allowed to warm to rt and stirred for 8 h. It was then stirred at 70 °C for 17 h and 90 °C for 2 h. The mixture was cooled to rt and the solvent was evaporated. Ether (50 mL) was slowly added to the oily-residue and excess sodium hydride was destroyed by the dropwise addition of water. The solution was acidified with 1 N HCl (50 mL) and extracted with ethyl acetate (2×100 mL). The combined organic phase was washed with water (100 mL) and dried (Na_2SO_4). Solvent was evaporated and the residue was purified by column chromatography on silica gel (90:10 dichloromethane:methanol) to yield the desired product (1.25 g, 51%) as a yellowish oil, which solidified upon extensive drying: mp 141–143 °C; IR (thin film) 3333, 2359, 1660 cm^{-1}; ^1H NMR (DMSO-d_6): δ 12.22 (br s, 1H), 7.50 (d, 1H, *J*=7.6 Hz), 7.45 (d, 1H, *J*=8.2 Hz), 7.40 (br s, 1H), 7.22 (s, 1H), 7.15–7.12 (m, 1H), 7.02 (t, 1H, *J*=7.5 Hz), 6.91 (br s, 1H), 4.34 (t, 2H, *J*=6.7 Hz), 3.62 (s, 2H), 2.55 (t, 2H, *J*=6.9 Hz); ^{13}C NMR (DMSO-d_6): δ 173.0, 171.9, 135.7, 127.6, 127.2, 121.2, 118.9, 118.6, 109.7, 107.2, 41.6, 35.8, 30.9; LRMS (EI): *m/z* 246 (M^+), 201 (100%), 130, 115; HRMS (EI) calcd for $C_{13}H_{14}N_2O_3$: 246.1004, found: 246.1003.

4.1.12. 1-Methyl-1*H*-indole (26). To a stirred solution of indole (4.68 g, 40 mmol) in acetone (120 mL) at 0 °C was added freshly powdered potassium hydroxide (11.22 g, 200 mmol). After stirring the mixture for 30 min at 0 °C, methyl iodide (11.35 g, 80 mmol) was added dropwise with vigorous stirring. The mixture was allowed to slowly reach rt and continued to stir for 18 h. The mixture was filtered to remove the insoluble materials and concentrated in vacuo. The residue was distilled at a reduced pressure (bp 123–125 °C/22–25 Torr) to give the desired product (4.72 g, 90%) as a colorless oil: (lit.[22] bp 118–120 °C/20 Torr); ^1H NMR ($CDCl_3$): δ 7.86–7.83 (m, 1H), 7.49–7.39 (m, 2H), 7.35–7.29 (m, 1H), 7.17 (d, 1H, *J*=3.2 Hz), 6.69–6.67 (m, 1H), 3.86 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 136.8, 128.9, 128.6, 121.6, 120.9, 119.3, 109.3, 100.9, 32.8.

4.1.13. 3-[2,5-Dihydro-4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-3-furanyl]-1*H*-indole-1-propanamide (28). To a stirred solution of 1-methylindole (656 mg, 5 mmol) in dichloromethane (50 mL) at 0 °C was added dropwise oxalyl chloride (635 mg, 5 mmol). After stirring the mixture for 1 h at 0 °C, the solvent was removed in vacuo. The residue was redissolved in dichloromethane (50 mL) and added dropwise

to a stirred solution of **25** (1.23 g, 5 mmol) and triethylamine (1.01 g, 10 mmol) in dichloromethane (20 mL). The mixture was stirred overnight at rt and the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel (90:10 dichloromethane:methanol) to give the desired product (640 mg, 31%) as a red solid: mp 218–220 °C; IR (thin film) 3194, 1816, 1749, 1673, 1612, 1529, 1257 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.96 (s, 1H), 7.83 (s, 1H), 7.55 (d, 1H, J=8.2 Hz), 7.48 (d, 1H, J=8.2 Hz), 7.42 (br s, 1H), 7.12–7.07 (m, 2H), 6.96 (br s, 1H), 6.91–6.88 (m, 1H), 6.76–6.69 (m, 3H), 4.46 (t, 2H, J=6.7 Hz), 3.89 (s, 3H), 2.58 (t, 2H, J=6.7 Hz); ^{13}C NMR (DMSO-d_6): δ 171.6, 166.5, 166.4, 136.7, 135.7, 134.4, 133.2, 127.9, 126.9, 125.7, 125.3, 122.3, 122.2, 121.5, 121.4, 120.3, 120.1, 110.6, 110.5, 104.4, 104.1, 42.4, 35.4, 33.1; LRMS (EI): m/z 413 (M^+), 260, 201 (100%), 158, 130, 72; HRMS (EI) calcd for $C_{24}H_{19}N_3O_4$: 413.1376, found: 413.1368.

4.1.14. 3-[2,5-Dihydro-4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-1*H*-pyrrol-3-yl]-1*H*-indole-1-propanamide (29). To a stirred solution of anhydride **28** (265 mg, 0.64 mmol) in DMF (3 mL) was added a 1,1,1,3,3,3-hexamethyldisilazane (1.03 g, 6.4 mmol) followed by methanol (102 mg, 3.2 mmol). The tightly closed mixture was stirred for 30 h at rt. The mixture was then poured into water (25 mL) and extracted with ethyl acetate (3×50 mL). The combined organic extracts were washed with water (50 mL). The separated organic phase was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (90:10 dichloromethane:methanol) to give the desired product (253 mg, 96%) as a red solid: mp >230 °C (dec); IR (thin film) 3189, 3044, 1750, 1701, 1661, 1606, 1530, 1336 cm^{-1}; ^1H NMR (DMSO-d_6): δ 10.94 (s, 1H), 7.84 (s, 1H), 7.73 (s, 1H), 7.50 (d, 1H, J=8.2 Hz), 7.42–7.40 (m, 2H), 7.04–7.01 (m, 2H), 6.96 (br s, 1H), 6.82 (d, 1H, J=7.9 Hz), 6.67–6.63 (m, 3H), 4.44 (t, 2H, J=6.7 Hz), 3.85 (s, 3H), 2.58 (t, 2H, J=6.7 Hz); ^{13}C NMR (DMSO-d_6): δ 173.0, 172.9, 171.8, 136.5, 135.5, 133.1, 132.0, 127.6, 126.7, 126.2, 125.8, 121.8, 121.7, 121.2, 121.1, 119.8, 119.6, 110.3, 110.1, 105.1, 104.7, 42.3, 35.6, 33.0; LRMS (ESI): m/z 413 $[M+H]^+$; HRMS (ESI) calcd for $C_{24}H_{21}N_4O_3$ [M+H]: 413.1614, found: 413.1596.

4.1.15. 5,6,7,13-Tetrahydro-13-methyl-5,7-dioxo-12*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-12-propanamide (22). A mixture of maleimide **29** (21 mg, 0.05 mmol) and palladium(II) trifluoroacetate (83 mg, 0.25 mmol) in DMF (3 mL) was heated at 90 °C for 2 h. The reaction mixture was then cooled, diluted with ethyl acetate (25 mL), and washed with 0.5 N HCl (50 mL). The organic phase was dried (Na_2SO_4) and filtered through Hyflo. The filtrate was concentrated in vacuo and the residue was purified by column chromatography on silica gel (90:10 dichloromethane: methanol) to give the desired product (6 mg, 30%) as a fluorescent yellow solid: mp >250 °C (dec); IR (thin film) 2921, 2853, 1715, 1645, 1254 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.14 (s, 1H), 9.14 (t, 2H, J=8.2 Hz), 7.91 (d, 1H, J=8.2 Hz), 7.82 (d, 1H, J=8.2 Hz), 7.68–7.63 (m, 2H), 7.45–7.41 (m, 2H), 7.24 (s, 1H), 6.80 (s, 1H), 5.01 (t, 2H, J=7.6 Hz), 4.26 (s, 3H), 2.41 (t, 2H, J=7.6 Hz); ^{13}C NMR (DMSO-d_6): δ 171.3, 170.8, 170.7, 144.7, 143.6, 133.2, 131.9, 127.6, 127.4, 124.7, 124.5, 122.9, 121.8, 121.3, 121.1, 120.8, 120.0, 119.4, 118.6, 112.3, 111.3, 44.7, 36.8, 33.7; LRMS (ESI): m/z 411 $[M+H]^+$; HRMS (ESI) calcd for $C_{24}H_{19}N_4O_3$ [M+H]: 411.1457, found: 411.1448.

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