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Synthesis of Potent Oxindole CDK2 Inhibitors

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Abstract—A series of oxindole CDK2 inhibitors was synthesized. These novel analogues have a saturated monosubstituted cyclic moiety at their C-4 position that mimics the ribofuranoside of ATP. This substitution afforded agents with increased potency relative to the parent indolinone and nanomolar range IC_{50} against the CDK2 enzyme and two cancer cell lines.
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Introduction

The *cell cycle*, is a term that describes collectively the events that take place during the proliferation of eukaryotic cells. The progression through the cell cycle is orchestrated by a set of Ser/Thr kinases called *cyclin-dependent kinases* (CDKs).[1] These kinases upon complexation with their activating proteins, called *cyclins*, phosphorylate and modulate the activity of many target substrates such as organizational proteins, transcription factors as well as proteins involved in the replication assembly and machinery of cells.[1] Among the nine CDK family members (CDK1-9) CDK1, CDK2, CDK4 and CDK6 are instrumental for cell cycle progression. This, in conjuction with findings that implicate aberrant CDK control in the majority of cancer cases,[2] has led to an intense search for inhibitors of these enzymes. Of particular interest for many research groups has been the inhibition of the CDKs with small molecules that compete with and displace from the CDK active site ATP, the cofactor these kinases use as source of phosphate. Indeed, in recent years, a multitude of chemical scaffolds and low molecular weight agents have been reported as ATP-competitive CDK inhibitors.[3] For a number of these inhibitors the oxindole core is a common structural feature. For instance, the natural product indirubin (**1**),[3,4] the potent and selective CDK2 analogue GW5181 (**2**)[3,5] and the CDK1 and CDK2 selective derivative SU9516 (**3**)[3,6] all share the oxindole core and belong in the broader oxindole class of ATP-competitive CDK inhibitors (Fig. 1).

In this paper we describe a new series of oxindole-type inhibitors that has excellent potency toward CDK2. This series began with lead **4d**, a compound that, along with the less potent analogues **4a**–**c** (Fig. 2, Table 1), was identified as a hit during screening.

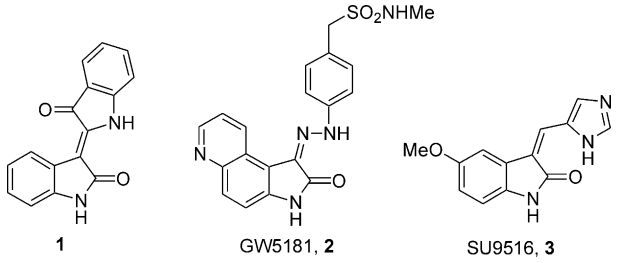

Figure 1. ATP-competitive inhibitors of CDKs from the oxindole class.

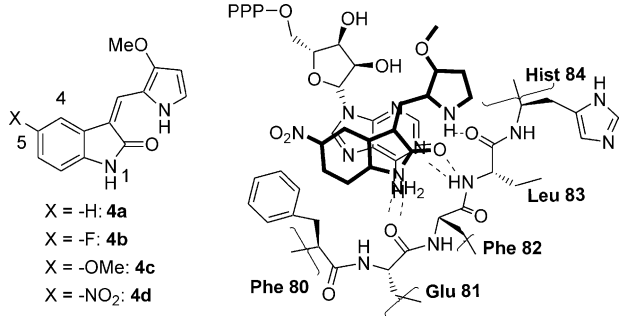

Figure 2. CDK2 screening hits **4a**–**d**. Overlay of lead **4d** with ATP in the CDK2 enzyme pocket. Interactions between CDK2 residues and the ribofuranoside of ATP have been omitted for clarity.

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Table 1. CDK2 inhibition and tumor cell growth inhibition by oxindoles **4a–d**

Compd	CDK2/Cyclin E (IC_{50} nM)[a]	RKO (IC_{50} nM)[a]	MDA MB435 (IC_{50} nM)[a]
4a	5,355	—[b]	—[b]
4b	847	11,921	>30,000
4c	209	3,860	8,080
4d	39	692	7,455

[a]IC_{50} values detemined by a single experiment run in duplicate.
[b]Not tested.

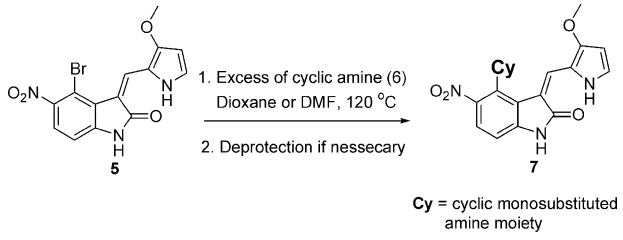
Chemistry and Results

Hypothesis for potency optimization. Our hypothesis for the potency optimization of lead **4d** was based on several known X-ray structures of CDK2 in complex with oxindole-type inhibitors and on the also known crystal structure of CDK2 in complex with ATP. This hypothesis relied on the premise that **4d** binds in the ATP pocket of CDK2, like other oxindoles, along the residues Glu81-Leu83 and in a donor–acceptor–donor motif (Fig. 2).[3–6]

An overlay of **4d** with ATP in the enzyme pocket at that orientation led us speculate further that the potency of this lead could be enhanced if a ribose surrogate was introduced at position 4 (Fig. 2).

Synthesis of C-4 substituted derivatives. The presence of a $-NO_2$ group at the C-5 position of **4d** and the commercial availability of many saturated cyclic amines that could serve as ribose mimics led us choose aromatic nucleophilic substitution as strategy for the synthesis of the desired 4-substituted inhibitors. Key starting material in our preparation of derivatives was the known 4-bromo-5-nitroindolone **5**.[7] This indolone was treated with a variety of cyclic amines, under the conditions shown in Scheme 1, to provide analogues **7a–o** (Table 2).

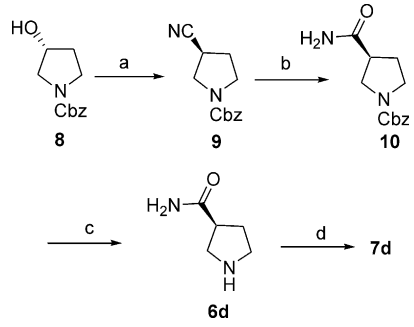
Among the inhibitors shown in Table 2, compounds **7a–c** and **7e–j** were accessed directly by the treatment of **5** with the corresponding commercially available cyclic amines. Oxindoles **7m–o** were synthesized by the treatment of **5** with the respective, also commercially available, *N*-Boc-monoprotected diamines followed by deprotection of the resulting *N*-Boc intermediates with 50% TFA in CH_2Cl_2. For the synthesis of inhibitors **7d**, **7k**, and **7l** the required cyclic amines were prepared. Cyclic amine **6d**, the reagent necessary for accessing **7d**, was synthesized from (*R*)-Cbz-3-hydroxypyrrolidine **8** in three steps (Scheme 2).



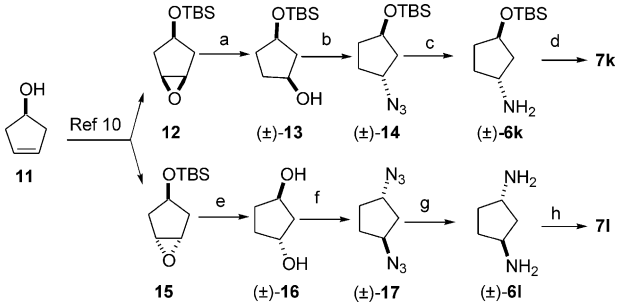
Scheme 1. General synthesis of oxindole inhibitors **7a–o**.

First, compound **8** was converted to (*S*)-cyanopyrrolidine **9** under the SN_2 cyanation conditions described by Gmeiner.[8] This nitrile was then reacted with Na_2CO_3 and 30% aqueous H_2O_2[9] to yield amide **10** that, in turn, upon a standard hydrogenolytic Cbz-deprotection was transformed to **6d** (Scheme 2). Amine **6d** was used crude in its subsequent reaction with **5**, under the conditions described, and led to oxindole inhibitor **7d**. Cyclic amines (±)-**6k** and (±)-**6l** were the precursors of the C-4 substituents of inhibitors **7k** and **7l**, respectively. Both of these amine intermediates were prepared from 3-cyclopenten-1-ol (Scheme 3).

In order to access (±)-**6k**, 3-cyclopenten-1-ol was converted to the *syn meso*-epoxide **12**[10] (Scheme 3). This epoxide was then hydrogenated at 50 psi in the presence of 10% Pd/C to the *syn*-alcohol (±)-**13** that, in turn, after a Mitsunobu reaction with diphenylphosphorylazide (DPPA)[11] yielded the *anti*-azide (±)-**14** (Scheme 3). From azide (±)-**14**, the desired amino-alcohol (±)-**6k** was obtained after hydrogenation. Further reaction of (±)-**6k** with oxindole **5**, under our standard conditions, followed by a silyl-group deprotection afforded **7k**. For the preparation of (±)-**6l**, 3-cyclopenten-1-ol was converted to the *anti meso*-epoxide **15**.[10] This epoxide, upon hydrogenation at 65 psi in the presence of 10% Pd/C, yielded the *anti*-1,3-cyclopentanediol (±)-**16** that was then treated with DPPA, under Mitsunobu conditions, to produce *anti*-diazide (±)-

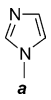
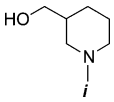
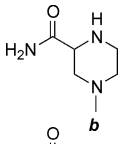
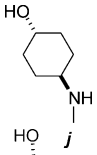
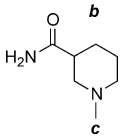
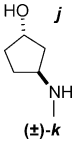
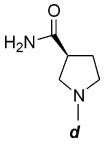
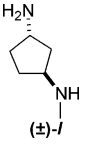
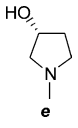
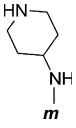
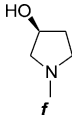
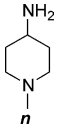
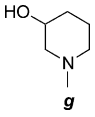
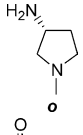
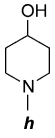
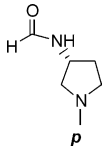


Scheme 2. Reagents and conditions: (a) MsCl, CH_2Cl_2, Et_3N, −20 to 0 °C, then Bu_4NCN, KCN, DMSO, 60 °C, 65%; (b) Na_2CO_3, 30% aq H_2O_2, acetone/H_2O 3:1, 57%; (c) H_2, 1 atm, 10% Pd/C, EtOH; (d) dioxane, **5**, 120 °C, 61%.



Scheme 3. Reagents and conditions: (a) H_2, 50 psi, 10% Pd/C, EtOH, 83%; (b) 1.1 equiv Ph_3P, 1.1 equiv DEAD, 1.1 equiv DPPA, THF, 72%; (c) H_2, 1 atm, 0.1 equiv PtO_2, EtOH, 76%; (d) dioxane, **5**, Hunig's base, 120 °C then 5% HF aq, CH_3CN, 18%; (e) H_2, 65 psi, 10% Pd/C, EtOH, 76%; (f) 3 equiv Ph_3P, 3 equiv DEAD, 3 equiv DPPA, THF, 80%; (g) H_2, 1 atm, 0.05 equiv PtO_2, EtOH/THF (2:1); (h) dioxane, **5**, 120 °C, 2%.

Table 2. CDK2/cyclin E and human cancer cell growth inhibition by analogues **7a**–**p**

Compd	C-4 (Cy)	CDK2/Cyclin E IC_{50} (nM)[a]	RKO IC_{50} (nM)[a]	MDA MB435 IC_{50} (nM)[a]	Compd	C-4 (Cy)	CDK2/Cyclin E IC_{50} (nM)[a]	RKO IC_{50} (nM)[a]	MDA MB435 IC_{50} (nM)[a]
7a		1,748	3,924	>30,000	**7i**		2[b]	124	441
7b		28	449	1,272	**7j**		6[b]	8,041	8,553
7c		6[b]	192	623	**7k**		5[b]	339	1,004
7d		5[b]	50	163	**7l**		5[b]	<15[b]	61
7e[c]		3[b]	32	88	**7m**		5[b]	24	139
7f		5[b]	37	104	**7n**		5[b]	<10[b]	<10[b]
7g		2[b]	105	250	**7o**		3[b]	<10[b]	<10[b]
7h		4[b]	<10[b]	46	**7p**		3[b]	32	33

[a]IC_{50} values detemined by a single experiment run in duplicate.
[b]Value at assay's detection limit.
[c]Evaluated further in a bromodeoxyuridine (BrdU) incorporation assay, see ref. 13.

17. Diamine (±)-**6l** was obtained from (±)-**17** upon hydrogenation and used crude[12] in its coupling with **5** to yield **7l**. The last inhibitor in Table 2, oxindole **7p**, was prepared from **7o** by stirring in neat methylformate.

Evaluation of C-4-substituted analogues. An overview of the potency data we obtained by screening derivatives **7a**–**p** against the CDK2/cyclin E holoenzyme and the RKO and MDA MB435 cancer cell lines indicates that the presence of a saturated cyclic moiety, a ribofuranose mimic, at C-4 does confer a beneficial effect on activity (Table 2). In the CDK2/cyclin E assay, for instance, the overwhelming majority of derivatives **7** was more active than lead **4d** and exhibited IC_{50} potencies that approached the detection limit of the assay. Alternatively, the imidazole-substituted analogue **7a** was less active than **4d**. This oxindole was synthesized as a negative control. It was expected to be less potent because an imidazole ring, due to its flat nature and lack of out-of-the-ring heteroatom substitution, is a poor surrogate for ribofuranose. Likewise, in the cellular assays compound **7a** had poor antiproliferative activity while most of the analogues **7** were consistently more potent than lead **4d**. The emerging SAR for the analogues in Table 2 appears to indicate that an amine-substituted saturated cyclic moiety is preferred over its corresponding amide- or hydroxy-substituted counterpart for overall potency (i.e., enzyme *and* antiproliferative potency). In this series, the most potent inhibitors were derivatives **7n** and **7o**.

Conclusion

In conclusion a series of potent CDK2 inhibitors was synthesized based on the oxindole derivative **4d** as a lead. The enzyme and cellular potencies of this lead were improved by the introduction of cyclic mono-substituted moieties that serve as ribofuranose surrogates at its C-4 position. This strategy yielded agents that inhibited CDK2 and the cellular proliferation of RKO and MDA MB435 cancer cells at low nanomolar concentrations.

Experimental

High resolution mass spectra under EI conditions were obtained with a VG AutoSpec Magnetic Sector spectometer. Under ES conditions, mass spectra were obtained with a Bruker FTMS (4.7 T) spectrometer while under FAB conditions mass spectra were obtained with a VG 70E Magnetic Sector spectrometer. All high resolution mass spectra were obtained at resolution of 10,000 at 5% valley. ^{1}H NMR spectra were taken with a 300 MHz Varian Mercury or a 400 MHz Varian Unity Spectrometer. Infrared spectra were taken with a Perkin Elmer Custom GX diffuse reflectance Fourier transform (DRIFTS) spectrometer using KBr or $CHCl_3$ and the spectra were ratioed against a blank spectrum of KBr or $CHCl_3$ respectively. Thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F-254 glass supported plates 0.25 mm thick. Column chromatography was performed with silica gel 60 (230–400 mesh). HPLC purifications were performed with a Rainin/Dynamax Model SD-1 system fitted with a Combi-HT SB-C18 column (100 mm long, 21.2 mm inner diameter size). Anhydrous solvents were obtained commercially and were used without further drying.

General procedure for the coupling of oxindole 5 with cyclic amines **6**. A solution of 1 equiv of **5**, 20–25 equiv of *N,N*-diisopropylethylamine, and 10 equiv of the corresponding cyclic amine **6** in DMF or dioxane was heated in a sealed tube at 120 °C. Upon consumption of the starting material, as judged by TLC, the mixture was cooled and partitioned between EtOAc and H_2O. The H_2O layer was extracted with EtOAc and the combined organic layer was dried over Na_2SO_4, filtered and concentrated. The coupling product was isolated after a silica gel column or HPLC purification followed by a precipitation out of THF with excess of pentane.

(*Z*)-4-Imidazolyl-3-(3-methoxy-1*H*-pyrrol-2-ylmethylene)-5-nitro-1,3-dihydro-indol-2-one (7a). A mixture of **5** (100 mg, 0.27 mmol), imidazole (190 mg, 2.79 mmol) and *N,N*-diisopropylethylamine (1 mL, 742 mg, 7.33 mmol) in DMF (6 mL) was treated as described in the general coupling procedure above. The crude product was purified on a silica gel column with a 0–100% EtOAc in hexane to 10% THF in EtOAc gradient. After a THF/pentane precipitation **7a** was isolated as an amorphous orange solid (35 mg, 37%). IR (KBr) 3141, 1671, 1619 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.75 (s, 3H), 5.83 (s, 1H), 6.04 (dd, 1H, J = 2.8, 2.0 Hz), 7.14 (d, 1H, J = 8.8 Hz), 7.25 (s, 1H), 7.40 (s, 2H), 7.85 (s, 1H), 7.97 (d, 1H, J = 7.6 Hz), 11.60 (s, 1H), 12.87 (s, 1H). HRMS (EI) for $C_{17}H_{13}N_5O_4$ (M^+): calcd, 351.0967; found, 351.0962.

(*Z*)-4-[3-(3-Methoxy-1*H*-pyrrol-2-ylmethylene)-5-nitro-2-oxo-2,3-dihydro-1*H*-indol-4-yl]-piperazine-2-carboxylic acid amide (7b). A mixture of **5** (120 mg, 0.33 mmol), piperazin-2-amide (425 mg, 3.29 mmol) and *N,N*-diisopropylethylamine (1 mL, 742 mg, 7.3 mmol) in DMF (10 mL) was treated as described in the general coupling procedure above. The crude product was purified on a silica gel column with a 0–100% EtOAc in hexanes to 0–100% THF in EtOAc to 0–5% MeOH in THF gradient. After a THF/pentane precipitation **7b** was isolated as an amorphous orange solid (72 mg, 53%). IR (KBr) 3434, 3328, 3187, 2946, 1675, 1610 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.81–3.19 (m, 7H), 3.60 (broad m, 1H), 3.89 (s, 3H), 6.13 (s, 1H), 6.79 (d, 1H, J = 8.0 Hz), 7.12 (s, 1H), 7.22 (s, 1H), 7.37 (s, 1H), 7.48 (d, 1H, J = 8.8 Hz), 8.68 (s, 1H), 11.23 (s, 1H), 12.92 (s, 1H). HRMS (ES+) for $C_{19}H_{20}N_6O_5$ ($M+H^+$): calcd, 413.1568; found, 413.1571.

(*Z*)-1-[3-(3-Methoxy-1*H*-pyrrol-2-ylmethylene)-5-nitro-2-oxo-2,3-dihydro-1*H*-indol-4-yl]-piperidine-3-carboxylic acid amide (7c). A mixture of **5** (150 mg, 0.42 mmol), nipecotamide (530 mg, 4.12 mmol) and *N,N*-diisopropylethylamine (1 mL, 742 mg, 7.3 mmol) in DMF (6 mL) was treated as described in the general procedure above. The crude product was purified on a silica gel column with a 0–100% EtOAc in hexanes gradient. After a THF/pentane precipitation **7c** was isolated as an amorphous orange solid (91 mg, 54%). IR (KBr) 3471, 3164, 2927, 2854, 1672, 1612 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42 (m, 1H), 1.70 (distorted d, 1H, J = 11.7 Hz), 2.04 (broad m, 2H), 2.65 (d, 1H, J = 12.4 Hz), 2.87–3.10 (m, 4H), 3.89 (s, 3H), 6.13 (s, 1H), 6.78 (d, 1H, J = 7.9 Hz), 6.84 (s, 1H), 7.28 (s, 1H), 7.37 (s, 1H), 7.47 (d, 1H, J = 7.9 Hz), 8.55 (s, 1H), 11.22 (s, 1H), 12.94 (s, 1H). HRMS (EI) for $C_{20}H_{21}N_5O_5$ (M^+): calcd, 411.1543, found 411.1545.

(+)-(*S*)-3-Cyanopyrrolidine-1-carboxylic-acid-benzylester (9). A solution of (*R*)-3-hydroxypyrrolidine-1-carboxylic acid benzylester (**8**) (6.5 g, 29.40 mmol) in THF (125 mL) was treated with methanesulfonyl chloride (6.21 g, 3.7 mL, 54.21 mmol) at −25 °C. After 3 min the mixture was warmed to 0 °C and after 45 min it was partitioned between EtOAc and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to the corresponding crude mesylate (9.09 g, 100%). This intermediate (4.1 g, 13.70 mmol) was dissolved in DMSO (30 mL) and then treated with KCN (1.9 g, 29.18 mmol) and tetrabutylammonium cyanide (3.7 g, 13.78 mmol). The resulting mixture was heated at 60 °C for 20 h then cooled, diluted with aq saturated $NaHCO_3$ and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered and concentrated to a residue that upon chromatography on a silica gel column with a 10–50% EtOAc in hexanes gradient afforded **9** as a light-yellow oil (2.1 g, 65%). IR ($CHCl_3$) 3023, 2960, 2891,

2249 (w), 1702 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 2.27 (m, 2H), 3.12 (m, 1H), 3.44–3.82 (m, 4H), 5.14 (s, 2H), 7.37 (m, 5H). HRMS (EI) for $C_{13}H_{14}N_2O_2$ (M^+): calcd, 230.1055; found, 230.1060. $[\alpha]_D^{25} = +27.9°$ (*c* 0.190, $CDCl_3$).

(+)-(*S*)-3-Carbamido-pyrrolidine-1-carboxylic acid benzyl-ester (10). To a solution of **9** (2.1 g, 9.12 mmol) in acetone (50 mL) was added H_2O (16 mL), 30% aq H_2O_2 (2.9 mL, 25.6 mmol) and Na_2CO_3 (3.2 g, 30.18 mmol). The resulting mixture was stirred for 18 h at room temperature and then partitioned between EtOAc and brine. The aqueous layer was extracted with EtOAc and the combined organic layer was dried over Na_2SO_4, filtered and concentrated to a residue that upon chromatography on a silica gel column with EtOAc as elution solvent afforded **10** as a white amorphous solid (1.3 g, 57%). IR ($CHCl_3$) 3387, 3300, 3212, 2962, 2882, 1686, 1664, 1623 cm^{-1}. ^1H NMR (300 MHz $CDCl_3$) δ 2.16 (m, 2H), 2.95 (broad m, 1H), 3.43 (distorted dd, 1H, *J* = 17.7, 7.8 Hz), 3.59 (dd, 1H, *J* = 11.1, 7.8 Hz), 3.70 (m, 2H), 5.13 (d, 2H, *J* = 0.9 Hz), 5.50 (broad m, 2H), 7.30–7.39 (m, 5H). HRMS (EI) for $C_{13}H_{16}N_2O_3$ (M^+): calcd, 248.1161; found, 248.1169. $[\alpha]_D^{25} = +1.3$ (*c* 0.160, $CDCl_3$).

(+)-(*S*)-(*Z*)-1-[3-(3-Methoxy-1*H*-pyrrol-2-ylmethylene)-5-nitro-2-oxo-2,3-dihydro-1*H*-indol-4-yl]-pyrrolidine-3-carboxylic acid amide (7d). A solution of **10** (1.20 g, 4.83 mmol), in EtOH (35 mL) was treated with of 10% Pd/C (260 mg) and the mixture was hydrogenated for 3 h under 1 atm of hydrogen. The slurry was then filtered and the catalyst was washed with a solution of 5% Et_3N in EtOH (60 mL). The combined filtrate was concentrated and the residue (crude amine **6d**) was dissolved in 15 mL of dioxane. To that solution was added **5** (120 mg, 0.33 mmol) and *N,N*-diisopropylethylamine (1 mL, 742 mg, 7.3 mmol) and the resulting mixture was treated as described in the general coupling procedure above. The crude product was purified on a silica gel column with a 0–100% EtOAc in hexanes to 20% THF in EtOAc gradient. After a THF/pentane precipitation **7d** was isolated as an amorphous orange solid (80 mg, 61%). IR (KBr) 3430, 3375, 3192, 2943, 2868, 1671, 1608 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 2.20 (m, 2H), 3.07–3.29 (m, 5H), 3.88 (s, 3H), 6.11 (distorted t, 1H, *J* = 2.8 Hz), 6.80 (d, 1H, *J* = 8.8 Hz), 6.93 (s, 1H), 7.36 (dd, 1H; *J* = 3.2, 2.8 Hz), 7.40 (s, 1H), 7.60 (d, 1H, *J* = 7.6 Hz), 8.05 (s, 1H), 11.24 (s, 1H), 12.79 (s, 1H). HRMS (EI) for $C_{19}H_{19}N_5O_5$ (M^+): calcd, 397.1386; found, 397.1375. $[\alpha]_D^{25} = +296.0°$ (*c* 0.075, DMF).

(−)-(*R*)-(*Z*)-4-(3-Hydroxy-pyrrolidinyl)-3-(3-methoxy-1*H*-pyrrol-2-ylmethylene)-5-nitro-1,3-dihydro-indol-2-one (7e). A solution of **5** (120 mg, 0.33 mmol), (*R*)-3-hydroxypyrrolidine (290 mg, 278 μL, 3.30 mmol) and *N,N*-diisopropylethylamine (1 mL, 742 mg, 7.3 mmol) in 3 mL of DMF was treated as described in the general coupling procedure above. The crude product was purified on a silica gel column with a 0–70% EtOAc gradient. After a THF/pentane precipitation **7e** was isolated as an amorphous orange solid (30 mg, 25%). IR (KBr) 3554, 3365, 3136, 2943, 2850, 1675, 1610 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 1.88 (ddd, 1H, *J* = 18.4, 12.8, 6.0 Hz), 2.23 (ddd, 1H, *J* = 19.6, 12.4, 6.8 Hz), 2.90 (dd, 1H, *J* = 8.8, 6.0 Hz), 3.13 (distorted q, 1H, *J* = 6.8 Hz), 3.21–3.35 (m, 2H), 3.88 (s, 3H), 4.48 (ddd, 1H, *J* = 16.4, 11.6, 5.6 Hz), 4.99 (d, 1H, *J* = 4.0 Hz), 6.12 (dd, 1H, *J* = 3.2, 2.8 Hz), 6.78 (d, 1H, *J* = 8.8 Hz), 7.35 (dd, 1H, *J* = 3.2, 2.8 Hz), 7.57 (d, 1H, *J* = 8.0 Hz), 7.99 (s, 1H), 11.23 (s, 1H), 12.87 (s, 1H). HRMS (EI) for $C_{18}H_{18}N_4O_5$ (M^+): calcd, 370.1277; found, 370.1275. $[\alpha]_D^{25} = -124.5°$ (*c* 0.090, DMF).

(+)-(*S*)-(*Z*)-4-(3-Hydroxy-pyrrolidinyl)-3-(3-methoxy-1*H*-pyrrol-2-ylmethylene)-5-nitro-1,3-dihydro-indol-2-one (7f). A solution of **5** (100 mg, 0.28 mmol), *N,N*-diisopropylethylamine (500 μL, 371 mg, 3.6 mmol) and (*S*)-3-hydroxypyrrolidine (240 mg, 231 μL, 2.80 mmol) in 10 mL of dioxane was treated as described in the general coupling procedure above. After purification (see preparation of **7e** above) and a THF/pentane precipitation **7f** was isolated as an orange amorphous solid. (54 mg, 53%). $[\alpha]_D^{25} = +132.0°$ (*c* 0.125, DMF).

(*Z*)-4-(3-Hydroxy-piperidinyl)-3-(3-methoxy-1*H*-pyrrol-2-ylmethylene)-5-nitro-1,3-dihydro-indol-2-one (7g). A solution of **5** (150 mg, 0.41 mmol), *N,N*-diisopropylethylamine (1 mL, 742 mg, 7.3 mmol) and 3-hydroxypiperidine (410 mg, 4.12 mmol) in DMF (10 mL) was treated as described in the general coupling procedure above. The crude product was purified on a silica gel column with a 0–70% EtOAc in hexane gradient. After a THF/pentane precipitation **7g** was isolated as an orange amorphous solid (110 mg, 69%). IR (KBr) 3356, 3149, 3007, 2943, 2857, 1664, 1609 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 1.16 (broad m, 1H), 1.68 (broad d, 1H, *J* = 11.6 Hz), 2.01 (broad m, 2H), 2.67 (distorted t, 1H, *J* = 10.8 Hz), 2.83–3.05 (m, 3H), 3.81–3.91 (s and m overlapping, 4H), 4.83 (d, 1H, *J* = 4.8 Hz), 6.13 (s, 1H), 6.78 (d, 1H, *J* = 8.8 Hz), 7.37 (t, 1H, *J* = 2.8 Hz), 7.47 (d, 1H, *J* = 8.4 Hz), 8.55 (s, 1H), 11.21 (s, 1H), 12.90 (s, 1H). HRMS (EI) for $C_{19}H_{20}N_4O_5$ (M^+): calcd, 384.1434; found, 384.1439.

(*Z*)-4-(4-Hydroxy-piperidinyl)-3-(3-methoxy-1*H*-pyrrol-2-ylmethylene)-5-nitro-1,3-dihydro-indol-2-one (7h). A solution of **5** (100 mg, 0.27 mmol), 4-hydroxypiperidine (400 mg, 3.95 mmol) and *N,N*-diisopropylethylamine (1 mL, 742 mg, 7.3 mmol) in dioxane (10 mL) was treated as described in the general coupling procedure above. The crude product was purified on a silica gel column with a 0–70% EtOAc in hexanes gradient. After a THF/pentane precipitation **7h** was isolated as an amorphous orange solid (70 mg, 67%). IR (KBr) 3543, 3135, 3059, 2943, 2859, 1668, 1608 cm^{-1}. ^1H NMR Mixture of equatorial-OH/axial-OH isomers in approximately 3:1 ratio respectively. (400 MHz, DMSO-d_6) δ 1.66 (broad m, 0.7H), 1.82 (broad s, 2.6H), 2.05 (broad m, 0.7H), 2.73 (broad m, 0.7H), 3.02 (broad s, 2.6H), 3.51 (broad m, 1.7H), 3.88 (s, 2.7H), 4.07 (s, 0.3H), 4.52 (s, 0.3H), 4.85 (s, 0.7H), 6.12 (s, 1H), 6.76 (d, 1H, *J* = 8.8 Hz), 7.36 (s, 1H), 7.45 (d, 1H, *J* = 8.8 Hz), 8.65 (s, 1H), 11.19 (s, 1H), 12.84 (broad s, 1H). HRMS (EI) for $C_{19}H_{20}N_4O_5$ (M^+): calcd, 384.1434; found, 384.1450.

(*Z*)-4-(3-Hydroxymethyl-piperidinyl)-3-(3-methoxy-1*H*-pyrrol-2-ylmethylene)-5-nitro-1,3-dihydro-indol-2-one (7i). A solution of **5** (150 mg, 0.41 mmol), *N,N*-diisopropylethylamine (1 mL, 742 mg, 7.30 mmol) and 3-piperidinemethanol (470 mg, 4.10 mmol) in DMF (10 mL) was treated as described in the general coupling procedure above. The crude product was purified on a silica gel column with a 0–70% EtOAc in hexanes gradient. After a THF/pentane precipitation **7i** was isolated as an amorphous orange solid. (95 mg, 58%). IR (KBr) 3367, 3188, 2938, 2851, 1667, 1609 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 0.97 (m, 1H), 1.68 (broad d, 1H, $J=12.0$ Hz), 1.84 (broad d, 1H, $J=12.8$ Hz), 2.03 (broad m, 2H), 2.63 (dd, 1H, $J=19.6$, 8.8 Hz), 2.90–3.22 (m, 5H), 3.87 (s, 3H), 4.49 (broad t, 1H), 6.12 (dd, 1H, $J=3.2$, 2.8 Hz), 6.77 (d, 1H, $J=7.6$ Hz), 7.36 (dd, 1H, $J=3.9$, 2.8 Hz), 7.46 (d, 1H, $J=7.6$ Hz), 8.65 (s, 1H), 11.20 (s, 1H), 12.92 (s, 1H). HRMS (EI) for $C_{20}H_{22}N_4O_5$ (M^+): calcd, 398.1590; found, 398.1591.

(*Z*)-4-(4-Hydroxy-cyclohexylamino)-3-(3-methoxy-1*H*-pyrrol-2-ylmethylene)-5-nitro-1,3-dihydro-indol-2-one (7j). A mixture of **5** (150 mg, 0.41 mmol), *trans*-4-aminocyclohexanol (805 mg, 6.99 mmol) and *N,N*-diisopropylethylamine (1 mL, 742 mg, 7.3 mmol) in dioxane (15 mL) was treated as described in the general coupling procedure above. The crude product was purified on a silica gel column with 0–70% EtOAc in hexane. After a THF/pentane precipitation **7j** was isolated as an amorphous red solid (73 mg, 45%). IR (KBr) 3521, 3346, 3135, 2932, 2856, 1678 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 1.04 (distorted dd, 2H, $J=22.4$, 10.8 Hz), 1.21 (dd, 2H, $J=23.2$, 10.8 Hz), 1.76 (broad d, 2H, $J=10.8$ Hz), 1.87 (broad d, 2H, $J=10.8$ Hz), 3.13 (broad m, 1H), 3.87 (s, 3H), 4.45 (d, 1H, $J=4.0$ Hz), 6.15 (s, 1H), 6.37 (d, 1H, $J=10.8$ Hz), 6.66 (d, 1H, $J=8.8$ Hz), 7.36 (s, 1H), 7.81 (s, 1H), 7.90 (d, 1H, $J=8.8$ Hz), 11.37 (s, 1H), 12.83 (s, 1H). HRMS (ES+) for $C_{20}H_{22}N_4O_5$ ($M+Na^+$): calcd, 421.1482; found, 421.1479.

(±)-*syn*-3-(*tert*-Butyl-dimethylsilanyloxy)-cyclopentanol (13). A slurry of *syn*-epoxide **12**[10] (2.15 g, 9.99 mmol) and 10% Pd/C (500 mg) in 70 mL of EtOH was hydrogenated with a Parr hydrogenator at 1 atm for 24 h and then at 50 psi for 24 h. Then another portion of 10% Pd/C (500 mg) was added and the mixture was hydrogenated at 50 psi for 24 h more. The catalyst was then filtered off and washed with THF (60 mL). The combined filtrate was concentrated and the residue was chromatographed on a silica gel column with a 0–100% Et_2O in hexanes gradient to afford (±)-**13** as a colorless liquid (1.81 g, 83%). IR ($CHCl_3$) 3510, 3008, 2956, 2931, 2858 cm^{-1}. ^1H NMR (300 MHz $CDCl_3$) δ 0.012 (s, 6H), 0.81 (s, 9H), 1.51–1.89 (m, 6H), 2.97 (d, 1H, $J=10.8$ Hz), 4.18 (broad m, 1H), 4.32 (m, 1H). HRMS (ES+) for $C_{11}H_{24}O_2Si$ ($M+Na^+$): calcd, 217.1618; found, 217.1619.

(±)-*anti*-3-Azido-1-(*tert*-butyl-dimethylsilanyloxy)-cyclopentane (14). To a solution of (±)-**13** (1.50 g, 6.93 mmol) and Ph_3P (1.99 g, 7.58 mmol) in THF (45 mL) at 0 °C was slowly added DEAD (1.32 g, 1.19 mL, 7.58 mmol). Then, after approximately 3–5 min, followed a dropwise addition of DPPA (2.09 g, 1.64 mL, 7.59 mmol). The resulting mixture was allowed to slowly warm up to room temperature and after overnight stirring was partitioned between Et_2O and H_2O. The water layer was extracted with Et_2O and the combined organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified on a silica gel column with a 0–20% Et_2O in hexanes gradient to afford (±)-**14** as colorless liquid (1.2 g, 72%). IR ($CHCl_3$) 2956, 2930, 2857, 2101 (s) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 0.01 (s, 6H), 0.83 (s, 9H), 1.56 (m, 2H), 1.84 (m, 3H), 2.07 (m, 1H), 4.05 (m, 1H), 4.31 (m, 1H).

(±)-*anti*-3-(*tert*-Butyl-dimethyl-silanyloxy)-cyclopentylamine (6k). A mixture of (±)-**14** (400 mg, 1.66 mmol) and PtO_2 (38 mg, 0.17 mmol) in EtOH (6 mL) was stirred under 1 atm of hydrogen for 2 h. The catalyst was then filtered off, washed with THF (approx. 40 mL) and the combined filtrate was evaporated to afford (±)-**6k** as a colorless liquid (270 mg, 76%). IR ($CHCl_3$) 3366, 2954, 2933, 2857 cm^{-1}. ^1H NMR (300 MHz $CDCl_3$) δ 0.01 (s, 6H), 0.82 (s, 9H), 1.22 (m, 1H), 1.46 (m, 2H), 1.81 (m, 1H), 1.99 (m, 2H), 3.52 (m, 1H), 4.38 (m, 1H). HRMS (ES+) for $C_{11}H_{25}NOSi$ ($M+H^+$): calcd, 216.1778; found, 216.1780.

(*Z*)-4-(3-Hydroxy-cyclopentylamino)-3-(3-methoxy-1*H*-pyrrol-2-ylmethylene)-5-nitro-1,3-dihydro-indol-2-one (7k). A mixture of **5** (120 mg, 0.33 mmol), (±)-**6k** (720 mg, 3.31 mmol) and *N,N*-diisopropylethylamine (500 μL, 371 mg, 3.60 mmol) in dioxane (5 mL) was treated as described in the general coupling procedure above. The coupling product was purified on a silica gel column with a 0–30% EtOAc in hexanes gradient and then dissolved in CH_3CN (5 mL). To this solution was added 5% aq HF (260 μL, 0.65 mmol) and the resulting mixture was stirred for 5 h and then partitioned between EtOAc and H_2O. The H_2O layer was washed with EtOAc and the combined organic layer was dried over Na_2SO_4, filtered and concentrated. Purification on a silica gel column with 0–70% EtOAc in hexanes followed by a THF/pentane precipitation afforded **7k** as a red amorphous solid (24 mg, 18%). IR (KBr) 3347, 3220, 2951, 1673 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 1.36 (m, 2H), 1.51 (dt, 1H, $J=12.8$, 6.8 Hz), 1.80 (m, 1H), 1.89 (m, 1H), 2.05 (m, 1H), 3.89 (s, 3H), 3.96 (m, 1H), 4.17 (broad m, 1H), 4.46 (d, 1H, $J=4.0$ Hz), 6.15 (distorted t, 1H, $J=2.8$ Hz), 6.45 (d, 1H, $J=11.6$ Hz), 6.67 (d, 1H, $J=8.8$ Hz), 7.36 (dd, 1H, $J=3.9$, 2.8 Hz), 7.91 (d, 1H, $J=8.8$ Hz), 7.95 (s, 1H), 11.37 (s, 1H), 12.87 (s, 1H). HRMS (EI) for $C_{19}H_{20}N_4O_5$ (M^+): calcd, 384.1434; found, 384.1434.

(±)-*anti*-1,3-Cyclopentanediol (16). A solution of *anti meso*-epoxide **15**[10] (3.23 g, 15.09 mmol) in EtOH (50 mL) was treated with 10% Pd/C (1.20 g) and the mixture was hydrogenated at 65 psi for 16 h. The catalyst was then filtered off and washed with THF (80 mL). The combined filtrate was concentrated and the residue was purified on a silica gel column with a 0–20% MeOH in CH_2Cl_2 gradient to afford (±)-**16** as a colorless oil (1.17 g, 76%). IR ($CHCl_3$) 3611, 3015, 2938 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 1.58 (m, 2H), 1.90 (t, 2H,

$J = 5.1$ Hz), 2.12 (m, 2H), 4.52 (broad m, 2H). HRMS (EI) for $C_5H_{10}O_2$ ($M-H^+$): calcd, 101.0602; found, 101.0602.

(±)-*anti*-1,3-Diazido-cyclopentane (17). To a 0 °C solution of (±)-**16** (0.85 g, 8.32 mmol) and Ph_3P (6.55 g, 24.97 mmol) in THF (80 mL) was slowly added DEAD (4.35 g, 3.95 mL, 24.97 mmol). Then, after 3–5 min, followed a dropwise addition of DPPA (6.87 g, 5.37 mL, 24.97 mmol) and the resulting solution was allowed to slowly warm up to room temperature and stirred overnight. The reaction mixture was then concentrated to a small volume (approx. 35 mL) via an atmospheric pressure distillation by means of a short path apparatus fitted with a 15 cm Vigreux column and then chromatographed on a silica gel column with a 0–20% Et_2O in pentane gradient. The product containing fractions were combined and the elution solvents were removed via an atmospheric pressure distillation, by means of a short path apparatus fitted with a 15 cm Vigreux column, to afford (±)-**17** as a light-yellow oil (1.02 g, 80%). IR ($CHCl_3$) 2945, 2103 (s) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 1.67 (m, 2H), 1.90 (t, 2H, $J = 6.0$ Hz), 2.02 (m, 2H); 4.03 (broad m, 2H).

(*Z*)-4-(3-Amino-cyclopentylamino)-3-(3-methoxy-1*H*-pyrrol-2-ylmethylene)-5-nitro-1,3-dihydro-indol-2-one (7l). To a solution of (±)-**17** (1.60 g, 10.50 mmol) in a 2:1 mixture of EtOH and THF (18 mL) was added Pt_2O (120 mg, 0.53 mmol) and the resulting slurry was hydrogenated under 1 atm of H_2 overnight. The catalyst was then filtered off, washed with THF (80 mL) and the combined filtrate was concentrated via an atmospheric pressure distillation by means of a short path apparatus fitted with a 15 cm Vigreux column to afford the crude diamine (±)-**6l**.[12] This diamine was then dissolved in dioxane (7 mL). Followed addition of oxindole **5** (80 mg, 0.22 mmol) and *N,N*-diisopropylethylamine (1 mL, 742 mg, 7.3 mmol) and the resulting mixture was treated as described in the general coupling procedure above. The crude coupling product was chromatographed with a reverse phase HPLC using a 30–90% CH_3CN in H_2O gradient that contained 0.05% TFA to afford a solid that was dissolved in THF and partitioned between EtOAc and aq 1 N NaOH. The EtOAc layer was washed with H_2O dried over Na_2SO_4, filtered and concentrated to **7l** that, after a THF/pentane precipitation, was isolated as a red amorphous solid (2 mg, 2%). IR (KBr) 3427, 2930, 1671 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 1.19 (m, 1H), 1.36 (m, 1H), 1.58 (m, 2H), 1.89 (m, 1H), 2.05 (m, 1H), 3.89–3.99 (s and m overlapping, 1H), 6.14 (s, 1H), 6.50 (d, 1H, $J = 11.6$ Hz), 6.60 (d, 1H, $J = 8.8$ Hz), 7.35 (t, 1H, $J = 2.8$ Hz), 7.90–7.93 (s and d overlapping, 2H), 12.87 (s, 1H). HRMS (EI) for $C_{19}H_{22}N_5O_4$ ($M+H^+$): calcd, 384.1666; found, 384.1672.

(*Z*)-3-(3-Methoxy-1*H*-pyrrol-2-ylmethylene)-5-nitro-4-(piperidinyl-4-amino)-1,3-dihydro-indol-2-one (7m). A mixture of **5** (150 mg, 0.41 mmol), 4-amino-1-(*tert*-butyloxycarbonyl)piperidine (1.20 g, 6.18 mmol) and *N,N*-diisopropylethylamine (1 mL, 742 mg, 7.3 mmol) in dioxane (15 mL) was treated as described in the general coupling procedure above. The coupling product was purified on a silica gel column with a 0–40% EtOAc in hexane and then dissolved at 0 °C in 6 mL of a 50% TFA in CH_2Cl_2 solution that also contained 300 μL of H_2O. This solution was stirred for 2 h and then partitioned between EtOAc and 3 N aq NaOH. The pH of the aqueous layer was adjusted to 12 by the addition of solid NaOH. The organic layer was then separated, washed with H_2O, dried over Na_2SO_4, filtered and concentrated to the crude product. Purification on a silica gel column with a 0–10% MeOH in CH_2Cl_2 gradient followed by a THF/pentane precipitation afforded **7m** as a red amorphous solid (40 mg, 25%). IR (KBr) 3333, 3127, 2940, 1673 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 1.27 (m, 2H), 1.84 (broad d, 2H, $J = 10.8$ Hz), 2.39 (m, 2H), 2.93 (broad d, 2H, $J = 11.6$ Hz), 3.22 (m, 1H), 3.86 (s, 3H), 6.14 (s, 1H), 6.40 (d, 1H, $J = 10.8$ Hz), 6.69 (d, 1H, $J = 8.8$ Hz), 7.36 (s, 1H), 7.86 (s, 1H), 7.91 (d, 1H, $J = 8.8$ Hz), 12.82 (s, 1H). HRMS (EI) for $C_{19}H_{21}N_5O_4$ (M^+): calcd, 383.1593; found, 383.1594.

(*Z*)-4-(4-Amino-piperidinyl)-3-(3-methoxy-1*H*-pyrrol-2-ylmethylene)-5-nitro-1,3-dihydro-indol-2-one (7n). A solution of **5** (120 mg, 0.33 mmol), 4-(*tert*-butyloxycarbonylamino)piperidine (1.20 g, 5.99 mmol) and *N,N*-diisopropylethylamine (2 mL, 1.5 g, 14.8 mmol) in dioxane (16 mL) was treated as described in the general coupling procedure above. The coupling product was purified on a silica gel column with a 0–60% EtOAc in hexanes and then dissolved at 0 °C in 5 mL of a 50% TFA in CH_2Cl_2 solution that contained 300 μL of H_2O. This solution was stirred for 2 h and then partitioned between EtOAc and 3 N aq NaOH. The pH of the aqueous layer was adjusted to 12 by the addition of solid NaOH. The organic layer was then separated, washed with H_2O, dried over Na_2SO_4, filtered and concentrated to the crude product. Purification on a silica gel column with a 0–10% MeOH in CH_2Cl_2 gradient followed by a THF/pentane precipitation afforded **7n** as an amorphous orange solid (42 mg, 32%). IR (KBr) 3350, 3289, 3170, 2949, 2865, 1669, 1608 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 1.75 (broad m, 4H), 2.65 (broad s, 1H), 3.01 (broad m, 4H), 3.91 (s, 3H), 6.13 (s, 1H), 6.76 (d, 2H, $J = 8.0$ Hz), 7.37 (s, 1H), 7.45 (d, 1H, $J = 8.8$ Hz), 8.60 (s, 1H), 12.94 (s, 1H). HRMS (EI) for $C_{19}H_{21}N_5O_4$ (M^+): calcd, 383.1593; found 383.1594.

(−)-(*R*)-(*Z*)-4-(3-Amino-pyrrolidinyl)-3-(3-methoxy-1*H*-pyrrol-2-ylmethylene)-5-nitro-1,3-dihydro-indol-2-one (7o). A solution of **5** (150 mg, 0.41 mmol), (*R*)-3-(*tert*-butyloxycarbonyl-amino)pyrrolidine (770 mg, 4.12 mmol) and *N,N*-diisopropylethylamine (1 mL, 742 mg, 7.3 mmol) in dioxane (16 mL) was treated as described in the general procedure above. The coupling product was purified on a silica gel column with a 0–100% EtOAc in hexanes gradient and then dissolved at 0 °C in 5 mL of a 50% TFA in CH_2Cl_2 solution that contained 200 μL of H_2O. After stirring for 2 h the mixture was partitioned between EtOAc and 3 N aq NaOH. The pH of the aqueous layer was adjusted to 12 by the addition of solid NaOH. The organic layer was then separated, washed with H_2O, dried over Na_2SO_4, filtered and concentrated to the crude product. Purification on a silica gel column with a 0–10% MeOH in CH_2Cl_2 gradient

followed by a THF/pentane precipitation afforded **7o** as an amorphous orange solid (87 mg, 57%). IR (KBr) 3343, 3133, 2942, 2846, 1673, 1608 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 1.72 (m, 1H), 2.23 (m, 1H), 2.78 (dd, 1H, $J=7.6$, 6.8 Hz), 3.14 (distorted dd, 1H, $J=14.4$, 6.8 Hz), 3.24 (m, 2H), 3.62 (m, 1H), 3.88 (s, 3H), 6.13 (s, 1H), 6.78 (d, 1H, $J=8.8$ Hz), 7.36 (s, 1H), 7.58 (d, 1H, $J=7.9$ Hz), 8.06 (s, 1H), 12.85 (s, 1H). HRMS (EI) for $C_{18}H_{19}N_5O_4$ (M^+): calcd, 369.1437; found, 369.1437. $[\alpha]_D^{25}=-110.0°$ (*c* 0.135, DMF).

(−)-(*R*)-(*Z*)-N-{1-[3-(3-Methoxy-1*H*-pyrrol-2-ylmethylene)-5-nitro-2-oxo-2,3-dihydro-1*H*-indol-4-yl]-pyrrolidin-3-yl}-formamide (7p). Oxindole **7o** (43 mg, 0.12 mmol) was dissolved in neat methyl formate (5 mL). This mixture was stirred at 50 °C for 10 h and then direclty concentrated to the crude product. Purification on a silica gel column with a 0–100% EtOAc in hexanes gradient followed by a THF/pentane precipitation afforded **7p** as an amorphous orange solid (42 mg, 91%). IR (KBr) 3204, 2944, 2853, 1677, 1609 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 1.89 (m, 1H), 2.36 (m, 1H), 2.95 (t, 1H, $J=8.0$ Hz), 3.22 (m, 2H), 3.90 (s, 3H), 4.56 (dd, 1H, $J=14.8$, 6.8 Hz), 6.11 (dd, 1H, $J=3.2$, 2.0 Hz), 6.81 (d, 1H, $J=7.6$ Hz), 7.37 (s, 1H), 7.62 (d, 1H, $J=8.8$ Hz), 8.04 (s, 2H), 8.32 (d, 1H, $J=6.0$ Hz), 11.27 (s, 1H), 12.82 (s, 1H). HRMS (EI) for $C_{19}H_{19}N_5O_5$ (M^+): calcd, 397.1386; found, 397.1387. $[\alpha]_D^{25}=-161.5°$ (*c* 0.130, DMF).

CDK2/cyclin E enzyme assay. Kinase assays were performed by FlashPlate™ assays (NEN™-Life Science Products) using a recombinant human cyclin E-CDK2 complex. GST-cyclin E (GST-cycE) and CDK2 cDNA clones in baculovirus vectors were provided by Dr. W. Harper at the Baylor College of Medicine, Houston, TX. Proteins were co-expressed in High Five™ insect cells and the complex was purified on glutathione Sepharose resin as described by Harper et al.[14] A 6x-Histidine tagged truncated form of retinoblastoma (Rb) protein (amino acid 386–928) was used as the substrate for the cycE-CDK2 assays (the expression plasmid was kindly provided by Dr. Veronica Sullivan, Department of Molecular Virology, Roche Research Centre, Welwyn Garden City, United Kingdom). The expression of the 62Kd protein was under the control of an IPTG inducible promoter in an M15 *E. coli* strain. Cells were lysed by sonication and purification was carried out by binding lysates at pH 8.0 to a Ni-chelated agarose column pre-treated with 1 mM imidazole. The resin was then washed several times with incrementally decreasing pH buffers to pH 6.0, and eluted with a 500 mM solution of imidazole. Eluted protein was dialysed against 20 mM HEPES pH 7.5, 30% glycerol, 200 mM NaCl, and 1 mM DTT. Purified Rb fusion protein stocks were quantitated for protein concentration, aliquoted, and stored at −70 °C. For the kinase assay, 96-well FlashPlates were coated with Rb protein at 10 μg/mL, using 100 μL per well. Plates were incubated at 4 °C overnight or at room temperature for 3 h on a shaker. To control for nonspecific phosphorylation, one row of wells was coated with 100 μL/well coating buffer (20 mM HEPES, 0.2 M NaCl). Plates were then washed twice with wash buffer (0.01% Tween 20 in phosphate-buffered saline). Compounds to be tested were added to the wells at 5× final concentration. Reactions were initiated by immediate addition of 40 μL reaction mix (25 mM HEPES, 20 mM $MgCl_2$, 0.002% Tween 20, 2 mM DTT, 1 μM ATP, 4 nM ^{33}P-ATP) and a sufficient amount of enzyme to give counts that are at least 10-fold above background. Plates were incubated at room temperature on a shaker for 30 min. Plates were washed four times with the wash buffer, sealed, and counted on the TopCount Scintillation Counter.

Tetrazolium dye proliferation assay. Proliferation was evaluated by the tetrazolium dye assay according to the procedure of Denizot and Lang.[15] Each cell line in its optimal medium is plated at the appropriate seeding density to give logarithmic growth over the course of the assay in a 96-well tissue culture plate. Plates are incubated overnight at 37 °C in a humidified incubator with 5% CO_2. The next day, test compounds are serially diluted to four times the final concentration in the appropriate medium containing 1.2% DMSO. One-fourth final volume of each dilution is added in duplicate to the plates containing cells. The same volume of 1.2% DMSO in medium is added to a row of control wells. Thus, the final concentration of DMSO in all wells is 0.3%. The plates are then returned to 37 °C for 5 days and then analyzed as follows: 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (thiazolyl blue; MTT; Sigma) is added to each well to yield a final concentration of 1 mg/mL. The plates are returned to the incubator for 2.5–3 h. The MTT-containing medium is removed and the resulting formazan metabolite is solubilized in 100% ethanol with shaking for 15 min at room temperature. Absorbances are read in a microtiter plate reader at a wavelength of 570 nm with a 650 nm reference. Percent inhibition (% INH) is calculated by subtracting the blank from all wells, then subtracting the ratio of the average absorbance of each duplicate (S_{AVE}) by the average of the controls (C_{AVE}) from 1.00. The final product is then multiplied by 100 (% INH $=(1.00-S_{AVE}/C_{AVE})\times 100$). The concentration at which 50% inhibition of cell proliferation (the IC_{50}) is determined from the linear regression of a plot of the logarithm of the concentration versus percent inhibition. Both cell lines used in the MTT assay were obtained from the ATCC. MDA MB435 cells (breast carcinoma) were grown in RPMI 1640 medium supplemented with 10% Fetal Bovine Serum (Gibco/BRL, Gaithersburg, MD) and 2 mM L-glutamine (Gibco/BRL). RKO cells (colorectal carcinoma) were grown in DMEM high glucose medium supplemented with 10% FCS and L-glutamine as indicated above.

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12. Diamine (±)-**6l** was fully characterized as its corresponding di-Boc derivative, (±)-*anti*-(3-*tert*-butyloxycarbonylamino)-cyclopentyl-carbamic acid *tert*-butylester. IR ($CHCl_3$) 3335, 2979, 1679 cm^{-1}. 300 MHz ^1H NMR ($CDCl_3$) δ 1.25–1.42 (s and m overlapping, 20H), 1.74 (t, 2H, $J = 6.9$ Hz), 2.06 (m, 2H), 3.97 (broad s, 2H), 4.42 (broad s, 2H). HRMS (ES+) for $C_{15}H_{28}N_2O_4$ ($M + Na^+$): calcd, 323.1941; found, 323.1944.
13. Compound **7e** was evaluated for cell cycle block in a bromodeoxyuridine (BrdU) incorporation assay. This assay assesses a compound's ability to block cell cycle progression from the G1 and into the S phase. The assay was preformed using a BrdU-ELISA kit (available from Roche Molecular Biochemicals, catalogue No. 1 647 229) and 1043SK human diploid fibroblasts synchronized at G_0. Treatment of cells with **7e** resulted in a reduction of incorporation of BrdU ($IC_{50} = 830$ nM) into cellular DNA. This level of inhibition of BrdU incorporation is indicative of a G1 cell cycle block and is consistent with the effect of a CDK2 inhibitor.
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