Synthesis of bisindolylmaleimides related to GF109203x and their efficient conversion to the bioactive indolocarbazoles

Sudipta Roy,[a] Alan Eastman[b] and Gordon W. Gribble*[a]

Received 26th May 2006, Accepted 30th June 2006
First published as an Advance Article on the web 21st July 2006
DOI: 10.1039/b607504e

From a structure–activity relationship perspective, the new indolocarbazoles **11** and **12** have been synthesized and evaluated biologically as novel Chk1 inhibitors. Compounds **11** and **12** were synthesized in high yield from indole *via* bisindolylmaleimides **18** and **24**.

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 at 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]

UCN-01 (**1**), the synthetic 7-hydroxy derivative of the non-selective PKC inhibitor staurosporine (**2**),[2] 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. 1).[3] Although UCN-01 is well recognized as a protein kinase C inhibitor,[4] this checkpoint inhibition was attributed to its ability to inhibit Chk1.[5] Unfortunately, UCN-01 binds avidly to human serum proteins thereby compromising its potential therapeutic activity.[6] Accordingly, we screened other indolocarbazoles to identify analogues with improved therapeutic potential. Initially, a K252a (**3**) analogue, ICP-1 (**4**), was synthesized and tested, and was found to overcome the problem of protein binding but it had considerably reduced potency.[7]

More recently, we found that Gö6976 (**5**) is a very potent checkpoint inhibitor even in the presence of human serum,[8] and this has also been attributed to the inhibition of Chk1.[9] Additionally, Gö6976 (**5**) abrogated S and G2 arrest at a concentration substantially lower than that required to inhibit PKC. Interestingly, UCN-01 (**1**) did not demonstrate this selectivity for checkpoint inhibition. Accordingly, we initiated a synthetic program to develop novel analogues rationally designed to overcome the obstacles observed with the other analogues. During our screening to identify novel inhibitors of Chk1, we found that ICP-103 (**6**) is also a potent checkpoint inhibitor.[10] Therefore, we have focused our investigation on this class of molecules as potential inhibitors of Chk1. We synthesized two nitrile analogues of ICP-103, ICP-106 (**7**) and ICP-109 (**8**), with variable lengths of the nitrile arm to investigate the effect of the nitrile chain-length on Chk1 activity (Fig. 2).[11] We find that ICP-106 (**7**) and ICP-109 (**8**) are less potent than ICP-103 (**6**) at abrogating DNA damage-induced cell cycle arrest. From this activity data for the nitrile analogues **7** and **8**, it was found that a three-carbon nitrile chain provided maximum activity. We then synthesized a novel amide analogue of ICP-103, ICP-112 (**9**), bearing the same number of carbons in the amide arm.[11] However, in this latter study we found that a cyano group is the more desirable functionality than an amide for activity since ICP-112 (**9**) was found to be less active than ICP-103 (**6**).

In the course of our structure–activity relationship studies on ICP-103 analogues, we have synthesized and tested two new amine analogues related to the known PKC inhibitor GF109203x[12] (Gö6850, **10**), ICP-121 (**11**) and ICP-125 (**12**) (Fig. 3). We wanted to further explore the SAR by replacing the nitrile with an amine. We decided to maintain the same three-carbon spacer between the indole nitrogen and the functional group nitrogen as in ICP-103 (**6**) due to its high activity compared to ICP-106 (**7**) and ICP-109 (**8**).

We herein describe the synthesis of compounds **11** and **12**, and briefly report the biological activity of these two novel indolocarbazoles.

Results and discussion

Towards the synthesis of **11**, we alkylated the indole nitrogen with 1,3-dibromopropane[13] in the presence of KOH to furnish **13** (Scheme 1). A small amount (10%) of 1-allylindole was also recovered from the reaction mixture. Compound **13** was then treated with di-*tert*-butyl-iminodicarboxylate and caesium carbonate to produce fully protected amine **14** in 99% yield. The key starting material **14** can be prepared alternatively using **15** with sodium hydride in DMF–THF. Compound **15** was synthesized

[a]*Department of Chemistry, Dartmouth College, Hanover, NH 03755, USA. E-mail: ggribble@dartmouth.edu; Fax: 603-646-3946; Tel: 603-646-3118*
[b]*Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH 03755, USA*

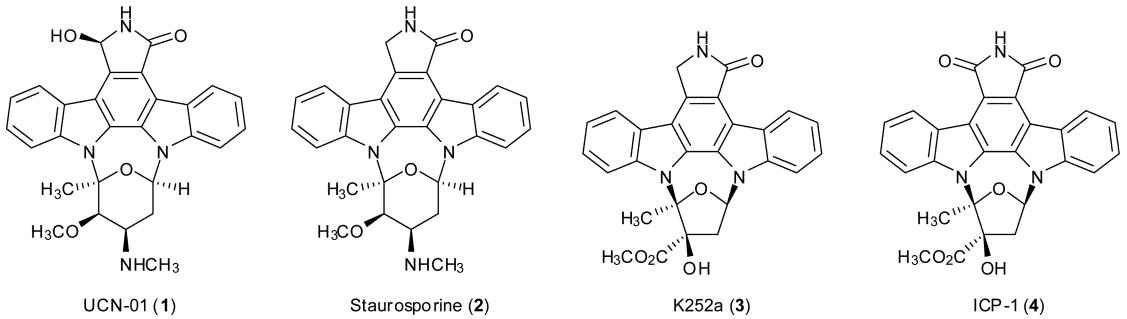


Fig. 1 Indolocarbazoles **1–4**.

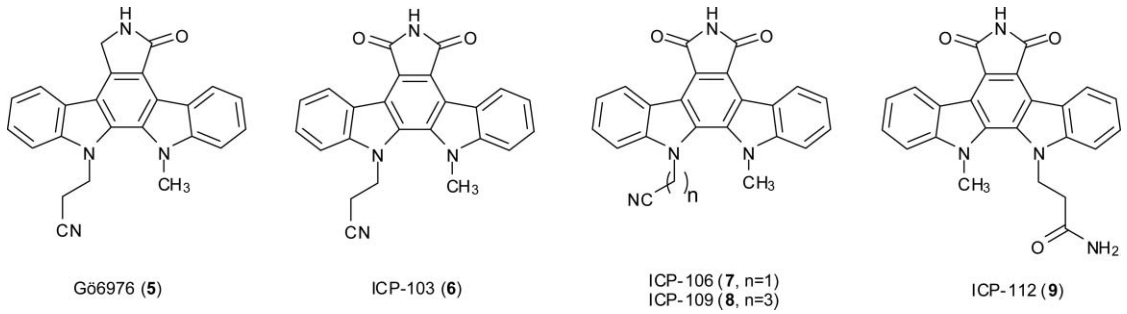


Fig. 2 Gö6976 analogues.

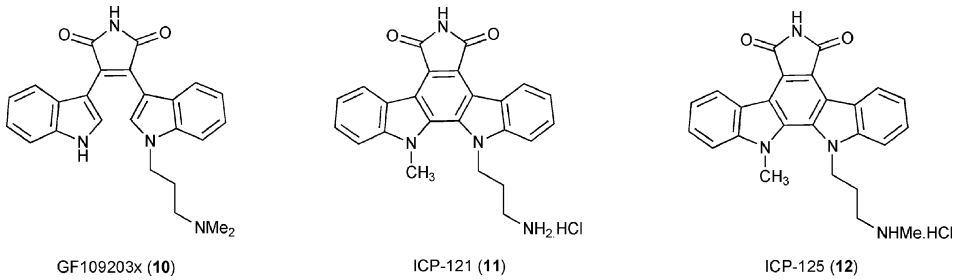
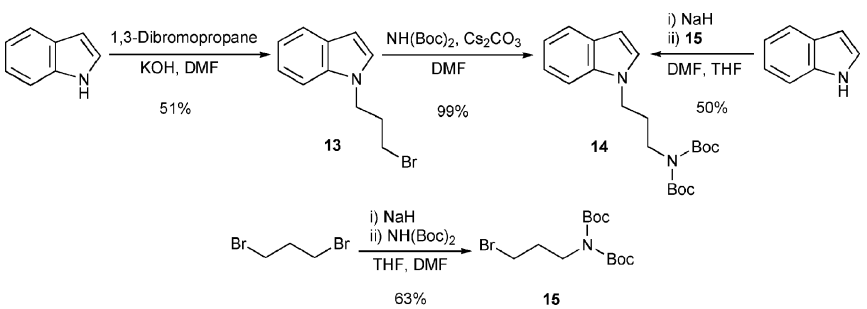


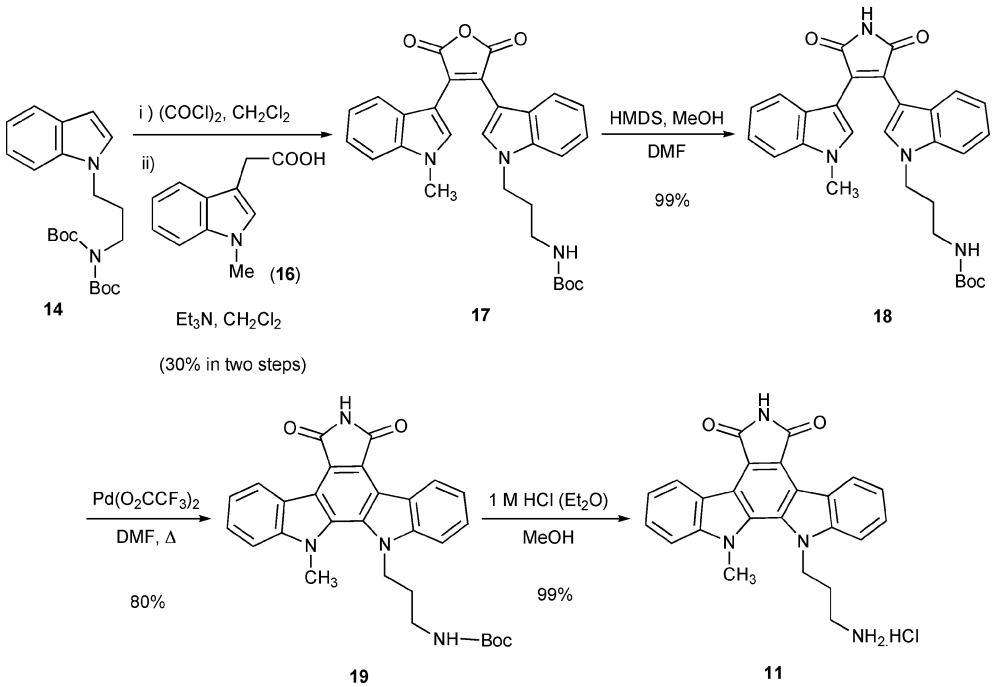
Fig. 3 GF109203x analogues.

from 1,3-dibromopropane and di-*tert*-butyl-iminodicarboxylate in the presence of sodium hydride.[14] *N*-Alkylation of indole-3-acetic acid using methyl iodide in the presence of excess sodium hydride gave 1-methylindole-3-acetic acid (**16**) in 94% yield.[10]

Amine **14** was treated with oxalyl chloride in dichloromethane to furnish the glyoxylyl chloride which was immediately treated with 1-methylindole-3-acetic acid (**16**) in the presence of triethylamine to produce anhydride **17** in 30% yield in two steps from **14** (Scheme 2).[15] Loss of one Boc group was observed during this reaction sequence. The anhydride **17** was subsequently converted to the imide **18** by exposure to HMDS and MeOH in DMF in 99% yield.[16] During our synthesis of ICP-106 (**7**), we found that the final oxidative cyclization was quite challenging for the bisindolylmaleimide with substituents present on both *N*-12 and *N*-13.[17] Low yields are registered in most cases and the isolation of the final product is difficult from the complex reaction mixture. However, in some cases, palladium(II) trifluoroacetate was found to be superior for this cyclization.[11] To our delight, heating



Scheme 1



Scheme 2

bisindolylmaleimide **18** in DMF in the presence of palladium(II) trifluoroacetate gave **19** in 80% yield. Finally, deprotection of the Boc-group using an ethereal solution of 1M HCl in methanol gave the target compound **11** in essentially quantitative yield.

Our target compound **12** was synthesized in a similar fashion. Boc-Protection of 3-bromopropylamine hydrobromide furnished **20** in 92% yield (Scheme 3).[18] Compound **20** was then used to alkylate indole to give compound **21**. Compound **21** was methylated with iodomethane in the presence of sodium hydride to produce **22**. Fully protected amine **22** was subjected to the coupling reaction with 1-methylindole-3-acetic acid (**16**) which produced the desired anhydride **23** in 32% yield. Conversion of anhydride **23** to imide **24** was achieved using HMDS and MeOH in 99% yield. Bisindolylmaleimide **24** was then subjected to the challenging oxidative cyclization reaction using palladium(II) trifluoroacetate. To our extreme satisfaction, we obtained the desired product **25** in excellent yield. This is the highest yield we have obtained so far for this otherwise capricious cyclization step. Finally, deprotection of the Boc group furnished the target compound **12**.

In work to be reported separately, we find that ICP-125 (**12**) exhibits high potency in an assay using flow cytometry analysis. Thus, ICP-125 (**12**) abrogates S phase arrest at 100 nM indicating the compound is inhibiting Chk1. However, ICP-121 (**11**) was tested up to 10 μM and found to be inactive in the same assay. These values can be compared to the efficacy of Gö6976 (**5**) of 30 nM and efficacy of ICP-103 (**6**) of 100 nM in the same assay.

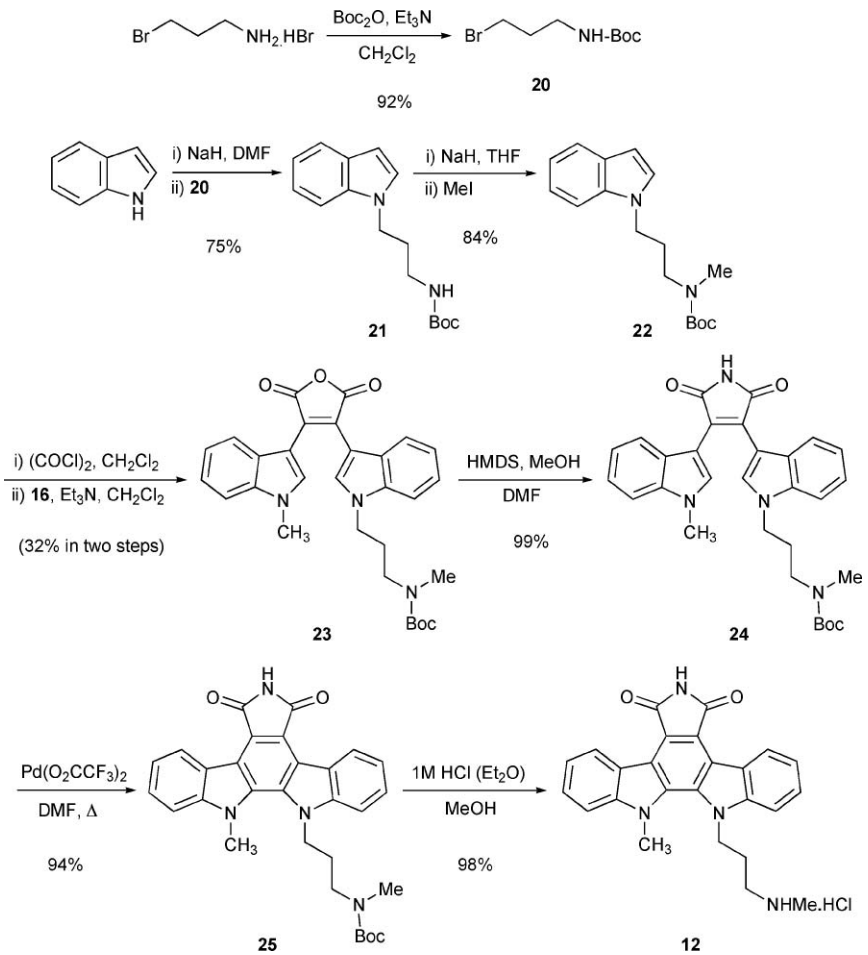
In summary, we have synthesized ICP-125 (**12**) and find it to be a potent Chk1 inhibitor. We have found that Pd(II) catalyzed oxidative cyclization is much more effective for bisindolylmaleimides bearing an amine group. Also, we find that a secondary amine or a nitrile are more desirable than a primary amine or amide on the chain. Work is in progress in our laboratory with other nitrogen-bearing functionalities and these will be reported in due course.

Experimental

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. All other solvents (analytical grade) including anhydrous solvents and reagents were used as received. All experiments were performed under a nitrogen atmosphere unless otherwise stated.

1-(3-Bromopropyl)-1*H*-indole (13)

To a stirred solution of indole (1.17 g, 10 mmol) and freshly powdered KOH (88%, 0.64 g, 10 mmol) in DMF (25 mL) was added 1,3-dibromopropane (6.06 g, 30 mmol) in DMF (25 mL) in one portion. The mixture was stirred at rt for 24 h. Water (100 mL) was added and extracted with ether (3 × 75 mL). The organic phase was washed with water (100 mL), dried ($MgSO_4$) and concentrated *in vacuo.* The residue was purified by column chromatography on silica gel (initially pet. ether; then 15 : 1 pet. ether : ether) to furnish the desired product (1.21 g, 51%) as a colorless oil: IR (thin film): 3051, 2939, 1611, 1511, 1461, 1314, 1258, 1229, 742 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.71–7.73 (m, 1H), 7.45 (d, 1H, J = 8.3 Hz), 7.29–7.32 (m, 1H), 7.19–7.22 (m, 2H), 6.58 (d, 1H, J = 2.9 Hz), 4.38 (t, 2H, J = 6.3 Hz), 3.35 (t, 2H, J = 6.3 Hz), 2.39 (2H, qn, J = 6.3 Hz); ^{13}C-NMR ($CDCl_3$): δ 135.9, 128.8, 128.1, 121.8, 121.2, 119.6, 109.4, 101.6, 44.0, 32.8, 30.7.



Scheme 3

A small amount of 1-allylindole was recovered as a colorless oil[19] (0.16 g, 10%) which came in earlier fractions during column chromatography: ^{1}H-NMR ($CDCl_3$): δ 7.78–7.82 (m, 1H), 7.44–7.47 (m, 1H), 7.33–7.38 (m, 1H), 7.24–7.30 (m, 1H), 7.20 (d, 1H, J = 3.2 Hz), 6.04–6.16 (m, 1H), 5.29–5.34 (m, 1H), 5.16–5.24 (m, 1H), 4.79–4.82 (m, 2H); ^{13}C-NMR ($CDCl_3$): δ 136.2, 133.6, 128.8, 128.0, 121.7, 121.1, 119.6, 117.3, 109.7, 101.5, 48.9.

Bis(1,1-dimethylethyl)(3-bromopropyl)imidodicarbonate (15)

To a stirred solution of di-*tert*-butyl-iminodicarboxylate (1.09 g, 5 mmol) in THF : DMF (40 mL, 3 : 1) was added sodium hydride (60% dispersion in mineral oil, 0.21 g, 5.25 mmol). The mixture was heated at 65 °C for 2.5 h, 1,3-dibromopropane (2.3 mL, 22.5 mmol) was added, and the mixture was stirred for 3 h. It was cooled to 0 °C and ether (50 mL) was added. Excess hydride was destroyed by the dropwise addition of water. The organic phase was washed with water (2 × 50 mL) and dried ($MgSO_4$). The solvent was evaporated and the residue was purified by column chromatography on silica gel (initially 98 : 2 hexanes : ether; then 1 : 1 hexanes : ether) to yield the desired product (1.06 g, 63%) as a colorless oil: IR (thin film): 3265, 2973, 1688, 1490, 1297, 1118, 1076 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 3.68–3.73 (m, 2H), 3.39 (t, 2H, J = 6.7 Hz), 2.08–2.18 (m, 2H); ^{13}C-NMR ($CDCl_3$): δ 152.6, 82.7, 45.4, 32.3, 30.6, 28.2.

Bis(1,1-dimethylethyl)[3-(1*H*-indolyl)propyl]imidodicarbonate (14)

From 13. Compound **13** (0.26 g, 1.1 mmol), di-*tert*-butyl-iminodicarboxylate (0.22 g, 1 mmol) and caesium carbonate (0.33 g, 1 mmol) in DMF (10 mL) were stirred for 6 h at 70 °C. Water (40 mL) was added and extracted with ethyl acetate (3 × 25 mL). The organic phase was washed with water (2 × 25 mL) and dried (Na_2SO_4). The solvent was evaporated and the residue was purified by column chromatography on silica gel (4 : 1 hexanes : ethyl acetate) to furnish the desired product (0.37 g, 99%) as a colorless oil.

From 15. To a stirred suspension of NaH (60% dispersion in mineral oil, 0.22 g, 5.5 mmol) in DMF (15 mL) at 0 °C was added dropwise a solution of indole (0.41 g, 3.5 mmol) in DMF (10 mL). After stirring the mixture at 0 °C for 30 min, a solution of **15** (1.59 g, 4.7 mmol) in DMF : THF (20 mL, 1 : 1) was added. The mixture was allowed to reach rt and stirring was continued for 36 h. It was then cooled to 0 °C and water (50 mL) was added very slowly. The aqueous layer was extracted with ethyl acetate (2 × 50 mL). The organic phase was washed with water (3 × 50 mL) and brine (50 mL), and dried (Na_2SO_4). The crude product was

purified by column chromatography on silica gel (4 : 1 hexanes : ethyl acetate) to yield the desired product (0.65 g, 50%) as a colorless oil.

IR (thin film): 2977, 2933, 1787, 1745, 1696, 1366, 1146, 1126, 740 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.62–7.64 (m, 1H), 7.33 (d, 1H, $J = 8.3$ Hz), 7.19–7.22 (m, 1H), 7.13 (d, 1H, $J = 2.9$ Hz), 7.08–7.12 (m, 1H), 4.16 (t, 2H, $J = 7.1$ Hz), 3.65 (t, 2H, $J = 7.1$ Hz), 2.14 (qn, 2H, $J = 7.3$ Hz), 1.46 (s, 18H); ^{13}C-NMR ($CDCl_3$): δ 152.6, 136.0, 128.8, 127.8, 121.6, 121.2, 119.4, 109.3, 101.4, 82.7, 44.3, 44.1, 29.7, 28.2; LRMS (EI): *m/z* 374 (M^+), 218, 201, 173, 144, 130 (100%); HRMS (EI): calcd for $C_{21}H_{30}N_2O_4$: 374.2206, found: 374. 2197.

1-Methyl-1*H*-indole-3-acetic acid (16)

To a stirred suspension of NaH (6.0 g, 150 mmol, 60% mineral oil dispersion) 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 stirring was continued 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}; ^{1}H-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.

1,1-Dimethylethyl(3-{3-[2,5-dihydro-4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-3-furanyl]-1*H*-indol-1-yl}propyl)carbamate (17)

To a stirred solution of **14** (0.37 g, 1.0 mmol) in dichloromethane (10 mL) at 0 °C was added dropwise oxalyl chloride (0.09 mL, 1.05 mmol). After stirring for 45 min at 0 °C, the solvent was evaporated *in vacuo.* The residue was redissolved in dichloromethane (10 mL) and added dropwise to a stirred solution of **16** (0.19 g, 1.0 mmol) and triethylamine (0.28 mL, 2 mmol) in dichloromethane (5 mL) at rt. The mixture was stirred at rt for 10 h. The solvent was evaporated and the crude residue was purified by column chromatography on silica gel (98 : 2 dichloromethane : methanol) to furnish the desired product (149 mg, 30%) as a dark red solid: mp 99–101 °C; IR (thin film): 2977, 1817, 1750, 1704, 1527, 1252, 1171, 740 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.98 (s, 1H), 7.88 (s, 1H), 7.52 (d, 1H, $J = 8.2$ Hz), 7.47 (d, 1H, $J = 8.2$ Hz), 7.07–7.11 (m, 2H), 6.97–7.00 (m, 2H), 6.78 (t, 1H, $J = 7.6$ Hz), 6.69–6.73 (m, 2H), 4.25 (t, 2H, $J = 6.7$ Hz), 3.89 (s, 3H), 2.90 (q, 2H, $J = 6.4$ Hz), 1.83 (qn, 2H, $J = 6.7$ Hz), 1.38 (s, 9H); LRMS (EI): *m/z* 499 (M^+), 425, 399 (100%), 356, 312, 283, 269, 249, 191, 158, 107, 77; HRMS (EI): calcd for $C_{29}H_{29}N_3O_5$: 499.2107, found: 499.2112.

1,1-Dimethylethyl(3-{3-[2,5-dihydro-4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-1*H*-pyrrol-3-yl]-1*H*-indol-1-yl}propyl)carbamate (18)

To a stirred solution of **17** (0.25 g, 0.5 mmol) in DMF (2.5 mL) at rt was added HMDS (1.1 mL, 5 mmol) and methanol (0.1 mL, 2.5 mmol). The flask was tightly sealed and the mixture was stirred at rt for 24 h. The mixture was poured into water (25 mL) and extracted with ethyl acetate (2 × 25 mL) and dried ($MgSO_4$). The solvent was evaporated and the residue was purified by column chromatography on silica gel (95 : 5 dichloromethane : methanol) to yield the desired product (246 mg, 99%) as a dark red solid: mp 112–114 °C; IR (thin film): 2969, 1755, 1703, 1610, 1531, 1333, 1170, 740 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.93 (s, 1H), 7.86 (s, 1H), 7.76 (s, 1H), 7.45 (d, 1H, $J = 8.2$ Hz), 7.40 (d, 1H, $J = 8.2$ Hz), 6.97–7.04 (m, 3H), 6.89 (d, 1H, $J = 7.9$ Hz), 6.61–6.70 (m, 3H), 4.23 (t, 2H, $J = 6.7$ Hz), 3.86 (s, 3H), 2.90 (q, 2H, $J = 6.1$ Hz), 1.83 (qn, 2H, $J = 6.6$ Hz, 1.38 (s, 9H); LRMS (ESI+): *m/z* 521 $[M + Na]^+$, 499 $[M + H]^+$; HRMS (ESI+): calcd for $C_{29}H_{31}N_4O_4$ [M + H]: 499.2345, found: 499.2341.

1,1-Dimethylethyl[12-(3-aminopropyl)-12,13-dihydro-13-methyl-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione]carbamate (19)

A mixture of **18** (25 mg, 0.05 mmol) and palladium(II) trifluoroacetate (84 mg, 0.25 mmol) in DMF (3 mL) was heated at 90 °C for 2.5 h. The mixture was cooled to rt and ethyl acetate (25 mL) was added. The organic phase was washed with 0.5 N HCl (50 mL), water (50 mL) and dried (Na_2SO_4). The solution was filtered through Hyflo and purified by column chromatography on silica gel (95 : 5 dichloromethane : methanol) to yield the desired product (20 mg, 80%) as a yellow fluorescent solid: mp 213–215 °C (dec); IR (thin film): 3203, 1755, 1698, 1575, 1450, 1317, 1163, 745 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 11.12 (s, 1H), 9.10–9.14 (m, 2H), 7.87 (d, 1H, $J = 8.2$ Hz), 7.75 (d, 1H, $J = 8.2$ Hz), 7.60–7.67 (m, 2H), 7.38–7.43 (m, 2H), 6.76 (t, 1H, $J = 5.3$ Hz), 4.79 (t, 2H, $J = 7.5$ Hz), 4.21 (s, 3H), 2.65 (q, 2H, $J = 6.1$ Hz), 1.67 (qn, 2H, $J = 6.9$ Hz), 1.24 (s, 9H); LRMS (ESI+): *m/z* 497 $[M + H]^+$; HRMS (ESI+): calcd for $C_{29}H_{29}N_4O_4$ [M + H]: 497.2189, found: 497.2169.

12-(3-Aminopropyl)-12,13-dihydro-13-methyl-5*H*-indolo[2,3-*a*]-pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione hydrochloride (11)

To a solution of **19** (15 mg, 0.03 mmol) in methanol (2 mL) at rt was added dropwise 1 M HCl in ether (9 mL). The mixture was stirred at rt for 5 h. The solvent was evaporated and the residue was recrystallized from methanol to furnish the desired product (12.8 mg, 99%) as a yellow solid: mp >300 °C (dec.); ^{1}H-NMR (DMSO-d_6): δ 11.18 (s, 1H), 9.16 (d, 1H, $J = 7.6$ Hz), 9.13 (d, 1H, $J = 7.9$ Hz), 7.97 (d, 1H, $J = 8.5$ Hz), 7.80 (d, 1H, $J = 8.2$ Hz), 7.65–7.70 (m, 5H), 7.44 (t, 2H, $J = 7.6$ Hz), 4.89 (t, 2H, $J = 7.3$ Hz), 4.25 (s, 3H), 2.46–2.49 (m, 2H), 1.78 (qn, 2H, $J = 7.6$ Hz); ^{13}C-NMR (DMSO-d_6): δ 170.8, 170.7, 144.9, 143.7, 133.3, 131.8, 127.7, 127.5, 124.9, 124.5, 122.9, 121.8, 121.5, 121.2, 121.1, 120.1, 119.6, 118.7, 112.4, 111.4, 45.6, 36.8; LRMS (ESI+): *m/z* 397 $[(M - HCl) + H]^+$; HRMS (ESI+): calcd for $C_{24}H_{21}N_4O_2$ [(M − HCl) + H]: 397.1665, found: 397.1662.

1,1-Dimethylethyl(3-bromopropyl)carbamate (20)

To a stirred solution of 3-bromopropylamine hydrobromide (2.20 g, 10 mmol) and Boc-anhydride (2.18 g, 10 mmol) in dichloromethane (50 mL) at 0 °C was added dropwise triethylamine (2.89 mL, 20 mmol). The solution was stirred at the same temperature for 15 min. The mixture was allowed to reach rt and stirred for 7 h. The mixture was washed with water (2 × 50 mL) and dried (Na_2SO_4). The solvent was evaporated and the residue was purified by column chromatography on silica gel (initially hexanes; then 1 : 1 hexanes : ethyl acetate) to furnish the desired product (2.19 g, 92%) as a colorless oil which slowly solidified at −4 °C: mp: 38–40 °C (lit.[21] 33–34 °C); IR (thin film): 3252, 2969, 1685, 1490, 1450, 1298, 1119, 1076 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 3.44 (t, 2H, $J = 6.6$ Hz), 3.27 (t, 2H, $J = 6.6$ Hz), 2.04 (qn, 2H, $J = 6.6$ Hz), 1.44 (s, 9H); ^{13}C-NMR ($CDCl_3$): δ 156.1, 32.9, 31.0, 28.6, 27.6.

1,1-Dimethylethyl[3-(1*H*-indol-1-yl)propyl]carbamate (21)

To a stirred solution of NaH (60% dispersion in mineral oil, 0.24 g, 6 mmol) in DMF (25 mL) at 0 °C was added dropwise indole (1.19 g, 5 mmol) dissolved in DMF (15 mL). After the addition, the mixture was heated at 80 °C for 1 h. It was then cooled to 0 °C. Then, a solution of **20** (1.31 g, 5.5 mmol) in DMF (10 mL) was added dropwise and stirred at 0 °C for 30 min. The mixture was then allowed to reach rt and stirring was continued for 18 h. The DMF was evaporated and the oily residue was dissolved in ethyl acetate (100 mL). The organic phase was washed with water (3 × 50 mL) and brine (50 mL), and dried (Na_2SO_4). The solvent was evaporated and the residue was purified by column chromatography on silica gel (2 : 1 hexanes : ethyl acetate) to furnish the desired product (1.03 g, 75%) as a colorless oil: IR (thin film): 3350, 2974, 2932, 1694, 1512, 1462, 1250, 1171, 742 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.74 (d, 1H, $J = 8.1$ Hz), 7.38–7.41 (m, 1H), 7.28–7.33 (m, 1H), 7.19–7.24 (m, 1H), 7.16 (d, 1H, $J = 3.3$ Hz), 6.59 (d, 1H, $J = 2.9$ Hz), 4.78 (brs, 1H), 4.16 (t, 2H, $J = 6.9$ Hz), 3.12 (m, 2H), 2.01 (qn, 2H, $J = 6.9$ Hz), 1.56 (s, 9H); ^{13}C-NMR ($CDCl_3$): δ 156.1, 135.9, 128.7, 127.9, 121.6, 121.1, 119.4, 109.3, 101.3, 79.4, 43.7, 38.2, 30.5, 28.5; LRMS (EI): *m/z* 274 (M^+), 218, 201, 156, 144, 130 (100%), 117; HRMS (EI): calcd for $C_{16}H_{22}N_2O_2$: 274.1681, found: 274.1685.

1,1-Dimethylethyl[3-(1*H*-indol-1-yl)propyl]methylcarbamate (22)

To a stirred solution of NaH (60% dispersion in mineral oil, 0.26 g, 6.4 mmol) in THF (20 mL) at 0 °C was added dropwise a solution of **21** (0.88 g, 3.2 mmol) in THF (10 mL). The mixture was stirred at the same temperature for 45 min. Then iodomethane (0.32 mL, 5.1 mmol) was added dropwise. After the addition, the solution was slowly allowed to warm to rt and stirred for 20 h. The solution was cooled to 0 °C and the excess hydride was destroyed by the dropwise addition of ice-cold water. Dichloromethane (100 mL) was added and the organic phase was washed with water (2 × 50 mL) and brine (50 mL), and dried (Na_2SO_4). The solvent was evaporated and the residue was purified by column chromatography on silica gel (2 : 1 hexanes : ethyl acetate) to furnish the desired product (0.77 g, 84%) as a yellowish oil: IR (thin film): 2974, 2930, 1694, 1482, 1464, 1393, 1365, 1170, 1147, 741 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.69–7.71 (m, 1H), 7.39 (d, 1H, $J = 8.3$ Hz), 7.24–7.30 (m, 1H), 7.14–7.20 (m, 2H), 6.56 (d, 1H, $J = 2.7$ Hz), 4.18 (t, 2H, $J = 7.2$ Hz), 3.32 (t, 2H, $J = 6.8$ Hz), 2.88 (s, 3H), 2.07–2.16 (m, 2H), 1.51 (s, 9H); ^{13}C-NMR ($CDCl_3$): δ 156.1, 136.1, 129.0, 127.9, 121.8, 121.3, 119.6, 109.5, 101.5, 79.9, 46.7, 44.1, 34.5, 28.7; LRMS (EI): *m/z* 288 (M^+), 232, 215, 156, 144, 131 (100%), 117; HRMS (EI): calcd for $C_{17}H_{24}N_2O_2$: 288.1838, found: 288.1844.

1,1-Dimethylethyl(3-{3-[2,5-dihydro-4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-3-furanyl]-1*H*-indol-1-yl}propyl)methylcarbamate (23)

To a stirred solution of **22** (1.44 g, 5 mmol) in dichloromethane (50 mL) at 0 °C was added dropwise oxalyl chloride (0.45 mL, 5.1 mmol). The mixture was stirred at the same temperature for 20 min. Then the solvent was evaporated and the residue was redissolved in dichloromethane (50 mL) and added dropwise to a stirred solution of **16** (0.95 g, 5 mmol) and triethylamine (1.52 mL, 10 mmol) in dichloromethane (20 mL) at rt. The solution was stirred for 12 h. The solvent was evaporated and the residue was purified by column chromatography on silica gel (98 : 2 dichloromethane : methanol) to furnish the desired product (0.81 g, 32%) as a dark-red solid: mp 81–83 °C; IR (thin film): 2973, 2932, 1816, 1752, 1689, 1528, 1255, 1147, 740 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.99 (s, 1H), 7.85 (s, 1H), 7.50 (t, 2H, $J = 9.3$ Hz), 7.06–7.14 (m, 2H), 7.01 (d, 1H, $J = 8.1$ Hz), 6.79 (t, 1H, $J = 7.5$ Hz), 6.67–6.73 (m, 2H), 4.22 (t, 2H, $J = 6.8$ Hz), 3.89 (s, 3H), 3.14 (m, 2H), 2.74 (s, 3H), 1.93 (m, 2H), 1.28–1.39 (m, 9H); LRMS (ESI+): *m/z* 536 $[M + Na]^+$, 514 $[M + H]^+$; HRMS (ESI+): calcd for $C_{30}H_{32}N_3O_5$ [M + H]: 514.2342, found: 514.2336.

1,1-Dimethylethyl(3-{3-[2,5-dihydro-4-(1-methyl-1*H*-indol-3-yl)-2,5-dioxo-1*H*-pyrrol-3-yl]-1*H*-indol-1-yl}propyl)methylcarbamate (24)

To a stirred solution of anhydride **23** (257 mg, 0.5 mmol) in DMF (2 mL) were added HMDS (1.1 mL, 5 mmol) and methanol (0.1 mL, 2.5 mL). The reaction flask was tightly closed and the mixture was stirred for 36 h. It was poured into cold water (50 mL) and extracted with ethyl acetate (3 × 50 mL). The organic phase was washed with water (2 × 50 mL) and dried (Na_2SO_4). The solvent was evaporated and the residue was purified by column chromatography on silica gel (95 : 5 dichloromethane : methanol) to furnish the desired product (253 mg, 99%) as an orange-red solid: mp 215–217 °C; IR (thin film): 3218, 2972, 1755, 1701, 1532, 1332, 1147, 739 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 10.94 (s, 1H), 7.86 (s, 1H), 7.74 (s, 1H), 7.39–7.46 (m, 2H), 6.99–7.06 (m, 2H), 6.94 (d, 1H, $J = 8.1$ Hz), 6.68–6.72 (m, 2H), 6.58–6.63 (m, 1H), 4.19 (t, 2H, $J = 6.8$ Hz), 3.85 (s, 3H), 3.13 (m, 2H), 2.73 (s, 3H), 1.92 (m, 2H), 1.29–1.39 (m, 9H); LRMS (ESI+): *m/z* 513 $[M + H]^+$; HRMS (ESI+): calcd for $C_{30}H_{33}N_4O_4$ [M + H]: 513.2502, found: 513.2501.

1,1-Dimethylethyl{12-(3-aminopropyl)-12,13-dihydro-13-methyl-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione}methylcarbamate (25)

A mixture of imide **24** (26 mg, 0.05 mmol) and palladium(II) trifluoroacetate (84 mg, 0.25 mmol) in DMF (3 mL) was heated at 90 °C for 2 h. The mixture was cooled and poured into ethyl acetate (25 mL). The organic phase was washed with 0.5 N HCl (50 mL),

water (2 × 50 mL), dried (Na_2SO_4) and filtered through Hyflo. The filtrate was concentrated and purified by column chromatography on silica gel (95 : 5 dichloromethane : methanol) to furnish the desired product (24 mg, 94%) as a yellow solid: mp 208–210 °C (dec.); IR (thin film): 3221, 2973, 1755, 1716, 1694, 1450, 1316, 1158, 744 cm^{-1}; ^{1}H-NMR (acetone-d_6): δ 9.84 (s, 1H), 9.18–9.22 (m, 2H), 7.79 (d, 1H, J = 8.2 Hz), 7.69 (d, 1H, J = 8.2 Hz), 7.58–7.64 (m, 2H), 7.34–7.39 (m, 2H), 4.84 (m, 2H), 4.27 (s, 3H), 2.87 (m, 2H), 2.64 (s, 3H), 1.83–1.89 (m, 2H), 1.26–1.41 (m, 9H); LRMS (ESI+): m/z 533 $[M + Na]^+$, 511 $[M + H]^+$; HRMS (ESI+): calcd for $C_{30}H_{30}N_4O_4Na$ [M + Na]: 533.2165, found: 533.2169.

12,13-Dihydro-12-methyl-13-{3-[(1-methyl)amino]propyl}-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione hydrochloride (12)

To a stirred solution of **25** (15 mg, 0.03 mmol) in methanol (2 mL) was added 1 M HCl in ether (9 mL). The mixture was stirred at rt for 5 h. Then the solvent was evaporated and the residue was purified by recrystallization from methanol to yield the desired product (13.2 mg, 98%) as a yellow solid: mp >290 °C (dec.); ^{1}H-NMR (DMSO-d_6): δ 11.17 (s, 1H), 9.16 (d, 1H, J = 7.9 Hz), 9.12 (d, 1H, J = 7.9 Hz), 8.61 (brs, 2H), 7.97 (d, 1H, J = 8.2 Hz), 7.79 (d, 1H, J = 8.2 Hz), 7.64–7.69 (m, 2H), 7.44 (t, 2H, J = 7.5 Hz), 4.88 (t, 2H, J = 7.5 Hz), 4.25 (s, 3H), 2.67 (m, 2H), 2.35 (s, 3H), 1.83–1.87 (m, 2H); ^{13}C-NMR (DMSO-d_6): δ 170.8, 170.7, 144.8, 143.6, 133.2, 131.7, 127.7, 127.5, 124.9, 124.5, 122.9, 121.8, 121.5, 121.2, 121.1, 120.2, 119.6, 118.7, 112.4, 111.4, 45.5, 45.4, 36.8, 32.3, 24.7; LRMS (ESI+): m/z 411 $[(M - HCl) + H]^+$; HRMS (ESI+): calcd for $C_{25}H_{23}N_4O_2$ $[(M - HCl) + H]$: 411.1821, found: 411.1817.

Acknowledgements

This investigation was supported in part by the donors of the Petroleum Research Fund (PRF), administered by the American Chemical Society, the National Institutes of Health (CA 82220), and Wyeth.

References

1 For a review on cell-cycle checkpoints, see: A. Eastman, *J. Cell Biochem.*, 2004, **91**, 223.
2 (*a*) G. W. Gribble and S. J. Berthel, in *Studies in Natural Product Chemistry*, ed. Atta-ur-Rahman, Elsevier, Amsterdam, 1993, vol. 12, p. 365; (*b*) M. Prudhomme, *Recent Pat. Anti-Cancer Drug Discov.*, 2006, **1**, 55; (*c*) M. Prudhomme, *Curr. Pharm. Des.*, 1997, **3**, 265; (*d*) U. Pindur and T. Lemster, *Recent Res. Dev. Org. Bioorg. Chem.*, 1997, **33**; (*e*) U. Pindur, Y.-S. Kim and F. Mehrabani, *Curr. Med. Chem.*, 1999, **6**, 29.
3 (*a*) R. T. Bunch and A. Eastman, *Clin. Cancer Res.*, 1996, **2**, 791; (*b*) R. T. Bunch and A. Eastman, *Cell Growth Differ.*, 1997, **8**, 779; (*c*) E. A. Kohn, N. D. Ruth, M. K. Brown, M. Livingstone and A. Eastman, *J. Biol. Chem.*, 2002, **277**, 26553.
4 (*a*) I. Takahashi, K. Asano, I. Kawamoto and H. Nakano, *J. Antibiot.*, 1989, **42**, 564; (*b*) I. Takahashi, Y. Saitoh, M. Yoshida, H. Sano, H. Nakano, M. Morimoto and T. Tamaoki, *J. Antibiot.*, 1989, **42**, 571.
5 (*a*) P. R. Graves, L. Yu, J. K. Schwarz, J. Gales, E. A. Sausville, P. M. O'Connor and H. Piwinica-Worms, *J. Biol. Chem.*, 2000, **275**, 5600; (*b*) E. C. Busby, D. F. Leistritz, R. T. Abraham, L. M. Karnitz and J. N. Sarkaria, *Cancer Res.*, 2000, **60**, 2108.
6 E. Fuse, H. Tanii, N. Kurata, H. Kobayashi, Y. Shimada, T. Tamura, Y. Sasaki, Y. Tanigawara, R. D. Lush, D. Headlee, W. D. Figg, S. G. Arbuck, A. M. Senderowicz, E. A. Sausville, S. Akinaga, T. Kuwabara and S. Kobayashi, *Cancer Res.*, 1998, **58**, 3248.
7 A. Eastman, E. A. Kohn, M. K. Brown, J. Rathman, M. Livingstone, D. H. Blank and G. W. Gribble, *Mol. Cancer Ther.*, 2002, **1**, 1067.
8 E. A. Kohn, C. J. Yoo and A. Eastman, *Cancer Res.*, 2003, **63**, 31.
9 Y. Ishimi, Y. Komamura-Kohno, H.-J. Kwon, K. Yamada and M. Nakanishi, *J. Biol. Chem.*, 2003, **278**, 24644.
10 S. Roy, A. Eastman and G. W. Gribble, *Synth. Commun.*, 2005, **35**, 595.
11 S. Roy, A. Eastman and G. W. Gribble, *Tetrahedron*, 2006, **62**, 7838.
12 (*a*) D. Toullec, P. Pianetti, H. Coste, P. Bellevergue, T. Grand-Perret, M. Ajakane, V. Baudet, P. Boissin, E. Boursier, F. Lucette Loriolle, L. Duhamel, D. Charon and J. Kirilovsky, *J. Biol. Chem.*, 1991, **266**, 15771; (*b*) D. Thomas, B. C. Hammerling, A.-B. Wimmer, K. Wu, E. Ficker, Y. A. Kuryshev, D. Scherer, J. Kiehn, H. A. Katus, W. Schoels and C. A. Karle, *Cardiovasc. Res.*, 2004, **64**, 467; (*c*) D. R. Alessi, *FEBS Lett.*, 1997, **402**, 121; (*d*) H. Sipma, L. Vanderzee, J. Vandenakker, A. Denhertog and A. Nelemans, *Br. J. Pharmacol.*, 1996, **119**, 730; (*e*) P. Hauss, F. Mazerolles, C. Hivroz, O. Lecomte, C. Barbat and A. Fischer, *Cell. Immunol.*, 1993, **150**, 439; (*f*) S. K. Lee and P. H. Stern, *Biochem. Pharmacol.*, 2000, **60**, 923.
13 W. Dehaen and A. Hassner, *J. Org. Chem.*, 1991, **56**, 896.
14 J. K. Dutton, J. H. Knox, X. Radisson, H. J. Ritchie and R. Ramage, *J. Chem. Soc., Perkin Trans. 1*, 1995, 2581.
15 P. D. Davis, R. A. Bit and S. A. Hurst, *Tetrahedron Lett.*, 1990, **31**, 2353.
16 P. D. Davis and R. A. Bit, *Tetrahedron Lett.*, 1990, **31**, 5201.
17 M. M. Faul, L. L. Winneroski and C. A. Krumrich, *J. Org. Chem.*, 1999, **64**, 2465.
18 M. I. Dawson, D. L. Harris, G. Liu, P. D. Hobbs, C. W. Lange, L. Jong, N. Bruey-Sedano, S. Y. James, X.-K. Zhang, V. J. Peterson, M. Leid, L. Farhana, A. K. Rishi and J. A. Fontana, *J. Med. Chem.*, 2004, **47**, 3518.
19 S. Bartlett and A. Nelson, *Org. Biomol. Chem.*, 2004, 2874.
20 H. R. Snyder and E. L. Eliel, *J. Am. Chem. Soc.*, 1948, **70**, 1703.
21 T. Boxus, R. Touillaux, G. Dive and J. Marchand-Brynaert, *Bioorg. Med. Chem.*, 1998, **6**, 1577.

