1-1-2007

Synthesis of novel 3'-spirocyclic-oxindole derivatives and assessment of their cytostatic activities

Sarah Yong
University of Wollongong, sry02@uow.edu.au

Alison T. Ung
University of Wollongong, alison_ung@uow.edu.au

Stephen G. Pyne
University of Wollongong, spyne@uow.edu.au

Brian W. Skelton
University of Western Australia

Allan H. White
University of Western Australia

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Abstract

The synthesis of some novel 3'-spirocyclic-oxindole compounds, based on the spiro[indole-3,5'-isoxazolidin]-2(1*H*)-one, the 2'*H*-spiro[indole-3,6'-[1,3]oxazinane]-2,2'(1*H*)-dione and the 2'*H*-spiro[indoline-3,3'-pyrrolo[1,2-*c*][1,3']oxazine]-1',2(1*H*)-dione heterocyclic structures, is described. These compounds were prepared from methyl α-(2-nitrophenyl)acrylate via [1,3]-dipolar cycloaddition reactions with two acyclic nitrones and one cyclic nitrone followed by reduction of the cycloadducts and then treatment with triphosgene. Two of these compounds showed significant cytostatic activity on three cancer cell lines with GI_{50} values of 2.6–4.1 μM on the human breast cancer cell line, MCF-7.

Keywords

Synthesis, novel, spirocyclic, oxindole, derivatives, assessment, their, cytostatic, activities, CMMB

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Publication Details

Yong, S., Ung, A. T., Pyne, S. G., Skelton, B. W. & White, A. H. (2007). Synthesis of novel 3"-spirocyclic-oxindole derivatives and assessment of their cytostatic activities. Tetrahedron, 63 5579-5586.

Synthesis of novel 3'-spirocyclic-oxindole derivatives and assessment of their cytostatic activities

Sarah R. Yong[a], Alison T. Ung[a]*, Stephen G. Pyne[a]*,

Brian W. Skelton[b] and Allan H. White[b]

[a]*Department of Chemistry, University of Wollongong, Wollongong, New South Wales, 2522, Australia*

[b]*Department of Chemistry, University of Western Australia, Crawley, Western Australia, 6009, Australia*

Abstract: The synthesis of some novel 3'-spirocyclic-oxindole compounds, based on the spiro[indole-3,5`-isoxazolidin]-2(1*H*)-one, the 2`*H*-spiro[indole-3,6`-[1,3]oxazinane]-2,2`(1*H*)-dione and the 2`*H*-spiro[indoline-3,3`-pyrrolo[1,2-*c*][1,3`]oxazine]-1`,2(1*H*)-dione heterocyclic structures, are described. These compounds were prepared from methyl α-(2-nitrophenyl)acrylate *via* [1,3]-dipolar cycloaddition reactions with two acyclic nitrones and one cyclic nitrone followed by reduction of the cycloadducts and then treatment with triphosgene. Two of these compounds showed significant cytostatic activity on three cancer cell lines with GI_{50} values of 2.6-4.1 μM on the human breast cancer cell line, MCF-7.

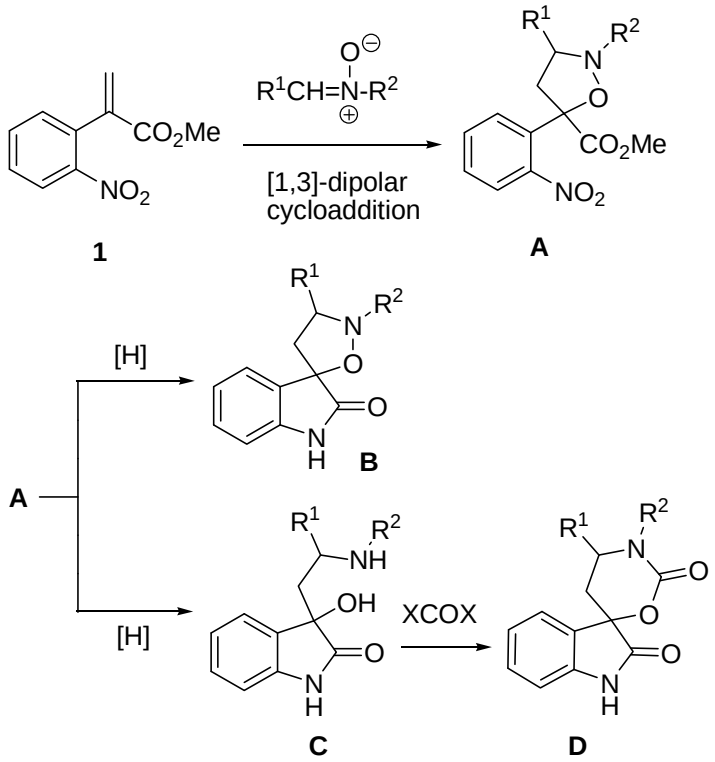
Key words: isoxazolidine, oxazinane, oxindole, nitrones, [1,3]-dipolar cycloaddition, spirocyclic compounds, cytotoxic activity.

Corresponding authors: e-mail: aung@uow.edu.au; spyne@uow.edu.au

1. Introduction

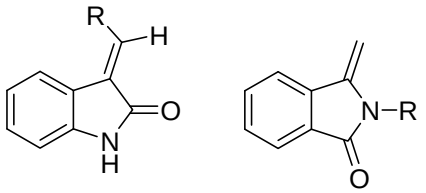
3'-Spirocyclo-oxindoles, of synthetic or natural origin, have a range of biological activities.[1] As part of a medicinal chemistry project we have been focusing on the synthesis of novel 3'-spirocyclo-oxindoles as scaffolds for new drug discovery.[2,3] As an extension of this project we required the synthesis of the novel 3'-spirocyclic-oxindoles of the type **B** and **D**. These were planned to be accessed from the isoxazoline intermediate **A** which in principle could be formed *via* a [1,3]-dipolar cycloaddition reaction between the acrylate **1**[2-4] and nitrones. Regioselective reduction of **A** would provide isoxazolidine spirocyclic oxindoles **B**, while further reduction would provide the amino-alcohol **C** which upon treatment with phosgene, or its equivalent, was expected to provide oxazinane spirocyclic oxindoles of the type **D** (Scheme 1).

Scheme 1



During the course of this project Parmar *et al.*[1f] reported the synthesis of isoxazolidine spirocyclic oxindoles, related to **B**, from the [1,3]-dipolar cycloaddition of the 3-methyleneindolone derivative **2a** (R = CO_2Et) with nitrones (Figure 1), while Williams,[5] Wang[1a] and Schreiber[6] have earlier

described [1,3]-dipolar cycloadditions of azomethine ylides to **2a** (R = CO_2Et, aryl and CO_2allyl) and their 5- and 6-substituted derivatives to provide novel spirocyclic structures. Some of these were found to be a new class of non-peptide, small molecule MDM2-p53 inhibitors, a relatively new target for cancer chemotherapy.[1a] In 1998, Melot[7] reported the synthesis of isoxazolidine spirocyclic isoindolines, compounds isomeric to **B**, using a nitrone cycloaddition reaction of 3-methyleneisoindolines **2b**.



2a **2b**

Figure 1

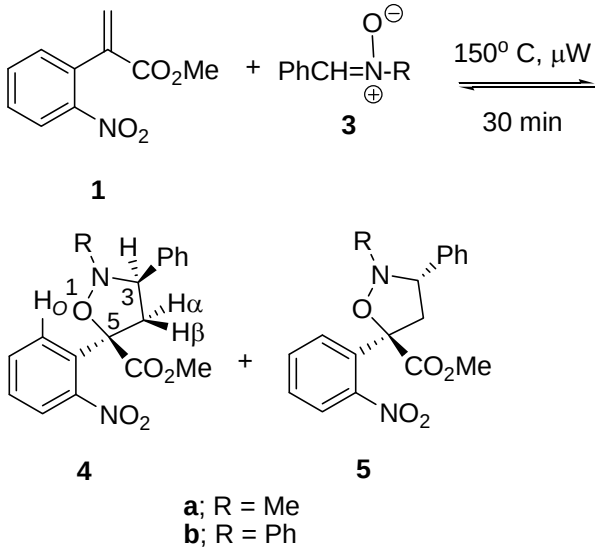
We report here our efforts for preparing novel spirocycles related to **B** and **D** and their stereochemistries and the cytostatic activities of some of these compounds against three cancer-cell lines.

2. Results and Discussion

Initial investigations on the [1,3]-dipolar cycloaddition of **1** with nitrones involved a study of the reaction of **1** and the nitrone **3a**. Heating a dichloromethane solution of **1** and **3a** (1.1 molar equiv.) at 60° C in a sealed tube for 4 d resulted in a mixture of **1**, **4a** and **5a**, from which pure samples of **4a** and **5a** could be isolated in yields of 26% and 20%, respectively after purification by column chromatography (Scheme 2). Heating a mixture of **1** and **3a** (1.1-1.2 molar equiv.) at 150° C in a microwave reactor in toluene solution, or in the absence of solvent, for 30 min resulted in the isolation of pure samples of **1**, **4a** and **5a**, in yields of 29%, 15% and 30%, respectively. In each case unreacted dipolarophile (**1**) was isolated. When pure cycloadduct **4a** was heated at 150° C in a microwave reactor without solvent for 30 min, a 42 : 30 : 28 mixture of **1**, **4a** and **5a**, respectively, was produced. This experiment clearly demonstrated the reversible nature of the cycloaddition reaction between **1** and **3a** and also helped to explain the lack of complete consumption of the

dipolarophile in these reactions. We assume that the nitrone **3a** was also generated in this experiment but it could not be detected from ^{1}H NMR analysis of the crude reaction mixture. We suspect that **3a** was unstable to the thermal conditions. Heating a mixture of **1** and nitrone **3b** (1.3 molar equiv.) at 150^o C in a microwave reactor in the absence of solvent resulted in *ca* 20 : 60 : 20 mixture of **1**, **4b** and **5b**, respectively from ^{1}H NMR analysis. Separation of this mixture by column chromatography gave pure samples of **1**, **4b** and **5b**, in yields of 11%, 58%, and 6%, respectively and a mixture of **1** and **5b** that was difficult to separate.

Scheme 2 (compounds **4** and **5** are racemic)



^{1}H NMR analysis clearly indicated that **4a,b** and **5a,b** were all 5-isoxazolidinecarboxylate regioisomers (with the expected three proton coupled spin system of H-3 and H4α and H-4β clearly evident) and not the alternative 4-isoxazolidinecarboxylate regioisomers. The structure of **4a** was unequivocally determined by a single crystal X-ray structural determination (Figure 2)[8] which indicated that **4a** was the 5-*exo* isomer and thus indicated that **5a** was the 5-*endo* isomer.

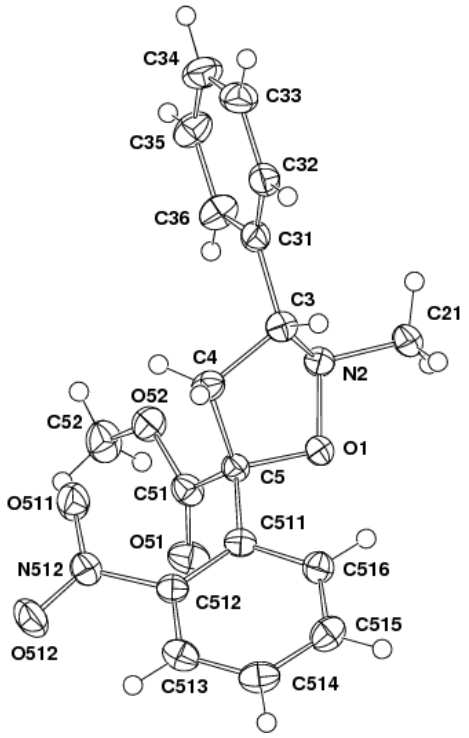
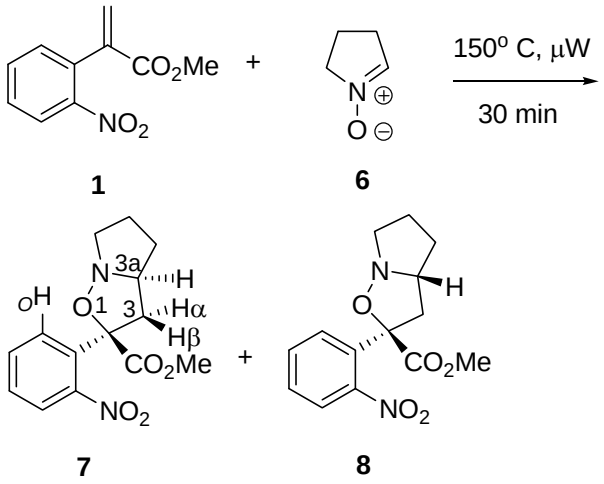


Figure 2. Molecular projection of **4a** (50% probability displacement amplitude ellipsoids for non-hydrogen atoms, hydrogen atoms having arbitrary radii of 0.1 Å/ O(1)-N(2) is 1.472(3) Å.

Heating a toluene solution of a mixture of **1** and the cyclic nitrone **6** at 150^{o} C for 30 min in a microwave reactor gave a *ca* 33 : 50 : 17 mixture of **1**, **7** and **8**, respectively (from ^{1}H NMR analysis). Separation of this mixture by column chromatography gave pure samples of **7** and **8**, in yields of 40%, and 6%, respectively and a mixture of **1** and **8** that was difficult to separate.

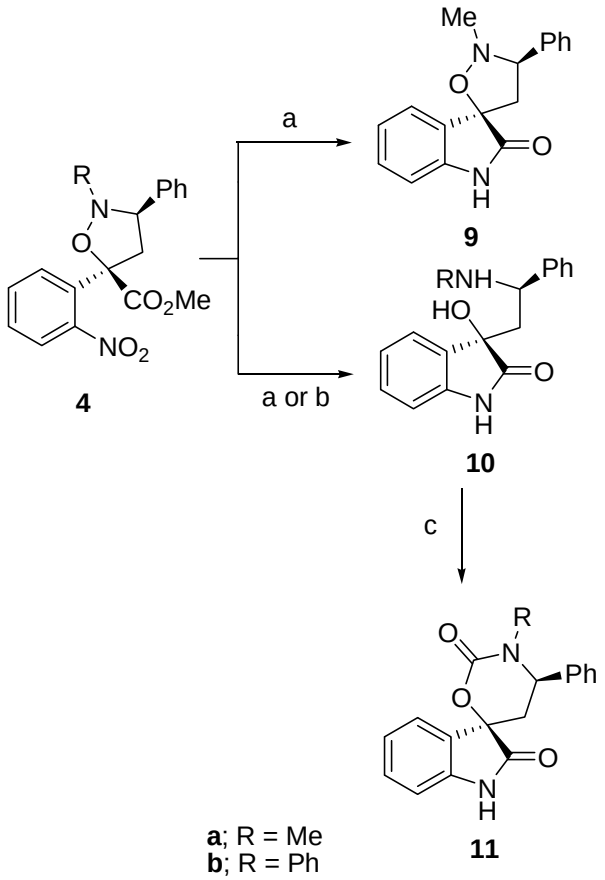
^{1}H NMR analysis clearly indicated that **7** and **8** were both pyrrolo[1,2-*b*]isoxazole-2-carboxylate regioisomers (with the expected three proton coupled spin system of H-3a and H-3α and H-3β clearly evident). The relative stereochemistry of these adducts was assigned based on NOESY studies. These studies showed cross-peaks between H_O and the most upfield H-3 proton (H-3α) for both compounds **7** and **8** while cross-peaks were observed between H-3a and H-3α in **7** and between H-3a and H-3β (in C_6D_6) in **8**. This analysis indicated that **7** was the *endo*-isomer and **8** the *exo*-isomer.

Scheme 3 (compounds **7** and **8** are racemic)



Treatment of **4a** over 10% Pd/C under a hydrogen atmosphere gave a mixture of the isoxazolidine spirocyclic oxindole **9a** and its further reduced product **10a** from which **9a** could be isolated in pure form in 24% yield (Scheme 4). The formation of the oxindole ring in **9a** was clearly evident from ^{13}C NMR analysis with a resonance at δ 179.4 for the oxindole carbonyl group. Treatment of **4b** under similar conditions gave **10b** in 55% yield. Treatment of **4a** or **4b** with activated zinc dust in glacial acetic acid under sonication conditions for 1 h provided **10a** and **10b**, in respective yields of 58% and 90% (Scheme 4). Treatment of these compounds with triphosgene under basic conditions (Et_3N) gave the oxazinane spirocyclic oxindoles **11a** and **11b** in respective yields of 61% and 68% (Scheme 4). The ^{13}C NMR spectra of these compounds clearly showed resonances for the oxindole (*ca* δ 170) and oxazinanone (*ca* δ 150) carbonyl groups.

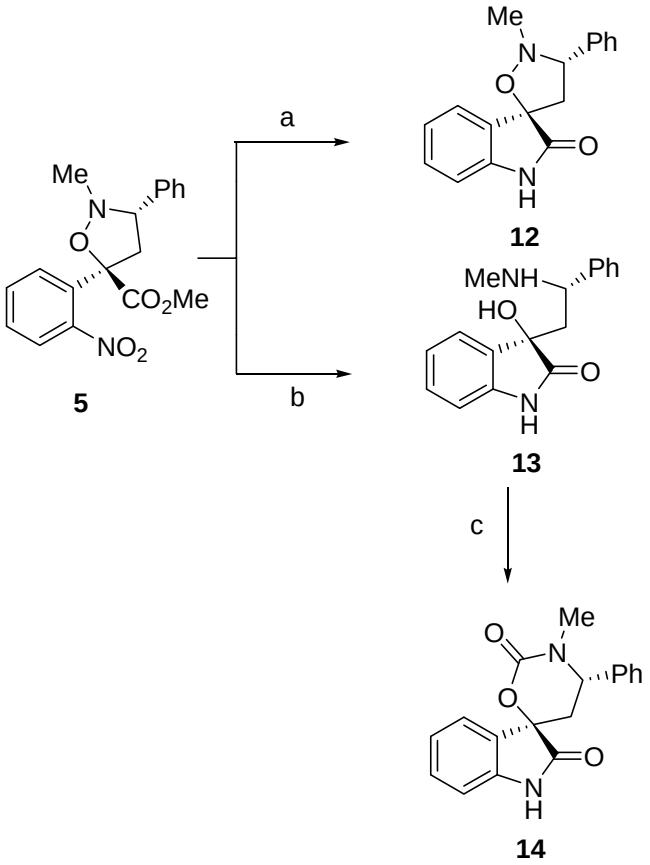
Scheme 4 (compounds **9**-**11** are racemic)[a]



[a] *Reagents and conditions*: (a) 10% Pd/C, H_2 (1 atm), EtOAc, rt, 18h; **9a** (24%), **10b** (55%). (b) Zn dust (10 equiv), HOAc, sonication, rt, 1 h; **10a** (58%), **10b** (90%). (c) triphosgene, Et_3N, THF, rt; **11a** (61%, 3 days), **11b** (68%, 2 days).

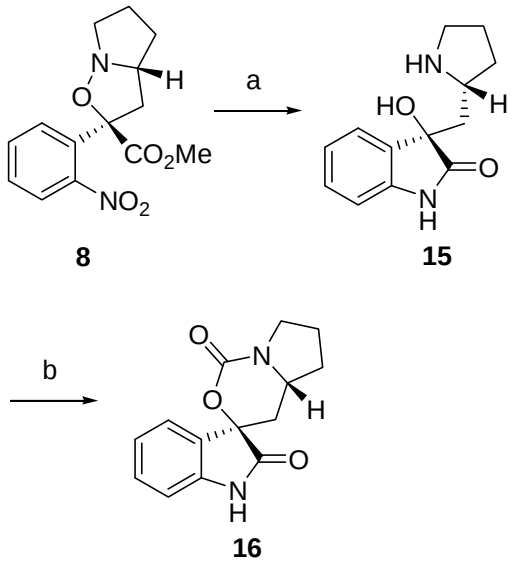
The compounds **12-16** were prepared in a similar fashion according to Schemes 5 and 6. All attempts at the hydrogenation of **7** or **8** over 10% Pd/C or the reduction of these compounds with zinc dust in glacial acetic acid under sonication conditions gave rise to complex reaction mixtures. However, hydrogenation/hydrogenolysis of **8** over $PdCl_2$ in methanol followed by treatment of the crude reaction mixture with triphosgene/Et_3N gave the desired tetracyclic spiro compound **16** in 25% overall yield (Scheme 6).

Scheme 5 (compounds **12-14** are racemic)[a]



[a] *Reagents and conditions*: (a) 10% Pd/C, H_2 (1 atm), EtOAc, rt, 18h; 54%. (b) Zn dust (10 equiv), HOAc, sonication, rt, 1 h, 94%. (c) triphosgene, Et_3N, THF, rt, 2 days, 51%.

Scheme 6 (compounds **15** and **16** are racemic)[a]



[a] *Reagents and conditions*: (a) $PdCl_2$, H_2 (1 atm), MeOH, rt, 3h. (b) triphosgene, Et_3N, THF, rt, 2 days, 25% overall from **8**.

3. Cytostaticity Studies

Cytostaticity studies against the cancer cell lines, H460 (human non small cell lung), MCF-7 (human breast) and SF-268 (human CNS) were performed at the Peter MacCallum Cancer Institute, Melbourne, Australia, using standard NCI protocols. Initially the percentage cell growth of cells incubated with 25 μM of compounds **4a**, **7**, **9**, **10b**, **11a**, **11b** and **14** was measured after 72 h. The results are presented in Table 1.

Table 1. Cytostatic studies on cancer cell lines.

Entry	Compound	Percentage Cell Growth		
		H460	**MCF-7**	**SF-268**
1	**4a**	64	97	78
2	**7**	2	7 (GI_{50} = 4.1 ± 0.2 μM)	18
3	**9**	0	1 (GI_{50} = 2.6 ± 0.1 μM)	14
4	**10b**	55	107	78
5	**11a**	63	79	77
6	**11b**	58	85	79
7	**14**	80	101	100

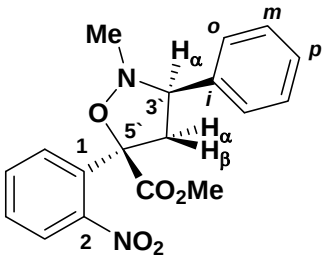
The initial screening indicated that only compounds **7** and **9** had an appreciable cytostatic activity against all three cell lines at 25 μM (Table 1, entries 2 and 3). The GI_{50} (concentration for 50% of growth inhibition) on these compounds on MCF-7 cells was 4.1 and 2.6 μM, respectively as extrapolated from duplicate studies of growth inhibition over a drug concentration of 0-25 μM (see Supporting Information). It was observed that **9** had a much sharper GI curve (see Figures A and B in Supporting Information) than **7** (see Figures C and D in Supporting Information), which may suggest that it has a narrow therapeutic index, that is there is a fineline between no activity and high toxicity. Furthermore at higher doses of **9**, cytotoxicity as opposed to cytostaticity was clearly demonstrated as fewer cells remained at the end of the assay than when at the beginning. Interestingly, while the isoxazolidine spirocyclic isoindoline **9** had the most cytostatic activity, its ring expanded analogues, **11a,b** having an oxazinane ring rather than a isoxazolidine showed little activity. This may be a function of the differences in ring size (5 versus 6) and/or the basicity of the respective nitrogen atoms.

In conclusion, the synthesis of some novel 3'-spirocyclic-oxindole compounds, based on the spiro[indole-3,5`-isoxazolidin]-2(1*H*)-one, the 2`*H*-spiro[indole-3,6`-[1,3]oxazinane]-2,2`(1*H*)-dione and the 2`*H*-spiro[indoline-3,3`-pyrrolo[1,2-*c*][1,3`]oxazine]-1`,2(1*H*)-dione heterocyclic structures has been achieved. These compounds were prepared from methyl α-(2-nitrophenyl)acrylate **1** *via* [1,3]-dipolar cycloaddition reactions with two acyclic nitrones and one cyclic nitrone followed by reduction of the cycloadducts and then treatment with triphosgene. Two

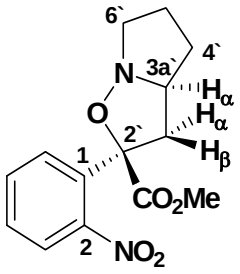
of these compounds showed significant cytostatic activity on three cancer cell lines with GI_{50} values of 2.6-4.1 μM on the human breast cancer cell line, MCF-7.

Experimental

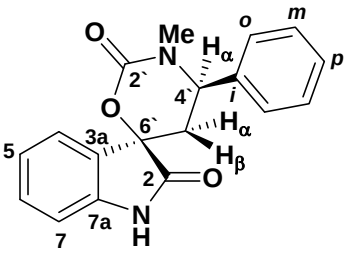
Petrol refers to the fraction of petroleum spirit with a boiling point of 40-60 °C. All ^{1}H NMR spectra were performed at 300 MHz and all ^{13}C NMR (DEPT) spectra at 75 MHz in $CDCl_3$ solution, unless otherwise noted. All spectra were referenced to $CDCl_3$ (^{1}H δ 7.26 ppm and ^{13}C NMR δ 77.00 ppm). ^{1}H NMR assignments were achieved with the aid of gCOSY, and in some cases NOESY and TOCSY experiments. ^{13}C NMR assignments were based upon DEPT, gHSQC and gHMBC experiments. All solvents were dried over anhydrous magnesium sulfate, unless stated otherwise. The atom numbering for compounds **4a** and **7** and **9** and their derivatives is as indicated below.



4a



7



9

Methyl (3`*R*\*, 5`*R)-2`-methyl-5`-(2-nitrophenyl)-3`-phenylisoxazolidine-5`-carboxylate (4a) and Methyl (3`*S*\*, 5`*R**)-2`-methyl-5`-(2-nitrophenyl)-3`-phenylisoxazolidine-5`-carboxylate (5a)**

The title compounds were prepared using two methods. **Method 1**: To a solution of **1** (110 mg, 5.3 × 10^{-4} mol) in anhydrous CH_2Cl_2 (1 mL), contained within a sealed tube was added nitrone **3a** (85.5

mg, 6.3×10^{-4} mol). The tube was sealed and the mixture was left stirring at 60 °C for 4 d. ^{1}H NMR analysis of the crude reaction mixture revealed the ratio of **4a** : **5a** : **1** was 59 : 33 : 8. The mixture was purified by column chromatography using 30% EtOAc:petrol as eluent to yield **4a** as a yellow oil (47.2 mg, 1.3×10^{-4} mol, 26%, R_f = 0.37 in EtOAc/petrol (1 : 9)) and **5a** as a yellow oil (36.8 mg, 1.1×10^{-4} mol, 20%, R_f = 0.16 in EtOAc/petrol (1 : 9)) and a mixture of **1** and **5a**. **Method 2**: A mixture of **1** (581.4 mg, 2.8 mmol) and nitrone **3a** (379 mg, 2.8 mmol) was placed in a sealed glass microwave reaction vessel. The mixture was subjected to microwave-assisted heating at 150 °C for 30 min (CEM microwave reactor with temperature and pressure control). ^{1}H NMR analysis of the crude reaction mixture revealed the ratio of **4a** : **5a** : **1** was 27 : 39 : 34. The mixture was purified by column chromatography using CH_2Cl_2/petrol/MeOH (1 : 4 : 0.1) as eluent to yield **4a** as off-white clear crystals (149.7 mg, 4.4×10^{-4} mol, 15%, m.p. 124-126 °C) and a mixture of **5a** and **1**. The mixture was further purified by column chromatography using 20% EtOAc:petrol as eluent to yield **5a** as a yellow oil (285 mg, 8.3×10^{-4} mol, 30%) and recovered **1** (168 mg, 8.0×10^{-4} mol, 29%).

4a: MS (EI) *m/z* 342 (19%) [$M^{\cdot+}$], 296 (9%), 220 (11%), 134 (88%); 118 (5%), 104 (89%); HRMS (EI) Calcd for $C_{18}H_{18}N_2O_5$ [$M^{\cdot+}$] 342.1216. Found: 342.1217. ^{1}H NMR (500 MHz) δ 8.22 (d, *J* 7.5 Hz, 1H, ArC<u>H</u>-6); 8.14 (d, *J* 7.5 Hz, 1H, ArC<u>H</u>-3); 7.74 (t, *J* 7.5 Hz, 1H, ArC<u>H</u>-5); 7.53 (t, *J* 7.5 Hz, 1H, ArC<u>H</u>-4); 7.49 (d, *J* 7.3 Hz, 2H, ArC<u>H</u>-*o*); 7.36 (t, *J* 7.3 Hz, 2H, ArC<u>H</u>-*m*); 7.32 (t, *J* 7.3 Hz, 1H, ArC<u>H</u>-*p*); 3.88 (bt, *J* 11.7 Hz, 1H, C<u>H</u>$_\beta$CH$_\alpha$-4`); 3.75 (s, 3H, CO$_2$C<u>H</u>$_3$); 3.54 (bs, 1H, C<u>H</u>$_\alpha$-3`); 2.73 (s, 3H, NC<u>H</u>$_3$); 2.62 (dd, *J* 13.5, 7.0 Hz, 1H, C<u>H</u>$_\alpha$CH$_\beta$-4`). ^{13}C NMR (125 MHz) δ 169.6 (<u>C</u>O$_2$); 146.3 (Ar<u>C</u>-2); 137.3 (Ar<u>C</u>-1); 136.9 (Ar<u>C</u>-*i*); 133.8 (Ar<u>C</u>H-5); 128.75 (Ar<u>C</u>H-4); 128.72 (Ar<u>C</u>H-*m*); 128.4 (Ar<u>C</u>H-*o*); 128.2 (Ar<u>C</u>H-*p*); 127.7 (Ar<u>C</u>H-6); 125.3 (Ar<u>C</u>H-3); 82.8 (<u>C</u>-5`); 73.4 (<u>C</u>H-3`); 53.0 (CO$_2$<u>C</u>H$_3$); 49.9 (<u>C</u>H$_2$-4`); 43.0 (N<u>C</u>H$_3$).

5a: MS (EI) *m/z* 342 (13%) [$M^{\cdot+}$], 296 (4%), 220 (9%), 134 (89%); 118 (28%), 104 (72%); HRMS (EI) Calcd for $C_{18}H_{18}N_2O_5$ [$M^{\cdot+}$] 342.1216. Found: 342.1220. ^{1}H NMR (500 MHz) δ 8.29 (dd, *J* 8.0, 1.5 Hz, 1H, ArC<u>H</u>-6); 8.14 (dd, *J* 8.0, 1.5 Hz, 1H, ArC<u>H</u>-3); 7.77 (dt, *J* 8.0, 1.5 Hz, 1H, ArC<u>H</u>-5); 7.51 (dt, *J* 8.0, 1.5 Hz, 1H, ArC<u>H</u>-4); 7.30-7.23 (m, 5H, ArC<u>H</u>-*o*, ArC<u>H</u>-*m* and ArC<u>H</u>-*p*); 3.97 (t, *J* 9.0, 7.5 Hz, 1H, C<u>H</u>$_\beta$-3`); 3.92 (dd, *J* 12.7, 6.5 Hz, 1H, C<u>H</u>$_\beta$CH$_\alpha$-4`); 3.73 (s, 3H, CO$_2$C<u>H</u>$_3$); 2.72 (s, 3H, NC<u>H</u>$_3$); 2.39 (dd, *J* 13.0, 9.5 Hz, 1H, C<u>H</u>$_\alpha$CH$_\beta$-4`). $^{13}$C NMR (125 MHz) δ 169.0 (<u>C</u>O$_2$); 146.3 (Ar<u>C</u>-2); 139.2 (Ar<u>C</u>-1); 137.1 (Ar<u>C</u>-*i*); 134.3 (Ar<u>C</u>H-5); 128.7 (Ar<u>C</u>H-*o*); 128.5 (Ar<u>C</u>H); 128.3 (Ar<u>C</u>H); 128.2 (Ar<u>C</u>H); 127.7 (Ar<u>C</u>H-*m*); 125.2 (Ar<u>C</u>H-3); 85.5 (<u>C</u>-5`); 74.1 (<u>C</u>H-3`); 53.0 (CO$_2$<u>C</u>H$_3$); 51.8 (<u>C</u>H$_2$-4`); 43.0 (N<u>C</u>H$_3$).

Methyl (3`*R*\*, 5`*R)-5`-(2-nitrophenyl)-2`,3`-diphenylisoxazolidine-5`-carboxylate (4b) and Methyl (3`*S*\*, 5`*R**)-5`-(2-nitrophenyl)-2`,3`-diphenylisoxazolidine-5`-carboxylate (5b)**

A mixture of **1** (133.7 mg, 6.5×10^{-4} mol) and nitrone **3b** (164.7 mg, 8.4×10^{-4} mol) was placed in a sealed glass microwave reaction vessel. The mixture was subjected to microwave-assisted heating at 150 °C for 30 min. ^{1}H NMR analysis of the crude reaction mixture revealed the ratio of **4b** : **5b** : **1** was 62 : 21 : 17. The mixture was purified by column chromatography using 0-10% EtOAc in petrol as eluent to yield **4b** as a bright yellow oil (151.3 mg, 3.7×10^{-4} mol, 58%, R_f = 0.72 in EtOAc/petrol (1 : 9)) and a mixture of **5b** and **1**. The mixture was further purified using a chromatotron® (0-2.5% EtOAc in petrol) to yield **5b** as a yellow oil (14.9 mg, 3.7×10^{-5} mol, 6%, R_f = 0.31 in EtOAc/petrol (1 : 9)) and recovered **1** (15.3 mg, 7.4×10^{-5} mol, 11%) and a mixture of **5b** and **1** (43.6 mg).

4b: MS (EI) *m/z* 404 (58%) [$M^{\cdot+}$], 345 (2%) [M^{+}-CO_2Me], 296 (7%), 220 (17%), 194 (21%), 180 (32%), 134 (26%), 104 (91%); HRMS (EI) Calcd for $C_{23}H_{20}N_2O_5$ [$M^{\cdot+}$] 404.1372. Found: 404.1357. ^{1}H NMR (500 MHz) δ 8.37 (dd, *J* 8.3, 1.3 Hz, 1H, ArC<u>H</u>-6); 8.14 (dd, *J* 8.3, 1.3 Hz, 1H, ArC<u>H</u>-3); 7.76 (dt, *J* 8.3, 1.3 Hz, 1H, ArC<u>H</u>-5); 7.52 (dt, 8.3, 1.3 Hz, 1H, ArC<u>H</u>-4); 7.31 (d, *J* 7.3 Hz, 2H, ArC<u>H</u>-*o*); 7.27 (t, *J* 7.3 Hz, 2H, ArC<u>H</u>-*m*); 7.24 (t, *J* 7.3 Hz, 1H, ArC<u>H</u>-*p*); 7.21 (t, *J* 8.0 Hz, 2H, ArC<u>H</u>-*m*`); 7.05 (d, *J* 8.0 Hz, 2H, ArC<u>H</u>-*o*`); 7.01 (t, *J* 8.0 Hz, 1H, ArC<u>H</u>-*p*`); 4.83 (dd, *J* 9.5, 7.5 Hz, 1H, C<u>H</u>$_\alpha$-3`); 4.12 (dd, *J* 13.5, 7.5 Hz, 1H, C<u>H</u>$_\beta$CH$_\alpha$-4`); 3.67 (s, 3H, C<u>H</u>$_3$); 2.54 (dd, *J* 13.5, 9.5 Hz, 1H, C<u>H</u>$_\alpha$CH$_\beta$-4`). ^{13}C NMR (125 MHz) δ 168.6 (<u>C</u>O$_2$Me); 149.3 (Ar<u>C</u>-*i*`); 146.5 (Ar<u>C</u>-2); 139.3 (ArC-*i*); 137.9 (Ar<u>C</u>-1); 134.3 (Ar<u>C</u>H-5); 128.6 (Ar<u>C</u>H-*m*`); 128.84 (Ar<u>C</u>H-*m*`); 128.82 (Ar<u>C</u>H-4); 128.1 (Ar<u>C</u>H-6); 127.9 (Ar<u>C</u>H-*p*); 127.0 (Ar<u>C</u>H-*o*); 125.2 (Ar<u>C</u>H-3); 123.8 (Ar<u>C</u>H-*p*`); 117.7 (Ar<u>C</u>H-*o*`); 85.6 (<u>C</u>-5`); 71.0 (<u>C</u>H-3`); 52.9 (<u>C</u>H$_3$); 51.9 (<u>C</u>H$_2$-4`).

5b: MS (EI) *m/z* 404 (52%) [$M^{\cdot+}$], 345 (2%) [M^{+}-CO_2Me], 296 (10%), 220 (18%), 194 (22%), 180 (39%), 134 (26%), 104 (92%). HRMS (EI) Calcd for $C_{23}H_{20}N_2O_5$ [$M^{\cdot+}$] 404.1372. Found: 404.1358. ^{1}H NMR δ 8.15 (dd, *J* 8.1, 1.2 Hz, 1H, ArC<u>H</u>-3); 8.12 (dd, *J* 7.8, 1.2 Hz, 1H, ArC<u>H</u>-6); 7.68 (dt, *J* 7.8, 1.2 Hz, 1H, ArC<u>H</u>-5); 7.53 (dt, *J* 8.1, 1.2 Hz, 1H, ArC<u>H</u>-4); 7.51 (d, *J* 6.9 Hz, 2H, ArC<u>H</u>-*o*); 7.36 (t, *J* 6.9 Hz, 2H, ArC<u>H</u>-*m*); 7.32 (t, *J* 6.9 Hz, 1H, ArC<u>H</u>-*p*); 7.20 (t, *J* 6.9 Hz, 2H, ArC<u>H</u>-*m*`); 7.02 (d, *J* 6.9 Hz, 2H, ArC<u>H</u>-*o*`); 7.02-6.98 (m, 1H, ArC<u>H</u>-*p*`); 4.37 (dd, *J* 9.3, 7.5 Hz, 1H, C<u>H</u>$_\beta$-3`); 3.93 (dd, *J* 13.5, 9.3 Hz, 1H, C<u>H</u>$_\beta$CH$_\alpha$-4`); 3.78 (s, 3H, C<u>H</u>$_3$); 2.89 (dd, *J* 13.5, 7.5 Hz, 1H, C<u>H</u>$_\alpha$CH$_\beta$-4`). ^{13}C NMR (125 MHz) δ 169.1 (<u>C</u>O$_2$Me); 148.5 (Ar<u>C</u>-*i*`); 146.5 (Ar<u>C</u>-2); 138.8 (Ar<u>C</u>-*i*); 136.9 (Ar<u>C</u>-1); 133.8 (Ar<u>C</u>H-5); 128.95 (Ar<u>C</u>H-4); 128.89 (Ar<u>C</u>H-*m*); 128.5 (Ar<u>C</u>H-*m*`); 128.0 (Ar<u>C</u>H-*p*); 128.1 (Ar<u>C</u>H-6); 127.6 (Ar<u>C</u>H-*o*); 125.4 (Ar<u>C</u>H-3); 123.5 (Ar<u>C</u>H-*p*`); 118.0 (Ar<u>C</u>H-*o*`); 84.2 (<u>C</u>-5`); 69.0 (<u>C</u>H-3`); 53.1 (<u>C</u>H$_3$); 50.9 (<u>C</u>H$_2$-4`).

Methyl (2`*R*\*, 3a`*S)-2`-(2-nitrophenyl)hexahydropyrrolo[1,2-*b*]isoxazole-2`-carboxylate (7) and Methyl (2`*R*\*, 3a`*R**)-2`-(2-nitrophenyl)hexahydropyrrolo[1,2-*b*]isoxazole-2`-carboxylate (8)**

To **1** (87.7 mg, 4.2×10^{-4} mol) in a sealed glass microwave reaction vessel was added a solution of nitrone **6** (72 mg, 8.5×10^{-4} mol) in anhydrous toluene (0.4 mL). The mixture was subjected to microwave-assisted heating at 150 °C for 30 min. ^{1}H NMR analysis of the crude reaction mixture revealed the ratio of **7** : **8** : **1** was 49 : 18 : 33. The crude mixture was purified by column chromatography using 20-100% EtOAc:petrol as eluent to yield **7** as a light-yellow crystalline solid (51.2 mg, 1.7×10^{-4} mol, 40%, R_f = 0.54 in EtOAc/petrol (3 : 7)) and **8** as a light yellow semicrystalline oil (7.3 mg, 2.5×10^{-5} mol, 6%, R_f = 0.22 in EtOAc/petrol (3 : 7)) and recovered **1** (35.6 mg, 1.7×10^{-4} mol, 40%).

7: MS (EI) *m/z* 292 (33%) [$M^{\cdot+}$], 257 (34%), 244 (52%), 233 (85%), 104 (96%); HRMS (EI) Calcd for $C_{14}H_{16}N_2O_5$ [$M^{\cdot+}$], 292.1059. Found: 292.1051. ^{1}H NMR δ 8.20 (dd, *J* 8.1, 1.5 Hz, 1H, ArC<u>H</u>-6); 8.13 (dd, *J* 8.1, 1.5 Hz, ArC<u>H</u>-3); 7.71 (dt, *J* 7.2, 1.5 Hz, 1H, ArC<u>H</u>-5); 7.47 (dt, *J* 7.2, 1.5 Hz, 1H, ArC<u>H</u>-4); 3.66 (s, 3H, OC<u>H$_3$</u>); 3.63-3.55 (m, 2H, C<u>H$_A$</u>CH$_B$-6` and C<u>H$_\alpha$</u>-3a`); 3.50 (dd, *J* 13.2, 3.6 Hz, 1H, C<u>H$_\beta$</u>CH$_\alpha$-3`); 3.05 (dt, *J* 13.5, 8.1 Hz, 1H, C<u>H$_B$</u>CH$_A$-6`); 2.58 (dd, *J* 13.2, 8.1 Hz, 1H, C<u>H$_\alpha$</u>CH$_\beta$-3`); 2.21-2.01 (m, 2H, C<u>H$_A$</u>CH$_B$-4` and C<u>H$_A$</u>CH$_B$-5`); 1.99-1.89 (m, 1H, C<u>H$_B$</u>CH$_A$-4`); 1.84-1.74 (m, 1H, C<u>H$_B$</u>CH$_A$-5`). $^{13}$C NMR δ 168.9 (<u>C</u>O$_2$); 146.1 (Ar<u>C</u>-2); 139.2 (Ar<u>C</u>-1); 134.2 (Ar<u>C</u>H-5); 128.5 (Ar<u>C</u>H-4); 128.4 (Ar<u>C</u>H-6); 125.3 (Ar<u>C</u>H-3); 87.5 (<u>C</u>-2`); 66.7 (<u>C</u>H$_\alpha$-3a`); 56.8 (<u>C</u>H$_2$-6`); 53.0 (O<u>C</u>H$_3$); 47.9 (<u>C</u>H$_2$-3`); 29.9 (<u>C</u>H$_2$-4`); 23.7 (<u>C</u>H$_2$-5`). $^{13}$C NMR ($C_6D_6$) 169.1 (<u>C</u>O$_2$); 147.0 (Ar<u>C</u>-2); 140.1 (Ar<u>C</u>-1); 133.7 (Ar<u>C</u>H-5); 129.4 (Ar<u>C</u>H-6); 128.0 (Ar<u>C</u>H-4); 125.1 (Ar<u>C</u>H-3); 87.9 (<u>C</u>-2`); 67.0 (<u>C</u>H-3a`); 57.0 (<u>C</u>H$_2$-6`); 52.5 (O<u>C</u>H$_3$); 48.5 (<u>C</u>H$_2$-3`); 30.2 (<u>C</u>H$_2$-4`); 24.1 (<u>C</u>H$_2$-5`).

8: MS (EI) *m/z* 292 (12%) [$M^{\cdot+}$], 257 (18%), 244 (25%), 233 (39%), 104 (49%); HRMS (ESI+ve) Calcd for $C_{14}H_{17}N_2O_5$ [MH^+] 293.1132. Found: 293.1130. ^{1}H NMR (500 MHz) δ 8.06 (dd, *J* 8.0, 1.3 Hz, 1H, ArC<u>H</u>-3); 8.01 (dd, *J* 8.0, 1.3 Hz, 1H, ArC<u>H</u>-6), 7.67 (dt, *J* 8.0, 1.3 Hz, 1H, Ar<u>C</u>H-5); 7.50 (dt, *J* 8.0, 1.3 Hz, 1H, Ar<u>C</u>H-4); 4.02-3.96 (m, 1H, C<u>H$_\beta$</u>-3a`); 3.82 (dd, *J* 13.0, 8.0 Hz, 1H, C<u>H$_\beta$</u>CH$_\alpha$-3`); 3.71 (s, 3H, O<u>C</u>H$_3$); 3.54 (ddd, *J* 13.8, 8.0, 4.0 Hz, 1H, C<u>H$_A$</u>CH$_B$-6`); 3.11 (dt, *J* 13.8, 8.0 Hz, 1H, C<u>H$_B$</u>CH$_A$-6`); 2.07 (dd, *J* 13.0, 4.0 Hz, 1H, C<u>H$_\alpha$</u>CH$_\beta$-3`); 2.11-2.02 (m, 1H, C<u>H$_A$</u>CH$_B$-5`); 1.94 (dt, *J* 13.0, 8.0 Hz, 1H, C<u>H$_A$</u>CH$_B$-4`); 1.84-1.76 (m, 1H, C<u>H$_B$</u>CH$_A$-5`); 1.46 (ddt, *J* 13.0, 9.0, 4.0 Hz, 1H, C<u>H$_B$</u>CH$_A$-4`). $^{13}$C NMR δ 169.7 (<u>C</u>O$_2$); 146.6 (Ar<u>C</u>-2); 136.9 (Ar<u>C</u>-1); 133.8 (Ar<u>C</u>H-5); 128.7 (Ar<u>C</u>H-4); 126.9 (Ar<u>C</u>H-6); 125.1 (Ar<u>C</u>H-3); 85.5 (<u>C</u>-2`); 66.9 (<u>C</u>H$_\beta$-3a`); 56.9 (<u>C</u>H$_2$-6`); 52.9 (O<u>C</u>H$_3$); 46.0 (<u>C</u>H$_2$-3`); 31.0 (<u>C</u>H$_2$-4`); 24.1 (<u>C</u>H$_2$-5`).

2`-Methyl-(3`*S, 5`*R*\*)-3`-phenylspiro[indole-3,5`-isoxazolidin]-2(1*H*)-one (9)**

To a solution of **4a** (54 mg, 1.6×10^{-4} mol) in EtOAc (1 mL) under an atmosphere of N_2 was added 10% Pd/C (9 mg). The vessel was then flushed with H_2 and left stirring under an atmosphere of H_2 (balloon) for 18 h. The crude mixture was filtered through a bed of celite, washed with EtOAc (3 × 50 mL) and the filtrate was evaporated *in vacuo*. The crude product was purified by column chromatography using 30-50% EtOAc in petrol as eluent to yield **9** as a yellow oil (10.6 mg, 3.8×10^{-5} mol, 24%, R_f = 0.18 in EtOAc/petrol (3 : 7)). MS (EI) *m/z* 280 (10%) [$M^{\cdot+}$], 263 (15%), 145 (37%) [M^+-$C_6H_5CHNCH_3O$], 134 (92%), 117 (42%); HRMS (EI) Calcd for $C_{17}H_{16}N_2O_2$ [$M^{\cdot+}$] 280.1212. Found: 280.1206. ^{1}H NMR (500 MHz) δ 8.79 (bs, 1H, NH); 7.57 (d, *J* 7.0 Hz, 2H, ArCH-*o*); 7.44 (d, *J* 7.5 Hz, 1H, ArCH-4); 7.39 (t, *J* 7.5 Hz, 2H, ArCH-*m*); 7.34 (d, *J* 7.5 Hz, 1H, ArCH-*p*); 7.28 (t, *J* 7.5 Hz, 1H, ArCH-6); 7.10 (t, *J* 7.5 Hz, 1H, ArCH-5); 6.94 (d, *J* 7.5 Hz, 1H, ArCH-7); 3.85 (bs, 1H, CH_α-3`); 3.01 (t, *J* 13.0 Hz, 1H, $CH_\beta CH_\alpha$-4`); 2.77 (dd, *J* 13.0, 6.5 Hz, 1H, $CH_\alpha CH_\beta$-4`); 2.71 (s, 3H, $NCH_3$). $^{13}$C NMR (125 MHz) δ 179.4 (C-2); 141.1 (ArC-7a); 137.0 (ArC-*i*); 130.5 (ArC-3a); 130.1 (ArCH-6); 128.8 (ArCH-*m*); 128.5 (ArCH-*o*); 128.4 (ArCH-*p*); 124.3 (ArCH-4); 123.1 (ArCH-5); 110.5 (ArCH-7); 80.5 (C-3); 74.5 (CH-3`); 49.3 (CH_2-4`); 43.7 (NCH_3).

(2*R, 3*R**)-3-Hydroxy-3-[2-(methylamino)-2-phenylethyl]-1,3-dihydro-2*H*-indol-2-one (10a)**

To a solution of **4a** (78.4 mg, 2.3×10^{-4} mol) in glacial AcOH (9.2 mL) was added activated Zn dust (150 g, 2.3 mmol). The mixture was sonicated for 1 h. The crude mixture was then filtered through a bed of celite and washed with EtOAc. The filtrate was washed with sat. Na_2CO_3 solution and then H_2O, then dried, filtered and evaporated *in vacuo*. The crude was purified by column chromatography using 10-30% EtOAc in petrol as eluent to yield **10a** as a yellow oil (37.6 mg, 1.3×10^{-4} mol, 58%, R_f = 0.16 in EtOAc/petrol (3 : 7)). MS (EI) *m/z* 282 (12%) [$M^{\cdot+}$], 206 (6%), 146 (13%), 134 (21%), 120 (91%), 104 (12%); HRMS (EI) Calcd for $C_{17}H_{18}N_2O_2$ [$M^{\cdot+}$] 282.1368. Found: 282.1365. ^{1}H NMR (500 MHz) δ 7.32 (t, *J* 7.3 Hz, 2H, ArCH-*m*); 7.30-7.26 (m, 1H, ArCH-*p*); 7.15-7.11 (m, 3H, ArCH-4 and ArCH-*o*); 7.08 (t, *J* 7.5 Hz, 1H ArCH-6); 6.71 (t, *J* 7.3 Hz, 1H ArCH-5); 6.67 (d, *J* 7.5 Hz, 1H ArCH-7); 4.70 (dd, *J* 7.5, 6.0 Hz, 1H, CH); 2.98 (dd, *J* 14.0, 8.0 Hz, 1H, CH_ACH_B); 2.76 (s, 3H, $NHCH_3$); 2.48 (dd, *J* 14.0, 6.0 Hz, 1H, CH_BCH_A). ^{13}C NMR (125 MHz) δ 175.7 (C-2); 145.4 (ArC-7a); 139.7 (ArC-*i*); 129.2 (ArCH-6); 128.9 (ArCH-*m*); 128.1 (ArCH–*p*); 126.8 (ArCH-*o*); 126.7 (ArC-3a and ArCH-4); 118.6 (ArCH-5); 118.4(ArCH-7); 79.4 (C-3); 61.8 (CH); 42.4 (CH_2); 28.8 ($NHCH_3$).

(2*R, 3*S**)-3-(2-Anilino-2-phenylethyl)-3-hydroxy-1,3-dihydro-2*H*-indol-2-one (10b)**

The title compound was prepared using two methods. **Method 1**: The title compound was prepared from **4b** (61 mg, 1.5×10^{-4} mol) using a similar method to that described for the preparation for **10a**. The crude product was purified by column chromatography using 20-40% EtOAc in petrol as eluent to yield **10b** as a yellow oil (28.6 mg, 8.3×10^{-5} mol, 55%, R_f = 0.66 in 40% EtOAc/petrol (2 : 3)). **Method 2**: The title compound was prepared from **4b** (72.7 mg, 1.8×10^{-4} mol) using a similar method to that described for the preparation for **10a**. After following the same workup procedure, the crude mixture was purified by column chromatography using 10-20% EtOAc in petrol as eluent to yield **10b** as a cream solid (55.5 mg, 1.6×10^{-4} mol, 90%) and purified further by recrystallision to yield **10b** as a cream solid (29.8 mg, 8.7×10^{-5} mol, 48%). MS (EI) *m/z* 344 (15%) [$M^{\cdot+}$], 148 (11%), 196 (46%), 120 (43%), 182 (92%), 104 (16%). HRMS (EI) Calcd for $C_{22}H_{20}N_2O_2$ [$M^{\cdot+}$] 344.1525. Found: 344.1505. ^{1}H NMR (500 MHz) δ 7.36 (d, *J* 8.5 Hz, 2H, ArC<u>H</u>-*o*`); 7.27-7.21 (m, 6H, ArC<u>H</u>-*m*`, ArC<u>H</u>-*o*, and ArC<u>H</u>-*m*); 7.20-7.15 (m, 2H, ArC<u>H</u>-*p* and ArC<u>H</u>-6); 7.09 (t, *J* 7.5 Hz, 1H, ArC<u>H</u>-*p*`) 6.94 (d, *J* 7.5 Hz, 1H, ArC<u>H</u>-4); 6.77 (d, *J* 7.5 Hz, 1H, ArC<u>H</u>-7); 6.71 (t, *J* 7.5 Hz, 1H, ArC<u>H</u>-5); 4.97 (dd, *J* 9.7, 6.0 Hz, 1H, C<u>H</u>$_\alpha$); 4.66 (bs, 1H, N<u>H</u>); 3.34 (dd, *J* 13.0, 6.0 Hz, 1H, C<u>H</u>$_\beta$CH$_\alpha$); 2.42 (dd, *J* 13.0, 9.7 Hz, 1H, C<u>H</u>$_\alpha$CH$_\beta$). ^{13}C NMR (125 MHz) δ 175.3 (<u>C</u>-2); 146.0 (Ar<u>C</u>-7a); 139.1 (Ar<u>C</u>-*i*); 136.8 (Ar<u>C</u>-*i*`); 129.5 (Ar<u>C</u>H-6); 128.8 (Ar<u>C</u>H-*m*`); 128.7 (Ar<u>C</u>H-*m*); 128.0 (Ar<u>C</u>H-*p*); 127.0 (Ar<u>C</u>H-*o*); 125.9 (Ar<u>C</u>H-4); 125.8 (Ar<u>C</u>H-*p*`); 124.4 (Ar<u>C</u>-3a); 123.4 (Ar<u>C</u>H-*o*`); 118.3(Ar<u>C</u>H-5); 118.0(Ar<u>C</u>H-7); 79.8 (<u>C</u>-3); 60.1 (<u>C</u>HPh); 43.5 (<u>C</u>H$_2$).

2`-Methyl-(3`*R, 5`*R*\*)-3`-phenylspiro[indole-3,5`-isoxazolidin]-2(1*H*)-one (12)**

The title compound was prepared from **5a** (78 mg, 2.3×10^{-4} mol) using a similar method to that described above for the preparation of **9**. However, the reaction was left for only 2 h. The crude product was purified by column chromatography using 30-50% EtOAc in petrol to yield **12** as a yellow oil (34.8 mg, 1.2×10^{-4} mol, 54%, R_f = 0.16 in 30% EtOAc/petrol (3 : 7)). MS (EI) *m/z* 280 (13%) [$M^{\cdot+}$], 263 (22%), 145 (58%), 134 (94%), 117 (63%); HRMS (ESI+ve) Calcd for $C_{17}H_{17}N_2O_2$ [MH^+] 281.1285. Found: 281.1293. ^{1}H NMR (500 MHz) δ 8.05 (bs, 1H, N<u>H</u>); 7.53 (d, *J* 7.5 Hz, 1H, ArC<u>H</u>-4); 7.50 (d, *J* 7.5 Hz, 2H, ArC<u>H</u>-*o*); 7.38 (d, *J* 7.5 Hz, 2H, ArC<u>H</u>-*m*); 7.33 (t, *J* 7.5Hz, 1H, ArC<u>H</u>-*p*); 7.25 (t, *J* 7.5 Hz, 1H, ArC<u>H</u>-6); 7.08 (t, *J* 7.5Hz, 1H, ArC<u>H</u>-5); 6.85 (d, *J* 7.5 Hz, 1H, ArC<u>H</u>-7); 4.23 (bm, 1H, C<u>H</u>$_\beta$-3`); 3.01 (dd, *J* 13.0, 6.0 Hz, 1H, C<u>H</u>$_\beta$CH$_\alpha$-4`); 2.79 (s, 3H, NC<u>H</u>$_3$); 2.74-2.70 (m, 1H, C<u>H</u>$_\alpha$CH$_\beta$-4`). ^{13}C NMR δ 178.2 (<u>C</u>-2); 140.7 (Ar<u>C</u>-7a); 137.7 (Ar<u>C</u>-*i*); 130.7 (Ar<u>C</u>-3a); 129.7 (Ar<u>C</u>H-6); 128.8 (Ar<u>C</u>H-*m*); 128.1 (Ar<u>C</u>H-*p*); 127.6 (Ar<u>C</u>H-*o*); 124.7 (Ar<u>C</u>H-4); 123.3 (Ar<u>C</u>H-5); 110.3 (Ar<u>C</u>H-7); 81.8 (<u>C</u>-3); 72.9 (<u>C</u>H-3`); 49.7 (<u>C</u>H$_2$-4`); 43.9 (N<u>C</u>H$_3$).

(2*R, 3*S**)-3-hydroxy-3-[2-(methylamino)-2-phenylethyl]-1,3-dihydro-2*H*-indol-2-one (13)**

The title compound was prepared from **5a** (49 mg, 1.43×10^{-4} mol) using a similar method to that described above for the synthesis of **10a**. Compound **13** was obtained as a yellow oil, which required no further purification (38 mg, 1.35×10^{-4} mol, 94%, R_f = 0.45 in EtOAc/petrol (3 : 7)). MS (EI) *m/z* 282 (31%) [$M^{\cdot+}$], 146 (18%), 134 (26%), 120 (89%), 104 (13%); HRMS (EI) Calcd for $C_{17}H_{18}N_2O_2$ [$M^{\cdot+}$], 282.1368. Found: 282.1366. ^{1}H NMR δ 7.39-7.34 (m, 3H, ArC<u>H</u>-*m* and ArC<u>H</u>-*p*); 7.26 (dd, *J* 7.0, 1.5 Hz, 2H, ArC<u>H</u>-*o*); 7.13 (dt, *J* 8.1, 1.5 Hz, 1H, ArC<u>H</u>-6); 6.89 (dd, *J* 8.1, 1.5 Hz, 1H, ArC<u>H</u>-4); 6.71 (t, 7.0 Hz, 2H, ArC<u>H</u>-5 and ArC<u>H</u>-7); 4.27 (dd, *J* 9.0, 6.0 Hz, 1H, C<u>H</u>); 3.15 (dd, *J* 13.2, 6.0 Hz, 1H, C<u>H</u>$_A$CH$_B$); 2.71 (s, 3H, NHC<u>H</u>$_3$); 2.34 (dd, *J* 13.2, 9.0 Hz, 1H, C<u>H</u>$_B$CH$_A$). ^{13}C NMR δ 176.1 (<u>C</u>-2); 146.0 (Ar<u>C</u>-7a); 138.7 (Ar<u>C</u>-*i*); 129.0 (Ar<u>C</u>H-*m*); 128.5 (Ar<u>C</u>H-*p*); 127.4 (Ar<u>C</u>H-*o*); 129.2 (Ar<u>C</u>H-6); 125.7 (Ar<u>C</u>H-4); 124.8 (Ar<u>C</u>-3a); 118.0 (Ar<u>C</u>H-5); 117.8 (Ar<u>C</u>H-7); 79.5 (<u>C</u>-3); 61.0 (<u>C</u>H); 43.5 (<u>C</u>H$_2$); 28.3 (NH<u>C</u>H$_3$).

3`-Methyl-(4`*R, 6`*R*\*)-4`-phenyl-2`*H*-spiro[indole-3,6`-[1,3]oxazinane]-2,2`(1*H*)-dione (11a)**

To a solution of **10a** (68.2 mg, 2.4×10^{-4} mol) in anhydrous THF (2 mL) was added triphosgene (21.5 mg, 7.2×10^{-5} mol) and anhydrous NEt_3 (0.07 mL, 4.8×10^{-4} mol). The mixture was stirred under N_2 for 7 d. The reaction mixture was diluted with EtOAc and the solution was washed successively with H_2O, sat. $NaHCO_3$ solution and brine and then dried and evaporated under reduced pressure. The crude product was purified by column chromatography using 30-100% EtOAc:petrol to yield **11a** as a white crystalline solid (45.9 mg, 1.5×10^{-4} mol, 61%, R_f = 0.25 in EtOAc/petrol (1 : 1), m.p. 200-204 °C). MS (EI) *m/z* 308 (67%) [$M^{\cdot+}$], 309 (15%) [MH^+], 251 (91%) [M^+-NMeCO], 206 (84%), 146 (94%) [M^+-$CH_2C_6H_5CHNCH_3CO$], 130 (80%) [M^+-$CH_2C_6H_5CHNCH_3CO_2$], 118 (38%), 102 (43%); HRMS (EI) Calcd for $C_{18}H_{16}N_2O_3$ [$M^{\cdot+}$] 308.1161. Found: 308.1161. ^{1}H NMR (500 MHz) δ 9.02 (bs, 1H, N<u>H</u>); 7.45 (t, *J* 7.5 Hz, 2H, ArC<u>H</u>-*m*); 7.39 (t, *J* 7.5 Hz, 1H, ArC<u>H</u>-*p*); 7.29-7.25 (m, 3H, ArC<u>H</u>-*o* and ArC<u>H</u>-6); 7.09-7.05 (m, 2H, ArC<u>H</u>-4 and ArC<u>H</u>-5); 6.89 (d, *J* 7.5 Hz, 1H, ArC<u>H</u>-7); 4.88 (dd, *J* 7.5, 7.0 Hz, 1H, C<u>H</u>$_\alpha$-4`); 3.09 (dd, *J* 15.0, 7.0 Hz, 1H, C<u>H</u>$_\beta$CH$_\alpha$-5`); 2.76 (s, 3H, NC<u>H</u>$_3$); 2.39 (dd, *J* 15.0, 7.0 Hz, 1H, C<u>H</u>$_\alpha$CH$_\beta$-5`). $^{13}$C NMR (125 MHz) δ 169.9 (<u>C</u>-2); 150.8 (<u>C</u>-2`); 138.8 (Ar<u>C</u>-*i*); 135.6 (Ar<u>C</u>-7a); 130.0 (Ar<u>C</u>H-6); 129.3 (Ar<u>C</u>H-*m*); 128.7 (Ar<u>C</u>H-*p*); 126.7 (Ar<u>C</u>H-*o*); 124.4 (Ar<u>C</u>H-4); 123.6 (Ar<u>C</u>H-5); 118.4 (Ar<u>C</u>-3a); 115.0 (Ar<u>C</u>H-7); 86.1 (<u>C</u>-3); 60.8 (<u>C</u>H-4`); 43.6 (C<u>H</u>$_2$-5`); 29.0 (N<u>C</u>H$_3$).

3`-Phenyl-(4`*R, 6`*R*\*)-4`-phenyl-2`*H*-spiro[indole-3,6`-[1,3]oxazinane]-2,2`(1*H*)-dione (11b)**

The title compound was prepared from **10b** (20.9 mg, 6.1×10^{-5} mol) using a similar method to that described above for the synthesis of **11a**. However, the reaction was left for only 2 d. The crude

product was then purified by column chromatography using 30-100% EtOAc in petrol as eluent to yield **11b** as a white crystalline solid (15.4 mg, 4.2×10^{-5} mol, 68%, $R_f = 0.73$ in MeOH:$CHCl_3$ (1 : 9), m.p. 256-258 °C). MS (EI) *m/z* 370 (21%) [$M^{\cdot+}$], 251 (65%) [M^+-PhNCO], 206 (46%), 180 (30%), 146 (89%), 130 (53%), 103 (32%); HRMS (EI) Calcd for $C_{23}H_{18}N_2O_3$ [$M^{\cdot+}$] 370.1317. Found: 370.1319. ^{1}H NMR (dDMSO, 500 MHz) δ 10.4 (s, 1H, NH); 7.50 (d, *J* 7.5 Hz, 1H, ArCH-4); 7.43 (d, *J* 7.5 Hz, 2H, ArCH-*o*`); 7.39 (d, *J* 7.5 Hz, 2H, ArCH-*o*); 7.33 (t, *J* 7.5 Hz, 1H, ArCH-6); 7.29 (t, *J* 7.5 Hz, 2H, ArCH-*m*); 7.26 (t, *J* 7.5 Hz, 2H, ArCH-*m*`); 7.20 (t, *J* 7.5 Hz, 1H, ArCH-*p*); 7.09 (t, *J* 7.5 Hz, 1H, ArCH-5); 7.08 (t, *J* 7.5 Hz, 1H, ArCH-*p*`); 6.91 (d, *J* 7.5 Hz, 1H, ArCH-7); 5.83 (dd, *J* 7.5, 7.0 Hz, 1H, $CH_\alpha$-4`); 3.37-3.30 (m, 1H, $CH_\alpha CH_\beta$-5`); 2.40 (dd, *J* 14.3, 7.5 Hz, 1H, $CH_\alpha CH_\beta$-5`). ^{13}C NMR (dDMSO, 125 MHz) δ 169.5 (C-2); 149.1 (C-2`); 140.2 (ArC-*i*); 136.6 (ArC-*i*`); 135.9 (ArC-7a); 130.0 (ArCH-6); 128.8 (ArCH-*m*); 128.6 (ArCH-*m*`); 127.9 (ArCH-*p*); 127.0 (ArCH-*o*); 125.9 (ArCH-*p*`); 123.8 (ArCH-4); 123.6 (ArCH-*o*`); 122.9 (ArCH-5); 119.7 (ArC-3a); 114.4 (ArCH-7); 84.2 (C-3); 58.8 (CH-4`); 42.5 (CH_2-5`).

3`-Methyl-(4`*S, 6`*R*\*)-4`-phenyl-2`*H*-spiro[indole-3,6`-[1,3]oxazinane]-2,2`(1*H*)-dione (14)**

The title compound was prepared from **13** (41.8 mg, 1.5×10^{-4} mol) using a similar method to that described above for the synthesis of **11a**. However, the reaction was left for only 2 d. The crude product was purified by column chromatography using 30-100% EtOAc in petrol as eluent to yield **14** as a white crystalline solid (23.5 mg, 7.6×10^{-5} mol, 51%, $R_f = 0.39$ in EtOAc/petrol (1 : 1), m.p. 244-248 °C). MS (EI) *m/z* 308 (43%) [$M^{\cdot+}$], 251 (72%), 206 (68%), 146 (94%), 130 (65%); HRMS (EI) Calcd for $C_{18}H_{16}N_2O_3$ [$M^{\cdot+}$] 308.1161. Found: 308.1166. ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 9.27 (bs, 1H, NH); 7.49-7.48 (m, 4H, ArCH-*o* and ArCH-*m*); 7.43-7.40 (m, 1H, ArCH-*p*); 7.35 (d, *J* 7.5 Hz, 1H, ArCH-4); 7.32 (dt, *J* 7.5, 1.2 Hz, 1H, ArCH-6); 7.07 (dt, *J* 7.5, 1.2 Hz, 1H, ArCH-5); 7.03 (d, *J* 7.5 Hz, 1H, ArCH-7); 4.96 (dd, *J* 7.5, 7.0 Hz, 1H, CH_β-4`); 3.27 (dd, *J* 15.0, 7.5 Hz, 1H, $CH_\beta CH_\alpha$-5`); 2.64 (s, 3H, NCH_3); 2.47 (dd, *J* 15.0, 7.5 Hz, 1H, $CH_\alpha CH_\beta$-5`). $^{13}$C NMR ($(CD_3)_2CO$) δ 171.0 (C-2); 150.2 (C-2`); 141.0 (ArC-*i*); 137.4 (ArC-7a); 130.6 (ArCH-6); 129.9 (ArCH-*m*), 129.3 (ArCH-*p*); 128.1 (ArCH-*o*); 124.5 (ArCH-4); 123.6 (ArCH-5); 121.7 (ArC-3a); 115.1 (ArCH-7); 85.0 (C-3); 61.4 (CH-4`); 43.9 ($CH_2$-5`); 28.6 (NCH_3).

(3*R, 3*R**)-3-Hydroxy-3-(pyrrolidin-2-ylmethyl)indol-2-one (15) and (3*R**, 4a`*R*\*)-2`*H*-spiro[indoline-3,3`-pyrrolo[1,2-*c*][1,3`]oxazine]-1`,2(1*H*)-dione (16)**

To a solution of **8** (41.3 mg, 1.4×10^{-4} mol) in anhydrous MeOH (2 mL) under an atmosphere of N_2 was added $PdCl_2$ (5.2 mg, 2.8×10^{-5} mol) and the vessel flushed with H_2 and left stirring for 3 h under a H_2 atmosphere (balloon). The crude mixture was then filtered through a bed of celite and the solid was washed with MeOH (10 mL). The solvent was evaporated *in vacuo*. The crude product was purified by column chromatography using 50-100% EtOAc in petrol as eluent, to yield material (**15**) that was impossible to analyse by NMR due to the broadening of all peaks, perhaps due to traces of palladium. To a solution of this material (28 mg, 1.2×10^{-4} mol) in anhydrous THF (1 mL) was added triphosgene (10.7 mg, 3.6×10^{-5} mol) and anhydrous NEt_3 (0.03 mL, 2.4×10^{-4} mol). The mixture was stirred under N_2 for 2 d. The crude was then washed with H_2O and extracted with EtOAc. The organic extracts were then successively washed with sat. $NaHCO_3$ solution and brine, dried and evaporated under reduced pressure. The crude product was purified by chromatotron® (0-4% MeOH in $CHCl_3$) to yield **16** as a brown semicrystalline oil (9.2 mg, 3.6×10^{-5} mol, 25% over 2 steps).

15: MS (EI) *m*/*z* 232 (10%) [$M^{\cdot+}$], 214 (6%) [M^{+}-H_2O], 149 (22%), 120 (34%), 86 (37%), 70 (77%), 43 (96%); HRMS (EI) Calcd for $C_{13}H_{16}N_2O_2$ [$M^{\cdot+}$] 232.1212. Found: 232.1206.

16: MS (EI) *m/z* 258 (70%) [$M^{\cdot+}$], 259 (19%) [MH^{+}], 214 (32%), 186 (24%), 174 (94%), 146 (94%), 133 (50%), 117 (35%), 104 (29%). HRMS (EI) Calcd for $C_{14}H_{14}N_2O_3$ [$M^{\cdot+}$] 258.1004. Found: 258.0997. ^{1}H NMR δ 7.34-7.29 (m, 2H, ArC<u>H</u>-6 and ArC<u>H</u>-4); 7.10 (dt, *J* 7.5, 1.0 Hz, 1H, ArC<u>H</u>-5); 6.91 (dd, *J* 7.5, 1.0 Hz, 1H, ArC<u>H</u>-7); 4.11-4.01 (m, 1H, C<u>H</u>$_\beta$-4a`); 3.63-3.52 (m, 1H, C<u>H</u>$_A$CH$_B$-7`); 3.20-3.12 (m, 1H, C<u>H</u>$_B$CH$_A$-7`); 3.00 (dd, *J* 13.3, 6.0 Hz, 1H, C<u>H</u>$_\beta$CH$_\alpha$-4`); 2.36 (dd, *J* 13.3, 6.0 Hz, 1H C<u>H</u>$_\alpha$CH$_\beta$-4`); 2.26-2.09 (m, 3H, C<u>H</u>$_A$CH$_B$-5` and C<u>H</u>$_2$-6`); 1.53-1.47 (m, 1H, C<u>H</u>$_B$CH$_A$-5`). ^{13}C NMR δ 171.5 (<u>C</u>-2); 151.6 (<u>C</u>-1`); 136.6 (Ar<u>C</u>-7a); 121.5 (Ar<u>C</u>-3a); 90.6 (<u>C</u>-3`); 131.2 (Ar<u>C</u>H-6); 124.3 (Ar<u>C</u>H-4); 124.7 (Ar<u>C</u>H-5); 115.6 (Ar<u>C</u>H-7); 59.5 (<u>C</u>H$_\beta$-4a`); 42.5 (<u>C</u>H$_2$-7`); 43.1 (<u>C</u>H$_2$-4`); 33.5 (<u>C</u>H$_2$-5`); 27.1 (<u>C</u>H$_2$-6`).

Acknowledgements

We thank the University of Wollongong for financial support and the Australian Research Council for a PhD scholarship to S.R.Y.

Supporting information

Details of the X-ray Crystal/refinement data and GI_{50} curves in duplicate for compounds **7** and **9** against an MCF-7 cell line (2 pages).

References

1. See for example, (a) Ding, K.; Lu, Y.; Nikolovska-Coleska, Z.; Wang, G.; Qiu, S.; Shangary, S.; Gao, W.; Qin, D.; Stuckey, J.; Krajewski, K.; Roller, P. P.; Wang, S., *J. Med. Chem.* **2006**, *49*, 3432-3435. (b) Kitajima, M.; Nakamura, T.; Kogure, N.; Ogawa, M.; Mitsuno, Y.; Ono, K.; Yano, S.; Aimi, N.; Takayama, H. *J. Nat. Prod.* **2006**, *69*, 715-718. (c) Bacher, N.; Tiefenthaler, M.; Sturm, S.; Stuppner, H.; Ausserlechner, M. J.; Kofler, R.; Konwalinka, G. *Br. J. Haematol.* **2006**, *132*, 615-622. (d) Mugishima, T.; Tsuda, M.; Kasai, Y.; Ishiyama, H.; Fukushi, E.; Kawabata, J.; Watanabe, M.; Akao, K.; Kobayashi, J. *J. Org. Chem.* **2005**, *70*, 9430-9435. (e) Ding, K.; Lu, Y.; Nikolovska-Coleska, Z.; Qiu, S.; Ding, Y.; Gao, W.; Stuckey, J.; Krajewski, K.; Roller, P. P.; Tomita, Y.; Parrish, D. A.; Deschamps, J. R.; Wang, S. *J. Am. Chem. Soc.* **2005**, *127*, 10130-10131. (f) Raunak; Kumar, V.; Mukherjee, S.; Poonam; Prasad, A. K.; Olsen, C. E.; Schaeffer, S. J. C.; Sharma, S. K.; Watterson, A. C.; Errington, W.; Parmar, V. S. *Tetrahedron* **2005**, *61*, 5687-5697. (g) Kang, T.-H.; Murakami, Y.; Matsumoto, K.; Takayama, H.; Kitajima, M.; Aimi, N.; Watanabe, H. *Eur. J. Pharmacol.* **2002**, *455*, 27-34. (g) Shimada, Y.; Goto, H.; Itoh, T.; Sakakibara, I.; Kubo, M.; Sasaki, H.; Terasawa, K., *J. Pharm. Pharmacol.* **1999**, *51*, 715-722. (h) Edmondson, S.; Danishefsky, S. J.; Sepp-Lorenzino, L.; Rosen, N. *J. Am. Chem. Soc.* **1999**, *121*, 2147-2155. (i) Cui, C.-B.; Kakeya, H.; Osada, H. *Tetrahedron* **1996**, *52*, 12651-12666. (j) Kornet, M. J.; Thio, A. P. *J. Med. Chem.* **1976**, *19*, 892-898.

2. Yong, S. R.; Williams, M. C.; Pyne, S. G.; Ung, A. T.; Skelton, B. W.; White, A. H.; Turner, P. *Tetrahedron* **2005**, *61*, 8120-8129.

3. Yong, S. R.; Ung, A. T.; Pyne, S. G.; Skelton, B. W.; White, A. H. *Tetrahedron* **2007**, *63*, 1191-1199.

4. Selvakumar, N.; Azhagan, A. M.; Srinivas, D.; Krishna, G. G. *Tetrahedron Lett.* **2002**, *43*, 9175-9178.

5. Sebahar, P. R.; Osada, H.; Usui, T.; Williams, R. M. *Tetrahedron* **2002**, *58*, 6311-6322.

6. Lo, M. M. C.; Neumann, C. S.; Nagayama, S.; Perlstein, E. O.; Schreiber, S. L. *J. Am. Chem. Soc.* **2004**, *126*, 16077-16086.

7. Rigolet, S.; Goncalo, P.; Melot, J. M.; Vebrel, J. *J. Chem. Res.* **1998**, 686-687.

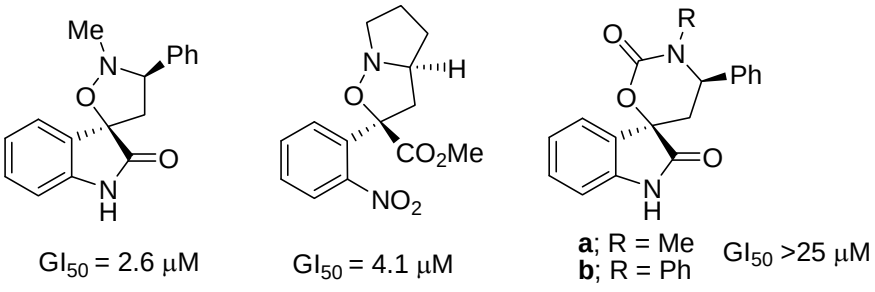
8. The CCDC deposition number for **4a** is 633506, see Supporting Information for crystal/refinement data.

GRAPHICAL ABSTRACT

Synthesis of novel 3'-spirocyclic-oxindole derivatives and assessment of their cytostatic activities

Sarah R. Yong, Alison T. Ung, Stephen G. Pyne, Brian W. Skelton and Allan H. White

Department of Chemistry, University of Wollongong, Wollongong, New South Wales, 2522, Australia and Department of Chemistry, University of Western Australia, Crawley, Western Australia, 6009, Australia



Synthesis of novel 3'-spirocyclic-oxindole derivatives and assessment of their cytostatic activities

Sarah R. Yong[a], Alison T. Ung[a]*, Stephen G. Pyne[a]*,

Brian W. Skelton[b] and Allan H. White[b]

[a]*Department of Chemistry, University of Wollongong, Wollongong, New South Wales, 2522, Australia*

[b]*Department of Chemistry, University of Western Australia, Crawley, Western Australia, 6009, Australia.*

Supporting Information

Structure determinations

Full spheres of CCD area-detector diffractometer data were measured (Bruker AXS instrument, ω-scans; monochromatic Mo Kα radiation; λ = 0.7107 Å) yielding $N_{t(otal)}$ reflections, these merging to N unique after 'empirical'/multiscan 'absorption correction' (proprietary software) (R_{int} cited), N_o ($I > 2\sigma(I)$) considered 'observed'. All reflections were used in the full matrix refinements on F^2, refining anisotropic displacement parameters for the non-hydrogen atoms, hydrogen atoms being included according to a riding model. Reflection weights were of the form $(\sigma^2(F^2) + n_w F^2)^{-1}$. Neutral atom complex scattering factors were employed within the SHELXL 97 and Xtal 3.7 program systems.

4a. $C_{18}H_{18}N_2O_5$, M = 342.3. Monoclinic, space group $P2_1$, a = 11.141(2), b = 6.866(1), c = 11.390(2) Å, β = 112.291(2)º, V = 806.$_2$ Å^3. D_c (Z = 2) = 1.41$_0$ g cm^{-3}. μ_{Mo} = 0.10 mm^{-1}; specimen: 0.65 x 0.10 x 0.10 mm; 'T'$_{min/max}$ = 0.84 ('empirical correction'. Monochromatic Kα radiation, λ = 0.7107$_3$ Å, $2\theta_{max}$ = 55°; 7451 CCD reflections merged to 2170 independent, 1902 with $I > 2\sigma(I)$; $R1$ = 0.054, $wR2$ = 0.014; S = 1.06 (weights: $(\sigma^2(F^2) + (0.1019P)^2 + 0.0404\,P)^{-1}$ ($P = (F_o + 2F_c)/3$)). $|\Delta\rho_{max}|$ = 0.50 e Å^{-3}. *T ca.* 170 K. CCDC 633506.

References

a G.M. Sheldrick, SHELXL 97. A program for crystal structure refinement, University of Göttingen, Göttingen, Germany, 1997.

b S.R. Hall, D.J. du Boulay, R. Olthof-Hazekamp (eds.), 'The Xtal 3.7 System', University of Western Australia, 2001.

CCDC deposition numbers: 622516 - 622523.

Figure. GI_{50} curves in duplicate for **9** ((**A)** and **B**)) and **7** ((**C**) and **D**)) against a MCF-7 cell line.