Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 14 (2006) 857–865
Bioorganic & Medicinal Chemistry

Synthesis of functionalised 2-aryl-5-nitro-1*H*-indoles and their activity as bacterial NorA efflux pump inhibitors

Siritron Samosorn,[a] John B. Bremner,[a,*] Anthony Ball[b] and Kim Lewis[b]

[a]*Institute for Biomolecular Science and Department of Chemistry, University of Wollongong, NSW 2522, Australia*
[b]*Department of Biology, Northeastern University, Boston, MA 02155, USA*

Received 27 August 2005; accepted 2 September 2005
Available online 3 October 2005

Abstract—In order to develop structure–activity relationships and to provide access to antibacterial agents for dual action studies, a variety of aryl group-substituted 2-aryl-5-nitro-1*H*-indoles were synthesized and the activity of the compounds assessed as inhibitors of the NorA multidrug resistance pump in the bacterium *Staphylococcus aureus*. The NorA protein from the major facilitator superfamily of efflux pumps confers resistance to a variety of structurally dissimilar antimicrobials such as norfloxacin, ethidium bromide, berberine and acriflavin. The compound [4-benzyloxy-2-(5-nitro-1*H*-2-yl)-phenyl]-methanol was the most potent pump inhibitor.
© 2005 Elsevier Ltd. All rights reserved.

1. Introduction

In the vast heterocyclic structural space, the indole nucleus occupies a position of major importance. Many indole derivatives, including fused derivatives, form the basis of a range of pharmaceuticals[1,2] and a high level of activity continues in the search for new indole-based medicinal agents.[3,4] In this context and as part of a wider programme on the development of dual action antibacterial agents, we have investigated the synthesis of 5-nitro-2-aryl-1*H*-indoles. The known compound 5-nitro-2-phenyl-1*H*-indole (INF55) is an inhibitor of the NorA efflux pump in the human pathogenic bacterium *Staphylococcus aureus*.[5] While some related 2-aryl derivatives (for example, an *o*-anisole group) have been predicted by CoMFA (3D-QSAR) analysis to be NorA pump inhibitors,[6] there is a need to access a greater range of 2-aryl indole derivatives with a variety of functionalisation in the aryl substituent group, particularly with respect to groups in the *ortho* position to the point of indole attachment in the aryl ring. Such derivatives would be of value in establishing structure–activity relationships in the pump inhibitors, and also in providing handles for linking other antibacterial agents for dual action studies. Several routes have been described for the synthesis of 5-nitro-2-phenyl-1*H*-indole, including a palladium-assisted reaction of 4-nitro-2-bromoaniline and α-piperidinostyrene,[7] the Fischer indole synthesis of acetophenone with phenylhydrazine and subsequent nitration,[8] and reaction of 4-nitroaniline and dimethyl-(2-oxo-2-phenyl-ethyl)-sulfonium bromide followed by cyclisation.[9] However, there are limitations with respect to functional group tolerances in these approaches. Another approach to such 2-aryl indoles involves a preformed indole nucleus and subsequent introduction of a functionalised 2-aryl group via N-acylation, palladium-induced oxidative cyclisation, and ring opening of the 2-nitro-6*H*-isoindolo[2,1-*a*]indol-6-one intermediate.

The NorA protein of *S. aureus* is a drug/proton antiporter belonging to the major facilitator family of transporters and extrudes chemically unrelated substances such as norfloxacin, ciprofloxacin, ethidium bromide, berberine and acriflavin.[10] The effects of functionalised INF55 can be reported by a decrease of the minimum inhibitory concentration (MIC) of berberine.[11] Berberine alkaloids are amphipathic cations, a preferred type of MDR transporter substrate.[10] Berberine is a plant secondary metabolite and a phytoalexin produced in response to stressors like microbial invasion.[12,13] Interestingly, Berberis plants that make berberine also produce an MDR inhibitor, 5′-methoxyhydnocarpin (5′-MHC).[14] The synergistic couple of berberine and 5′-MHC produces an effective antimicrobial action. Designing inhibitors to block resistance conferred by

Keywords: Indoles; 5-Nitro-2-phenyl-1*H*-indole derivatives; NorA efflux pump inhibitors; Antibacterial agent potentiators.
* Corresponding author. Tel.: +61 2 4221 4255; fax: +61 2 4221 4287; e-mail: john_bremner@uow.edu.au

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.09.019

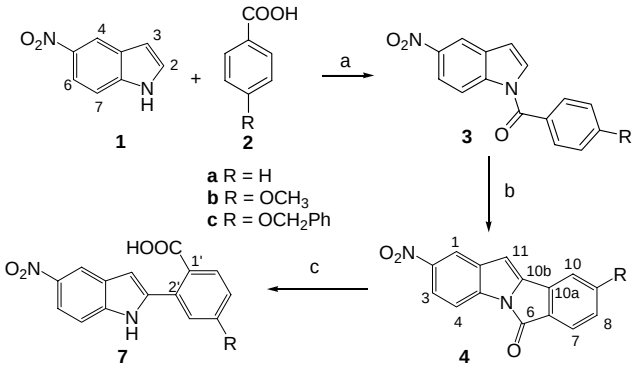
multidrug transporters could be an attractive strategy for developing a new anti-infective.

Application of this methodology, with subsequent functional group manipulations, to the synthesis of new aryl-group functionalised 5-nitro-2-aryl-1*H*-indoles, together with their assessment as NorA pump inhibitors, are now described in this article.

2. Results and discussion

2.1. Synthesis of 2-aryl-5-nitro-1*H*-indoles

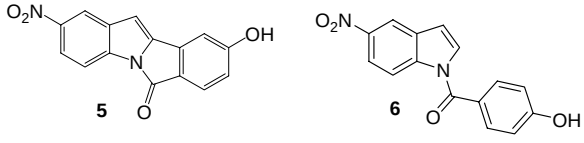
Commercially available 5-nitro-1*H*-indole (**1**) was used as the starting point for the syntheses followed by direct *N*-acylation using the carboxylic acids **2**, and then palladium cyclisation and hydrolytic ring opening of **4** to the carboxylic acid derivatives **7** (Scheme 1).



Scheme 1. *Reagents*: (a) DCC, DMAP, DCM; (b) $Pd(OAc)_2$, AcOH; (c) *t*-BuOK, *t*-BuOH, H_2O.

The direct *N*-acylation of **1** with carboxylic acids **2a**–**2c**, which were prepared according to the reported procedure,[15] afforded **3a**–**3c** in high yields. The intramolecular ring-closure of the N-acylated indoles **3a**–**3c** to the isoindolo-indolones **4a**–**4c** (Scheme 1) was accomplished via a palladium(II) acetate-promoted oxidative intramolecular cyclisation, following the method for the intramolecular ring-closure of 1-aroylindoles reported previously by Itahara.[16] The compounds **4a**–**4c** were obtained in moderate yields. The structure of **4a** was confirmed by NMR spectroscopic analysis and mass spectrometry. The ^{1}H NMR spectrum revealed a distinctive signal attributed to H-11 at δ 6.79. In **4b**, this signal appeared at δ 6.77.

In the case of the cyclisation of **3c**, **4c** was obtained in 20% yield together with the unexpected products **5** and **6** in 22% and 35% yields, respectively.

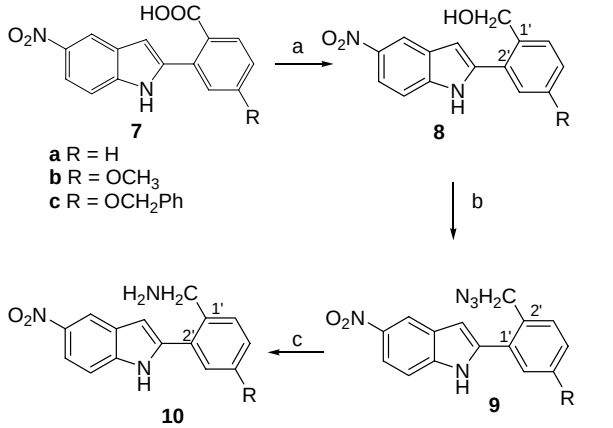


Debenzylation can be accomplished under acidic conditions at reflux.[17] Therefore, the cleavage of the benzyloxy substituent in **3c** and **4c** could both occur together with the cyclisation of **3c**. Compound **5** can be converted to **4c** in good yield by benzylation of the phenoxide anion with benzyl bromide. The structure of **4c** was confirmed by the presence of a singlet signal attributed to H-11 at δ 6.79 in the ^{1}H NMR spectrum.

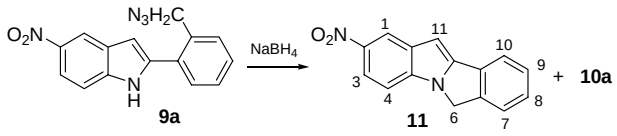
Ring-opening by amide hydrolysis[18] of compounds **4a**–**4c** then afforded the acids **7a**–**7c** in high yield. The ^{13}C NMR spectra of these acids revealed a signal ascribed to the carbonyl carbon of the carboxylic acids in the range of δ 167.9–171.7.

Further selective functional group manipulations in the indole derivatives were based on the carboxylic acids **7**, starting with carboxylic acid group reduction to the corresponding alcohols **8** without affecting the nitro substituent. Subsequently, conversion of the alcohols to the azides **9** and then the amines **10** was also established; no indolic NH protection was required in these sequences (Scheme 2). Selective reduction of the benzoic acid derivatives **7a**–**7c** to the corresponding alcohols **8a**–**8c** was achieved with borane in tetrahydrofuran.[19,20] The reaction was remarkably facile and proceeded in high yield. The ^{1}H NMR analysis of **8a**–**8c** confirmed the formation of the benzyl alcohol derivatives with a distinctive signal ascribed to the methylene protons in the range of δ 4.63–4.77, and the loss of the signal assigned to the carbonyl carbon in the starting materials **7a**–**7c** was observed in the ^{13}C NMR spectra.

Treatment of alcohols **8a**–**8b** with sodium azide and triphenylphosphine[21] afforded the azides **9a**–**9b** in moderate yields. Reduction of the azides **9a**–**9b** to the amines **10a**–**10b** was achieved using $NaBH_4$. The initial reduction attempt involved the reduction of **9a** with $NaBH_4$ in THF with dropwise addition of MeOH.[22] Two products were obtained, one of which was the amine **10a**, and the other was the new cyclised product**11** (Scheme 3). The ^{1}H NMR spectrum of **11** revealed the absence of a signal which could be ascribed to the indolic NH proton and the appearance of a downfield singlet integrating for two protons at δ 5.12, which was attributed to



Scheme 2. *Reagents*: (a) BH_3-THF; (b) NaN_3, PPh_3, CCl_4-DMF; (c) $NaBH_4$, $HS(CH_2)_3SH$.



Scheme 3. Reduction of azide **9a** with $NaBH_4$.

the methylene protons (H-6). The ^1H NMR spectrum of **10a** showed an upfield signal integrating for two protons at δ 3.99 as a singlet, which was assigned to the methylene protons adjacent to the amine group, and a signal ascribed to the indolic NH was apparent at δ 13.63.

When reduction of the azides **9a**–**9b** was undertaken using $NaBH_4$ and 1,3-propanedithiol[23] in *i*-PrOH and triethylamine at low temperature, to avoid cyclisation, the amines **10a**–**10b** were obtained in high yields.

Since the palladium-induced oxidative cyclisation of **3c** gave **4c** in low yield and two by-products **5** and **6**, the synthesis of benzyloxy analogues of the azide and amine derivatives from **4c** was omitted.

The various 2-aryl-5-nitro-1*H*-indoles prepared were then evaluated as inhibitors of the NorA MDR efflux pump in *S. aureus*.

2.2. Assessment of NorA MDR pump inhibitory activity

The NorA inhibitory activity of synthetic indole derivatives **7a**–**b**, **8a**–**c**, **9a**–**b** and **10a**–**c** was assayed in the wild type *S. aureus* 8325-4, in a mutant deleted in NorA (K1758), and in a strain overexpressing the pump from a recombinant plasmid (K2361). The inhibitors did not have a direct activity (MICs $\geqslant$ 50 μg/mL), except for **9a** (MIC 3 μg/mL) and **10b** (25 μg/mL) against *S. aureus* K1758. The MIC of berberine alone against the *S. aureus* strains was 15 μg/mL for K1758, 125 μg/mL for 8325-4 and >500 μg/mL for K2361 (Table 2). All inhibitors, with the exception of **7a**, increased *S. aureus* susceptibility to berberine (Table 1). Compound **8c** had the highest activity against the strain overexpressing the NorA pump. At a concentration of 0.8 μg/ml, it potentiated berberine more than 15-fold.

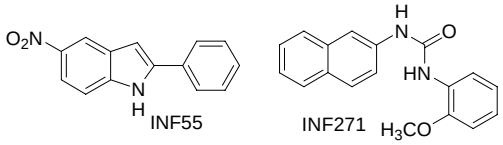
Table 1. Potentiation of berberine by MDR inhibitors in *Staphylococcus aureus*

Compound	MIC (μg/mL) of inhibitor		
	Δ*norA* K1758 plus berberine (3 μg/mL)	WT 8325-4 plus berberine (30 μg/mL)	$NorA^{++}$ K2361 plus berberine (30 μg/mL)
7a	>50	>50	>50
8a	6.25	12.5	12.5
9a	0.8	6	3
7b	12.5	12.5	>50
8b	3	12.5	6.25
9b	0.4	1.5	1.5
8c	0.4	0.4	0.8
10a	<0.2	0.4	12.5
10b	0.8	0.4	12.5
INF271	3	0.8	3
INF55	0.8	0.2	3

Inhibitors were added to *S. aureus* cells growing in MH broth in the presence of sub-inhibitory amounts of berberine and inhibition of growth was measured by recording the optical density after 18 h of incubation.

Compound **9b** was almost as active as **8c**, and both showed higher activity as compared to the known inhibitors INF271 and INF55. Compounds **10a** and **10b** were highly active against the strain deleted in NorA and the wild type, but relatively ineffective against K2361 overexpressing NorA. This suggests that these two inhibitors are more effective in blocking some additional pumps in *S. aureus*, rather than NorA.

The nature of the functional group in the 2-aryl substituent is clearly significant in determining relative potency. With a neutral group at the 1′ position in the aryl ring and an ether substituent in the 4′ position, an increase in potency was observed. In the case of the primary amines **10a** and **10b**, the presence of the 4-methoxy group in the latter decreased potency as an inhibitor. Protonation of the primary amino group at physiological pH may be a factor in determining potency between the strains in the case of **10a**.



Next, we tested the ability of the most potent inhibitor, **8c** to potentiate a variety of chemically unrelated antimicrobial compounds, which are substrates of NorA. Compound **8c** was comparable in its potency to the known inhibitor INF55 (Table 2). Potentiation of a number of substrates was highly significant, for example, the MIC of berberine changed from >500 μg/mL in a strain overexpressing NorA to 2 μg/mL in the presence of **8c**; and the potency of the fluoroquinolone antibiotic norfloxacin increased 16-fold.

Potentiation of berberine accumulation by **8c** into cells of *S. aureus* was measured by following fluorescence of berberine bound to DNA (Fig. 1). The rate of berberine accumulation increased sharply in the presence of **8c** in all strains tested. The initial accumulation rate was higher with **8c** as compared to INF55.

3. Conclusions

The synthesis of a range of new 2-aryl-5-nitroindole derivatives was achieved using a palladium-mediated cyclisation to establish the crucial indole C2-aryl C bond formation. These compounds were then shown to be effective NorA MDR pump inhibitors, apart from an acid derivative. Overall, compounds **8a**–**8c**,

Table 2. Potentiation of antimicrobials by an indole-based MDR inhibitor **8c**.

	MIC (μg/mL)	MIC (μg/mL)plus **INF55** (5 μg/mL)	Fold change	MIC (μg/mL)plus **8c** (5 μg/mL)	Fold change
Wild-type (8325-4)					
Berberine	125	1	125	1	125
Ciprofloxacin	1	0.1	10	0.1	10
Ethidium bromide	4	0.03	130	0.1	40
Norfloxacin	1	0.25	4	0.25	4
$NorA^{++}$ (K2361)					
Berberine	~ 500	2	250	2	250
Ciprofloxacin	4	0.5	8	0.5	8
Ethidium bromide	31	0.5	62	0.1	310
Norfloxacin	16	2	8	1	16
Δ*norA* (K1758)					
Berberine	15	0.5	30	0.5	30
Ciprofloxacin	0.24	0.1	2.4	0.12	2
Ethidium bromide	0.24	0.03	8	0.03	8
Norfloxacin	0.24	0.5	0.5	0.24	No change

Conditions as described in legend to Table 1.

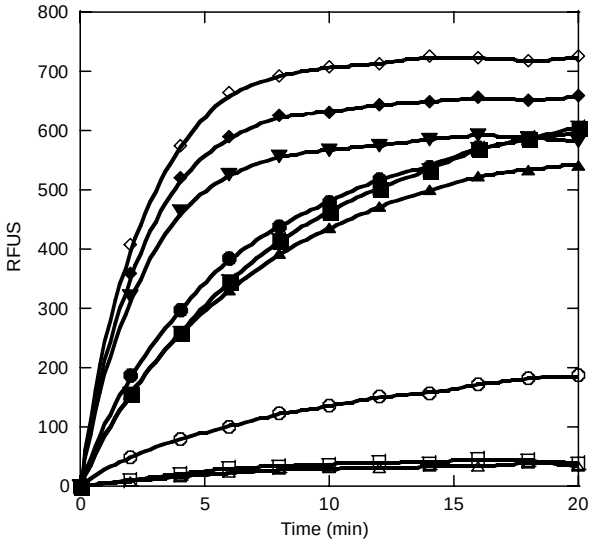


Figure 1. Accumulation of berberine in *S.aureus*. Accumulation of berberine in *S. aureus* cells. The uptake of berberine with no addition (○ □ Δ) or with the addition of INF55 (● ■ ▲); **8c** (◊ ♦ ▼); in Δ*norA* (○ ● ◊), WT (□ ■ ♦) and $NorA^{++}$ (Δ ▲▼) was measured by increase in fluorescence, and is expressed as relative fluorescence units (RFUs). Berberine was present at a concentration of 30 μg/mL and INF55 and **8c** were added at 10 μg/mL.

9a–**9b** and **10a**–**10b** potentiated the action of the antibacterial agent berberine by blocking the NorA MDR pump in *S. aureus*. The alcohol **8c** was the most effective inhibitor in the 2-aryl-5-nitroindole series against all strains of *S. aureus*. Compound **8c** also potentiated the action of other antibiotics (e.g., ciprofloxacin) in *S. aureus*.

4. Experimental

4.1. General chemistry methods

All starting materials were used without further purification. Tetrahydrofuran (THF) and diethyl ether were distilled from sodium in the presence of benzophenone. Other solvents used were of AR grade, and were used as received, except for dichloromethane (DCM), which was of LR grade and was distilled before use. The term petroleum spirit (PS) refers to petroleum spirit within the boiling range of 40–60 °C. Flash chromatography was performed under medium pressure using silica gel 60 (230–400 mesh, Merck); this silica gel was also used with vacuum liquid chromatography (VLC). Preparative TLC was done on Merck Silica gel 60 F_{254} with a thickness of 0.2 mm on aluminium sheet. Reactions were monitored by thin-layer chromatography (TLC) on Merck Silica gel 60 F_{254} with a thickness of 0.2 mm on aluminium sheet, and the compounds were detected by examination under ultraviolet light and by exposure to iodine vapour. Organic solvents were dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure with a Büchi rotary evaporator. Solvent ratios are vol/vol. All compounds were judged to be greater than 95 % purity by ^{1}H NMR and TLC analysis. High resolution (EI) MS (for M^+) was run using a VG Autospec spectrometer operating at 70 eV and a source temperature of 250 °C with PFK reference. The ^{1}H and ^{13}C NMR were determined at 299.92 and 75.42 MHz with a Varian Unity-300 spectrometer, and at 499.91 and 125.71 MHz with Varian Inova-500 spectrometer. Unless otherwise stated, the spectra were obtained from solutions in $CDCl_3$ and referenced to TMS (proton) and the chloroform mid-line (77) (carbon). Chemical shifts of the outer peaks are given for specified multiplet patterns in the ^{1}H NMR spectra. The assignments were made by standard gradient correlation spectroscopy (gCOSY), gradient heteronuclear single quantum correlation (gHSQC) and gradient heteronuclear multiple bond correlation (gHMBC) spectroscopy. The same superscript assignments may be reversed for the signals designated in the same compound. The infrared spectrum was measured (KBr disc) with an AVATAR 370 FT-IR spectrometer. The melting point (mp) determinations were recorded with a Reichert melting point apparatus and are reported uncorrected.

4.2. Synthesis

4.2.1. Preparation of 4-benzyloxybenzoic acid 2c.[24,25] To a suspension of *p*-hydroxybenzoic acid (3.0 g, 22.8 mmol) and dry K_2CO_3 (7.0 g, 50.0 mmol) in dry DMF (60 mL) was added dropwise benzyl bromide (5.4 mL, 45.8 mmol) under a nitrogen atmosphere. The reaction mixture was heated (with a reflux condensor attached) at 80 °C for 6 h. The mixture was evaporated, added to ice water (500 mL) and acidified with 5 M HCl to pH1. The suspension was filtered, washed several times with H_2O, then dried and chromatographed on silica gel by VLC (60% DCM in PS) to give benzyl 4-(benzyloxy) benzoate (5.62 g, 81%) as an off-white solid, mp 93–95 °C. To the suspension of the dibenzylated product (5.0 g, 15.7 mmol) in MeOH (50 mL) was added 30% aqueous solution of KOH (10 mL) and heated at reflux for 6 h. The solvent was evaporated, added to ice water (300 mL) and acidified to pH1. The white precipitate was filtered, washed thoroughly with water and then 20% DCM in PS (100 mL) to remove the dibenzylated starting material, and dried to give the acid **2c** (3.25 g, 91%) as white needles, mp 185–187 °C (lit.[25] 187–190 °C).

4.2.2. N-Acylation of 5-nitro-1*H*-indole (1): typical procedure. To a solution of indole **1** (0.2 mmol), DMAP (0.2 mmol) and carboxylic acid **2a**, **2b**, **or 2c** (0.4 mmol) in DCM (2 mL) at 0 °C under a nitrogen atmosphere were added a stirred solution of DCC (0.4 mmol) in DCM (1 mL). The solution was warmed to rt and stirred for 4–6 h, and was monitored by TLC until no starting material remained. The resulting suspension was concentrated in vacuo. The residue was treated with MeOH (20 mL), the mixture filtered and the precipitate was then washed with MeOH and dried to give the desired product (**3a**–**3b**). The filtrate was concentrated and the residue was recrystallised from MeOH. In the case of **3c**, a different workup procedure was used. After evaporation of the solvent, the residue was chromatographed on silica gel (40% DCM in PS) to obtain **3c**.

4.2.2.1. 1-Benzoyl-5-nitro-1*H*-indole (3a). Yield: 48.4 mg (91%); mp 158–159 °C (Lit.[15] 158–159 °C).

4.2.2.2. 1-(4-Methoxybenzoyl)-5-nitro-1*H*-indole (3b). Yield: 56.1 mg (95%); mp 199–200 °C (lit.[15] 199–200 °C).

4.2.2.3. 1-(4-Benzyloxybenzoyl)-5-nitro-1*H*-indole (3c). Yield: 64.7 mg (87%) as off-white needles, mp 177–179 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.19 (s, 2H, OCH_2), 6.77 (dd, J = 3.8, 0.8 Hz, 1H, H-3), 7.12 (d, J = 9.0 Hz, 2H, H-3′), 7.36–7.48 (m, 5H, ArH), 7.55 (d, J = 3.6 Hz, 1H, H-2), 7.77 (d, J = 8.7 Hz, 2H, H-2′), 8.25 (dd, J = 9.3, 2.1 Hz, 1H, H-6), 8.41 (d, J = 9.0 Hz, H-7), 8.54 (d, J = 2.1 Hz, 1H, H-4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 70.3 (OCH_2), 108.3 (C3), 115.0 (2C, C3′), 116.2 (C7), 117.2 (C4), 119.6 (C6), 125.4 (C1′), 127.5 (2C, C3′), 128.4 (2C, C4″), 128.8 (C2″), 130.4 (C3a), 130.5 (C2), 132.1 (2C, C2′), 135.8 (C1″), 139.1 (C7a), 144.2 (C5), 162.5 (C4′), 167.9 (CO). HMRS (EI); *m*/*z* calcd for $C_{22}H_{16}N_2O_4$ $[M]^+$: 372.1110; found: 372.1105.

4.2.3. Palladium cyclisation of compounds 3a–3c: typical procedure. A solution of **3a**, **3b**, **or 3c** (0.75 mmol) and palladium (II) acetate (0.75 mmol) in glacial acetic acid (20 mL) was heated at 110 °C under a nitrogen atmosphere for 11–20 h (**3a**, 11 h; **3b**, 16 h; **3c**, 20 h). The reaction was monitored by TLC until there was no change in the concentration of cyclised product. The reaction was allowed to cool to rt. The black suspension was filtered through Celite and washed with acetone and then the filtrate was evaporated. The residue was added to ice water (200 mL), the precipitate filtered, dried and chromatographed on silica gel by VLC (40–60% DCM in PS) to give the desired products (**4a**–**4c**).

4.2.3.1. 2-Nitro-isoindolo[2,1-*a*]indol-6-one (4a). Yield: 100.1 mg (50%) as pale yellow needles, mp 268.8 °C (decomposed). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.79 (d, J = 0.6 Hz, 1H, H-11), 7.43–7.49 (ddd, J = 8.4, 5.9, 2.6 Hz, 1H, H-9) 7.62-7.65 (m, 2H, H-10, H-8), 7.84 (dt, J = 7.5, 0.9 Hz, 1H, H-7), 7.98 (d, J = 8.7 Hz, 1H, H-4), 8.23 (dd, J = 8.7, 2.1 Hz, 1H, H-3), 8.41 (d, J = 2.1 Hz, 1H, H-1). ^{13}C NMR (125 MHz, DMSO-d_6): δ 104.2 (C11), 112.6 (C4), 118.8 (C1), 121.8 (C3), 122.8 (C7), 125.6 (C10), 130.2 (C9)[a], 132.5 (C10a), 133.8 (C11a), 134.6 (C6a), 135.1 (C8)[a], 136.1 (C4a), 141.1 (C10b), 143.9 (C2), 162.0 (C6). HRMS (EI); *m*/*z* calcd for $C_{15}H_8N_2O_3$ $[M]^+$: 264.0535; found: 264.0533.

4.2.3.2. 9-Methoxy-2-nitro-isoindolo[2,1-*a*]indol-6-one (4b). Yield: 163.7 mg (74%) as pale yellow needles, mp 238–240 °C. ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD): δ 3.95 (s, 3H, OCH_3), 6.77 (s, 1H, H-11), 6.91 (dd, J = 8.7, 2.1 Hz, 1H, H-8), 7.11 (d, J = 2.7 Hz, 1H, H-10), 7.74 (d, J = 8.4 Hz, 1H, H-7), 7.95 (d, J = 8.7 Hz, 1H, H-4), 8.20 (dd, J = 8.7, 2.1 Hz, 1H, H-3), 8.40 (d, J = 2.1 Hz, 1H, H-1). ^{13}C NMR (75 MHz, $CDCl_3$ + CD_3OD): δ 55.8 (OCH_3), 103.0 (C11), 107.7 (C10), 112.6 (C4), 115.1 (C8), 118.3 (C1), 121.7 (C3), 125.0 (C10a), 127.5 (C7), 134.1 (C11a), 136.3 (C4a), 136.5 (C6a), 140.8 (C10b), 144.0 (C2), 162.3 (CO), 165.1 (C9). HRMS (EI); *m*/*z* calcd for $C_{16}H_{10}N_2O_4$ $[M]^+$: 294.0641; found: 294.0648.

4.2.3.3. 9-Benzyloxy-2-nitro-isoindolo[2,1-*a*]indol-6-one (4c). Yield: 51.5 mg (20%) as pale yellow needles, mp > 250 °C. IR (KBr): ν_{max} 1736, 1615, 1597, 1516, 1342, 1245, 1130, 1077 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD): δ 5.21 (s, 2H, OCH_2), 6.79 (s, 1H, H-11), 7.01 (dd, J = 8.7, 2.1 Hz, 1H, H-8), 7.22 (d, J = 2.1 Hz, 1H, H-10), 7.43-7.50 (m, 5H, ArH), 7.76 (d, J = 8.7 Hz, 1H, H-7), 7.97 (d, J = 8.7 Hz, 1H, H-6), 8.22 (dd, J = 8.7, 2.4 Hz, 1H, H-3), 8.42 (d, J = 2.1 Hz, 1H, H-1) ^{13}C NMR ($CDCl_3$ + CD_3OD or DMSO-d_6) spectrum showed only methine and methylene carbon signals without quaternary carbon signals due to precipitation whilst the experiment was underway. ^{13}C NMR (125 MHz, DMSO-d_6): δ 70.9 (CH_2), 105.4, 109.9, 113.1, 117.5, 119.7, 126.6, 128.2, 128.7, 129.4 (>1C). HRMS (EI); *m*/*z* calcd for $C_{22}H_{14}N_2O_4$ $[M]^+$: 370.0954; found: 370.0944.

4.2.3.4. 9-Hydroxy-2-nitro-isoindolo[2,1-*a*]indol-6-one (5). Yield: 47.5 mg (22%) as pale yellow needles, mp 199-201 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.82 (dd, J = 8.6, 2.3 Hz, 1H, H-8), 7.07 (s, 1H, H-11), 7.16 (d, J = 2.1 Hz, 1H, H-10), 7.60 (d, J = 8.7 Hz, 1H, H-7), 7.81 (d, J = 9.0 Hz, 1H, H-4), 8.13 (dd, J = 8.7, 2.4 Hz, 1H, H-3), 8.45 (d, J = 1.8 Hz, 1H, H-1). ^{13}C NMR (75 MHz, DMSO-d_6): δ 103.6 (C11), 109.6 (C10), 112.1 (C4), 116.7 (C8), 118.6 (C1), 121.5 (C3), 122.7 (C10), 127.6 (C7), 134.1 (C11a), 135.9 (C4a), 136.2 (C6a)[a], 140.6 (C10b)[a], 143.4 (C2), 161.6 (CO), 163.9 (C9). HRMS (EI); *m*/*z* calcd for $C_{15}H_8N_2O_4$ $[M]^+$: 280.0484; found: 280.0480.

4.2.3.5. 1-(4-Hydroxybenzoyl)-5-nitro-1*H*-indole (6). Yield: 73.7 mg (35%) as a pale yellow solid, mp 201–203 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.92 (d, J = 3.3 Hz, 1H, H-3), 6.91–6.93 (m, 2H, ArH), 7.64–7.67 (m, 2H, ArH), 7.73 (d, J = 3.3 Hz, 1H, H-2), 8.17 (dd, J = 9.3, 2.4 Hz, 1H, H-6), 8.27 (d, J = 9.0 Hz, 1H, H-7), 8.60 (d, J = 1.8 Hz, 1H, H-4), 10.53 (br s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 108.1 (C3), 115.6 (2 × ArCH), 115.8 (C7), 117.3 (C1′), 119.3 (C6), 123.0 (C4), 130.3 (C2), 131.7 (C3a), 132.5 (2 × ArCH), 138.6 (C7a), 143.4 (C5), 162.0 (C4′), 167.6 (CO). HRMS (EI); *m*/*z* calcd for $C_{15}H_{10}N_2O_4$ $[M]^+$: 282.0641; found: 282.0638.

4.2.4. Conversion of 5 to 4c. To a suspension of **5** (200.0 mg, 0.71 mmol) and caesium carbonate (230.0 mg, 0.70 mmol) in dry DMF (20 mL) was added dropwise with stirring benzyl bromide (0.1 mL, 0.84 mmol). The reaction was heated at 80 °C under a nitrogen atmosphere for 7 h. The reaction was allowed to cool to rt and then filtered. The precipitate was washed thoroughly with water and dried to give a pale yellow solid **4c** (196.1 mg, 74%). ^{1}H NMR and HRMS (EI) data for **4c** were the same as those noted for this compound above.

4.2.5. Ring opening of compounds 4a–4c: typical procedure. A solution of *t*-BuOH (192 mL for **4a** or **4b**; 22.5 mL for **4c**) and H_2O (19.2 mL **4a** or **4b**; 2.3 mL for **4c**) containing *t*-BuOK (37.9 mmol **4a** or **4b**; 4.45 mmol for **4c**) was added to **4a** or **4b** (3.79 mmol; 0.45 mmol for **4c**) and heated at 82 °C for 12 h. The mixture was evaporated and added to ice water (400 mL **4a** or **4b**; 50 mL for **4c**). The solution was acidified to pH 1 with 5 M HCl and then saturated with solid NaCl. The solution was stirred vigorously for 2 h and then extracted with diethyl ether (3 × 400mL **4a** or **4b**; 3 × 50 mL for **4c**). The combined ether extract was dried and evaporated to give the desired acids (**7a**–**7c**).

4.2.5.1. 2-(5-Nitro-1*H*-indol-2-yl)benzoic acid (7a). Yield: 1.016 g (95%) as a pale yellow solid, mp 250–252 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.98 (s, 1H, H-3), 7.33–7.45 (m, 2H, H-4′, H-5′), 7.52 (d, J = 9.0 Hz, 1H, H-7), 7.67 (br.d, J = 7.2 Hz, 1H, H-3′), 7.75 (br d, J = 7.5 Hz, 1H, H-6′), 7.94 (dd, J = 9.03, 2.4 Hz, 1H, H-6), 8.52 (d, J = 2.4, H-4). ^{13}C NMR (75 MHz, DMSO-d_6): δ 102.2 (C3), 117.8 (C7), 116.3 (C6), 116.8 (C4), 127.5 (C3a), 127.8 (C5′), 128.5 (C2), 128.7 (C4′), 128.9 (C6′), 129.9 (C3′), 138.5 (C2′), 139.3 (C7a), 140.5 (C5), 142.2 (C1′), 171.7 (CO). HRMS (EI); *m*/*z* calcd for $C_{15}H_{10}N_2O_4$ $[M]^+$: 282.0641; found: 282.0643.

4.2.5.2. 4-Methoxy-2-(5-nitro-1*H*-indol-2-yl)benzoic acid (7b). Yield: 1.113 g (94%) as a pale yellow solid, mp 206–208 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.86 (s, 3H, OCH_3), 6.78 (dd, J = 2.1, 0.6 Hz, 1H, H-3), 7.08 (dd, J = 7.8, 2.7 Hz, 1H, H-5′), 7.11 (s, 1H, H-3′), 7.50 (d, J = 8.7 Hz, 1H, H-7), 7.89 (d, J = 7.8 Hz, 1H, H-6′), 8.00 (dd, J = 9.0, 2.4 Hz, 1H, H-6), 8.55 (d, J = 2.4 Hz, 1H, H-4), 12.07 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.7 (OCH_3), 103.3 (C3), 111.5 (C7), 114.0 (C5′), 116.4 (C3′), 116.7 (C6), 117.1 (C4), 123.8 (C2), 127.4 (C3a), 132.3 (C6′), 134.1 (C1′)[a], 139.6 (C7a), 140.7 (C5), 141.5 (C2′)[a], 161.3 (C4′), 167.9 (CO). HRMS (EI); *m*/*z* calcd for $C_{16}H_{12}N_2O_5$ $[M]^+$: 312.0746; found: 312.0749.

4.2.5.3. 4-Benzyloxy-2-(5-nitro-1*H*-indol-2-yl)benzoic acid (7c). Yield: 167.0 mg (96%) as a pale yellow solid, m.p. 223–235 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.24 (s, 2H, OCH_2), 6.78 (s, 1H, H-3), 7.16 (dd, J = 8.6, 2.6 Hz, 1H, H-5′), 7.24 (d, J = 2.7 Hz, 1H, H-3′), 7.30–7.50 (m, 5H, ArH), 7.52 (d, J = 9.0 Hz, 1H, H-7), 7.85 (d, J = 8.7, 1H, H-6′), 8.00 (dd, J = 8.9, 2.3 Hz, 1H, H-6), 8.55 (d, J = 2.4 Hz, H-4), 12.20 (s, 1H, NH), 12.57 (s, 1H, COOH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 69.6 (OCH_2), 103.2 (C3), 111.5 (C7), 114.6 (C5′), 116.6 (C6), 117.0 (C3′), 117.1 (C4), 124.1 (C2), 127.3 (C3a), 127.7 (2 × ArCH), 128.0 (ArCH), 128.4 (2 × ArCH), 132.1 (C6′), 133.8 (C2′), 136.4 (C1″), 139.6 (C7a), 140.6 (C5), 141.1 (C1′), 160.2 (C5′), 167.9 (CO). HRMS (EI); *m*/*z* calcd for $C_{22}H_{16}N_2O_5$ $[M]^+$: 388.1059; found: 388.1069.

4.2.6. Selective reduction of acids 7a–7c: typical procedure. To a solution of **7a** or **7b** (3.54mmol; 0.52 mmol for **7c**) in dry THF (90 mL for **7a** or **7b**; 15 mL for **7c**) was slowly added 1 M BH_3-THF complex solution (7.1 mL, 7.1 mmol for **7a** or **7b**; 1.0 mL, 1.0 mmol for **7c**) at 0 °C under a nitrogen atmosphere. After vigorous stirring for 2 h at room temperature, the excess hydride was carefully destroyed by slowly adding a solution of 50% THF in H_2O (20 mL for **7a** or **7b**; 2 mL for **7c**) until no gas bubbling was observed in the reaction mixture. The aqueous layer was saturated with anhydrous K_2CO_3. The THF layer was separated and the aqueous layer was extracted with diethyl ether (3 × 20mL). The combined THF and ether extract was dried, then evaporated, and the residue was chromatographed on silica gel by VLC (2% MeOH in DCM) to give the desired alcohols (**8a**–**8c**).

4.2.6.1. [2-(5-Nitro-1*H*-indol-2-yl)-phenyl]-methanol (8a). Yield: 940.5 mg (99%) as bright yellow needles, mp 132-134 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.77 (s, 2H, CH_2O), 6.91 (d, J = 1.2 Hz, 1H, H-3), 7.36–7.47 (m, 4H, H-7, ArH), 7.79 (br d, J = 7.8 Hz, 1H, ArH), 8.12 (dd, J = 8.9, 2.4 Hz, 1H, H-6), 8.61 (d, J = 2.4 Hz, 1H, H-4), 10.92 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 65.1 (CH_2), 103.5 (C3), 111.2

(C7), 117.5 (C6), 117.6 (C4), 127.8 (C3a), 128.8 (ArCH), 129.4 (ArCH), 130.3 (ArCH), 131.1 (ArCH), 132.8 (C2), 135.8 (C2′), 139.6 (C7a), 141.4 (C1′), 141.8 (C5). HRMS (EI); *m*/*z* calcd for $C_{15}H_{12}N_2O_3$ $[M]^+$: 268.0848; found: 268.0838.

4.2.6.2. [4-Methoxy-2-(5-nitro-1*H*-indol-2-yl)-phenyl]-methanol (8b). Yield: 997.8 mg (95%) as bright yellow needles, mp 206–208 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (s, 3H, OCH_3), 4.70 (s, 2H, CH_2OH), 6.91 (d, J = 1.8 Hz, 1H, H-3), 6.92 (dd, J = 8.4, 2.7 Hz, 1H, H-5′), 7.30 (d, J = 2.7 Hz, 1H, H-3′), 7.34 (d, J = 8.7 Hz, 1H, H-6′), 7.46 (d, J = 8.7 Hz, 1H, H-7), 8.10 (dd, J = 8.7, 2.1 Hz, 1H, H-6), 8.62 (d, J = 2.1 Hz, 1H, H-4), 11.02 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.5 (OCH_3), 64.6 (CH_2O), 103.5 (C3), 111.3 (C7), 113.9 (C5′), 115.7 (C3′), 117.6 (C6), 117.7 (C4), 127.7 (C3a), 128.3 (C2), 132.6 (C6′), 134.2 (C1′)[a], 139.6 (C2′)[a], 141.4 (C7a), 141.8 (C5), 160.1 (C4′). HRMS (EI); *m*/*z* calcd for $C_{16}H_{14}N_2O_4$ $[M]^+$: 298.0954; found: 298.0941.

4.2.6.3. [4-Benzyloxy-2-(5-nitro-1*H*-indol-2-yl)-phenyl]-methanol (8c). Yield: 163.5 mg (85%) as bright yellow needles, mp 70–72 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.63 (s, 2H, C**H**$_2$OH), 5.08 (s, 2H, OCH_2), 6.80 (dd, J = 2.0, 0.8 Hz, 1H, H-3), 6.90 (dd, J = 8.4, 2.7 Hz, 1H, H-5′), 7.23–7.42 (m, 8H, H-7, H-3′, H-6′, ArH), 8.03 (dd, J = 9.0, 2.1 Hz, 1H, H-6), 8.54 (d, J = 2.4 Hz, 1H, H-4), 10.92 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 64.6 (CH_2OH), 70.2 (OCH_2), 103.5 (C3), 111.3 (C7), 114.7 (C5′), 116.7 (C6′), 117.6 (C6), 117.7 (C4), 112.4 (2 × ArCH), 127.7 (C2), 128.2 (ArCH), 128.5 (C3a), 128.7 (2 × ArCH), 132.6 (C3′), 134.2 (C1″), 136.4 (C2′), 139.6 (C7a), 141.3 (C5), 141.9 (C1′), 159.3 (C4′). HRMS (EI); *m*/*z* calcd for $C_{22}H_{18}N_2O_4$ $[M]^+$: 374.1267; found: 374.1256.

4.2.7. Synthesis of azides 9a–9b: typical procedure. A mixture of **8a** or **8b** (1.06 mmol), sodium azide (1.16 mmol) and triphenyl phosphine (2.08 mmol) in a solution of 25% CCl_4 in DMF (10 mL) was heated at 90 °C under a nitrogen atmosphere for 5 h. The reaction mixture was then cooled to room temperature, quenched by adding H_2O (10 mL) and stirred for 10 min. The mixture was diluted with diethyl ether (40 mL) and washed thoroughly with H_2O. The organic layer was dried, concentrated, and then chromatographed on silica gel by VLC (30% DCM in PS) to give the azides (**9a**–**9b**).

4.2.7.1. 2-(2-Azidomethyl-phenyl)-5-nitro-1*H*-indole (9a). Yield: 231.0 mg (74%) as bright yellow needles, mp 146–148 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.40 (s, 2H, CH_2N_3), 6,86 (dd, J = 2.1 Hz, 0.6 Hz, 1H, H-3), 7.44–7.54 (m, 4H, H-7, ArH), 7.69 (dd, J = 6.2, 1.7 Hz, 1H, ArH), 8.13 (dd, J = 9, 2.1 Hz, 1H, H-6), 8.61 (d, J = 2.1 Hz, 1H, H-4), 9.53 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$,): δ 54.0 (CH_2N_3), 104.7 (C3), 111.1 (C7), 117.8 (C6), 117.9 (C4), 127.8 (C3a), 129.2 (ArCH), 129.5 (ArCH), 130.7 (ArCH), 131.2 (ArCH), 132.1 (C2), 132.3 (C1′), 139.4 (C7a), 140.0 (C2′), 142.1 (C5). HRMS (EI); *m*/*z* calcd for $C_{15}H_{12}N_5O_2$ $[M]^+$: 294.0991; found: 294.0987.

4.2.7.2. 2-(2-Azidomethyl-5-methoxy-phenyl)-5-nitro-1*H*-indole (9b). Yield: 209.88 mg (61%) as bright yellow needles, mp 138–140 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.79 (s, 3H, OCH_3), 4.26 (s, 2H, CH_2), 6.78 (dd, J = 2.1, 0.9 Hz, 1H, H-3), 6.89 (dd, J = 8.7, 2.7 Hz, 1H, H-4′), 7.12 (d, J = 2.7 Hz, 1H, H-6′), 7.29 (d, J = 8.7 Hz, 1H, H-3′), 7.37 (d, J = 8.4 Hz, 1H, H-7), 8.03 (dd J = 9.0, 2.1 Hz, 1H, H-6), 8.52 (d, J = 2.1 Hz, 1H, H-4), 9.77 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 53.6 (CH_2), 55.5 (OCH_3), 104.6 (C3), 111.2 (C7), 114.2 (C5′), 116.1 (C6′), 117.7 (C4), 117.9 (C6), 124.6 (C2), 127.7 (C3a), 132.6 (C3′), 133.5 (C1′)[a], 139.5 (C7a), 140.0 (C2′)[a], 142.3 (C5), 160.1 (C5′). HRMS (EI); *m*/*z* calcd for $C_{16}H_{13}N_5O_3$ $[M]^+$: 323.1018; found: 323.1024.

4.2.8. Reduction of azides 9a–9b: typical procedure. To a solution of **9a or 9b** (0.34 mmol), triethylamine (TEA) (0.09 mL, 0.68 mmol) and 2 drops of 1,3-propanedithiol (ca 0.1 mL, 1.0 mmol) in 35% MeOH in *i*-PrOH (12 mL) was added sodium borohydride (128.6 mg, 3.4 mmol) at 0 °C. After 2 h, more sodium borohydride (64.3 mg, 1.7mmol) was added and the mixture was stirred for a further 10 min. The reaction mixture was then evaporated, the residue was added to H_2O (50 mL) and the mixture was extracted with 40% diethyl ether in PS (2 × 40 mL). The aqueous layer was basified to pH 11 with a saturated NaOH solution and extracted with DCM (3 × 50 mL). The combined DCM extract was dried, concentrated and then chromatographed on silica gel by VLC (4% MeOH in DCM). The amine products (**10a**–**10b**) were washed with 40% diethyl ether in PS to remove traces of 1,3-propanedithiol.

4.2.8.1. 2-(5-Nitro-1*H*-indol-2-yl)-benzylamine (10a). Yield: 81.6 mg (90%) as a brown yellow solid, mp 127–129 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.99 (s, 2H, CH_2), 6.87 (br s, 1H, H-3), 7.26–7.46 (m, 4H, H-7, ArH), 7.78 (d, J = 7.5 Hz, 1H, ArH), 8.08 (dd, J = 8.7, 2.4 Hz, 1H, H-6), 8.61 (d, J = 2.1 Hz, 1H, H-4), 13.63 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 46.0 (CH_2), 102.5 (C3), 111.2 (C7), 117.1 (C6), 117.6 (C4), 127.9 (C3a), 128.5 (ArCH), 128.7 (ArCH), 130.3 (ArCH), 131.4 (ArCH), 133.1 (C2), 136.4 (C2′), 139.6 (C7a), 141.4 (C5), 142.7 (C1′). HRMS (EI); *m*/*z* calcd for $C_{15}H_{12}N_5O_2$ $[M]^+$: 267.1008; found: 267.0095.

4.2.8.2. 4-Methoxy-2-(5-nitro-1*H*-indol-2-yl)-benzylamine (10b). Yield: 91.0 mg (91%) as a yellow solid, mp 170–178 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.86 (s, 3H, OCH_3), 3.91 (s, 2H, CH_2), 6.84 (br s, 1H, H-3), 6.86 (dd, J = 7.9, 2.6 Hz, 1H, H-5′), 7.23 (d, J = 8.1 Hz, 1H, H-6′), 7.28 (s, 1H, H-6′), 7.40 (d, J = 9.3 Hz, 1 H, H-7), 8.06 (dd, J = 9.3, 2.4 Hz, 1H, H-6), 8.59 (d, J = 2.1 Hz, 1H, H-4), 13.66 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 42.5 (CH_2), 55.3 (OCH_3), 103.4 (C3), 111.9 (C7), 114.1 (C5′), 114.6 (C3′), 116.7 (C6), 117.0 (C4), 127.6 (C3a), 129.0 (C2), 131.8 (C6′), 132.9 (C1′)[a], 139.6 (C2′)[a], 140.7 (C5), 141.4 (C7a), 158.6 (C4′). HRMS (EI); *m*/*z* calcd for $C_{16}H_{15}N_3O_3$ $[M]^+$: 297.1113; found: 297.1101.

4.2.9. Reduction of azide 9a with $NaBH_4$ in THF/MeOH. To a mixture of **9a** (260.0 mg, 0.89 mmol) and sodium borohydride (25.0 mg, 0.66 mmol) in dry THF (10 mL) heated at reflux was slowly added MeOH (1.0 mL) and the mixture was stirred for 2 h. Further sodium borohydride (25.0 mg, 0.66 mmol) was then added and stirring was continued for 3 days. The mixture was cooled to room temperature and then 1 M HCl (3 mL) was added until the gas bubbling ceased. The mixture was basified to pH11 with a saturated NaOH solution and then extracted with DCM (3×20 mL). The combined DCM extract was dried, concentrated and then chromatographed on silica gel by VLC (PS, PS and DCM, then MeOH and TEA) to give starting material **9a** (70.2 mg, 0.24 mmol; eluent: 30% DCM in PS), 2-nitro-6*H*-isoindolo[2,1-*a*]indole (**11**) (30.1 mg, 14%; eluent: 40% DCM in PS) as a yellow solid and the benzylamine **10a** (70.6 mg, 30%; eluent: 30:1:2 DCM/MeOH/TEA) as a brown solid.

Compound **11**: mp 234–236 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.12 (s, 2H, H-6), 6.75 (s, 1H, H-11), 7.33 (d, $J = 9.0$ Hz, 1H, H-4), 7.38–7.51 (m, 3H, ArH), 7.74 (d, $J = 7.2$ Hz, 1H, ArH), 8.09 (dd, $J = 9.0$, 2.1 Hz, 1H, H-3), 8.58 (d, $J = 2.1$ Hz, 1H, H-1). ^{13}C NMR (75 MHz, $CDCl_3$): δ 48.8 (C6), 93.6 (C11), 108.8 (C4), 117.3 (C3), 118.7 (C1), 121.6 (ArCH) 123.7 (ArCH), 128.3 (ArCH), 128.6 (ArCH), 132.0 (C10b), 136.6 (C11a), 141.3 (C6a, C10a), 141.6 (C2), 147.1 (C4a). HRMS (EI); *m/z* calcd for $C_{15}H_{10}N_2O_2$ $[M]^+$: 250.0742; found: 250.0732.

4.3. Bacterial strains and growth conditions

The following bacterial strains were used in this study: *S. aureus* 8325-4[26] (wild-type), K1758[27] (ΔnorA) and K2361 (K1758 with pK364:norA). Cells were grown in Mueller–Hinton broth (MH) with aeration at 37 °C. Growth of K1758 was supplemented with erythromycin (25 μg/mL) and K2361 with chloramphenicol (25 μg/mL).

4.3.1. Antimicrobial compounds. Erythromycin, ciprofloxacin, chloramphenicol, berberine chloride, ethidium bromide and norfloxacin were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Penelope Markham provided INF271. INF55 was obtained from ChemBridge (San Diego, CA). Erythromycin and chloramphenicol were dissolved in ethanol at 10 mg/mL and diluted in water to desired concentrations. Ciprofloxacin was dissolved in water. Norfloxacin was dissolved in 0.01 N NaOH. Berberine was dissolved in methanol at 10 mg/mL and diluted in DMSO. INF55, INF271 and all synthetic indoles were dissolved in DMSO at 10 mg/mL and diluted in DMSO to desired concentrations. For MIC determination the final concentration of DMSO never exceeded 2.5%.

4.3.2. Determination of antimicrobial susceptibility. Cells (10^5/mL) were inoculated into Mueller–Hinton broth and dispensed at 0.2 mL/well in 96-well microtitre plates. MICs were determined in triplicate by serial 2-fold dilution of the test compounds. The MIC was defined as the concentration of an antimicrobial or antimicrobial plus inhibitor that completely inhibited cell growth during an 18 h incubation at 37 °C. Growth was assayed with a microtitre plate reader (Spectramax PLUS384; Molecular Devices) by monitoring absorption at 600 nm.

4.3.3. Berberine uptake assay[28]. Cells were cultured with aeration at 37 °C to an optical density at 600 nm (OD_{600}) of 1.5, pelleted by centrifugation for 2 min at 9000 RPMs and washed with fresh MH broth. The above procedure was repeated twice. The cells were then resuspended to an OD_{600} of 0.4 in MH broth. Assays were performed in 96-well flat-bottomed white plates (NUNC) in a final volume of 200 μL. Berberine was added at 30 μg/mL and inhibitor when present was added at 10 μg/mL. Fluorescence was measured with a Spectramax GeminiXS spectrofluorometer (Molecular Devices) at a 355-nm excitation wavelength and a 517-nm emission wavelength.

Acknowledgments

We wish to thank the University of Wollongong, Australia, and Srinakharinwirot University, Thailand, for supporting this work. The award of UPA and IPRS scholarships (Siritron Samosorn) is also gratefully acknowledged. Kim Lewis and Anthony Ball were supported by Grant R21 AI059483-01 from the NIH. We thank Glenn Kaatz for kindly providing *S. aureus* K1758 and K2361. We also thank Penelope Markham for providing INF271.

References and notes

1. Zhu, Y.-F.; Chen, C.; Struthers, R. S. In *Annual Reports in Medicinal Chemistry*; Doherty, A. M., Ed.; Elsevier Academic Press: Amsterdam, 2004; Vol. 39, pp 99–110.
2. Lednicer, D. The Organic Chemistry of Drug Synthesis; Wiley: New York, 1999; Vol. 6, pp 124–132.
3. Aygun, A.; Pindur, U. *Curr. Med. Chem.* **2003**, *10*, 1113.
4. Lobo, A. M.; Prabhakar, S. *J. Heterocycl. Chem.* **2002**, *39*, 429.
5. Markham, P. N.; Westhaus, E.; Klyachko, K.; Johnson, M. E.; Neyfakh, A. A. *Antimicrob. Agents Chemother.* **1999**, *43*, 2404.
6. Markham, P. N.; Mulhearn, D. C.; Neyfakh, A. A.; Crich, D.; Jaber, M.-R.; Johnson, M. E. WO Patent 0032196, 2000.
7. Kasahara, A.; Izumi, T.; Kikuchi, T.; Lin, X. *J. Heterocycl. Chem.* **1987**, *24*, 1555.
8. Noland, W. E.; Rush, K. R.; Smith, L. R. *J. Org. Chem.* **1966**, *31*, 65.
9. Gupta, K. C.; Manglum, P. *J. Indian Chem. Soc.* **1988**, *65*, 223.
10. Lewis, K. *J. Mol. Microbiol. Biotechnol.* **2001**, *3*, 247.
11. Belofsky, G.; Diamond, P.; Lewis, K.; Tegos, G.; Ekart, J. *J. Nat. Prod.* **2004**, *67*, 481.
12. Dixon, R. *Nature* **2001**, *411*, 843.
13. Darvill, A.; Albersheim, P. *Annu. Rev. Plant Physiol.* **1984**, *35*, 243.

14. Stermitz, F. R.; Lorenz, P.; Tawara, J. N.; Zenewicz, L. A.; Lewis, K. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 1433.
15. Bremner, J. B.; Samosorn, S.; Ambrus, J. I. *Synthesis* **2004**, 2653.
16. Itahara, T. *Heterocycles* **1986**, *24*, 2557.
17. Partington, S. M.; Watt, C. I. F. *J. Chem. Soc. Perkin. Trans. II* **1988**, 983.
18. Itahara, T. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 305.
19. Yoon, N. M.; Pak, C. S.; Brown, H. C.; Krishnamurthy, S.; Stocky, T. P. *J. Org. Chem.* **1973**, *38*, 2786.
20. Yoon, N. M.; Cho, B. T. *Tetrahedron Lett.* **1982**, *23*, 2475.
21. Reddy, G. V. S.; Rao, G. V.; Subramanyam, R. V. K.; Iyengar, D. S. *Synth. Commun.* **2000**, *30*, 2233.
22. Soai, K.; Yokoyama, S.; Ookawa, A. *Synthesis* **1987**, 48.
23. Pei, Y.; Wickham, B. O. S. *Tetrahedron Lett.* **1993**, *34*, 7509.
24. Okumura, M.; Maekawa, Y.; Mizuno, H.; Yagiyu, O. EP Patent 0312604, 1988.
25. Drzewinski, W.; Dabrowski, R.; Czuprynski, K. *Polish J. Chem.* **2002**, *76*, 273.
26. Novick, R. P. *Virology* **1963**, *33*, 155.
27. Price, C. T. D.; Kaatz, G. W.; Gustafson, J. E. *Int. J. Antimicrob. Agents* **2002**, *20*, 206.
28. Tegos, G.; Stermitz, F. R.; Lomovskaya, O.; Lewis, K. *Antimicrob. Agents Chemother.* **2002**, *46*, 3133.