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SYNTHESIS OF BERBERINE—EFFLUX PUMP INHIBITOR HYBRID ANTIBACTERIALS

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This article describes the compact synthesis of two isomeric dual-action hybrid antimicrobials where the 13-position of the antibacterial berberine has been linked via 3′- and 4′-methylene bridges to INF55 (5-nitro-2-phenylindole), an inhibitor of the bacterial NorA multidrug-resistance pump.

Keywords: Antibacterial; 2-arylindole; berberine; dual-action; hybrid; INF55

INTRODUCTION

A recent promising strategy for combating bacterial drug resistance arising from multidrug-resistance (MDR) efflux pumps is to covalently link a pump inhibitor to an antibacterial agent that is normally a pump substrate.[1,2] Dual-action hybrid antibacterials of this type potentially carry the advantage of synchronous and equimolar delivery of an antibacterial and an efflux pump inhibitor to sites of infection[3] and may slow the onset of resistance since they challenge bacteria to acquire resistance phenotypes at two independent targets.[4]

In the first demonstration of this concept we synthesized a prototype hybrid antibacterial SS14 **1**,[1] which contained the antibacterial alkaloid berberine substituted at its 13-position with a non-cleavable 2′-CH_2-linkage to 5-nitro-2-phenyindole (INF55), a known inhibitor of the NorA MDR pump. In recent work, we have compared the antimicrobial activity and conformations of **1** with its 3′- and 4′-substituted regioisomers **2** and **3** (Figure 1) in order to probe the effects of varying the relative orientation of the INF55 and berberine components in hybrids.[5] This article describes the synthesis of the two new dual-action hybrid antibacterials **2** and **3**.

RESULTS AND DISCUSSION

Our previous synthesis of **1**[1] involved pre-assembling 2′-bromomethyl-5-nitro-2-phenylindole and subsequently reacting this with 8-allyldihydroberberine[6] as the final step. It was envisaged that similar reactions of 8-allyldihydroberberine with the analogous 3′- and 4′-bromomethyl-5-nitro-2-phenylindoles (**8** and **9**,

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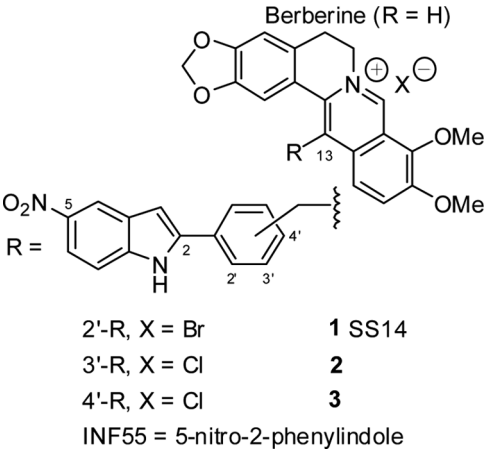
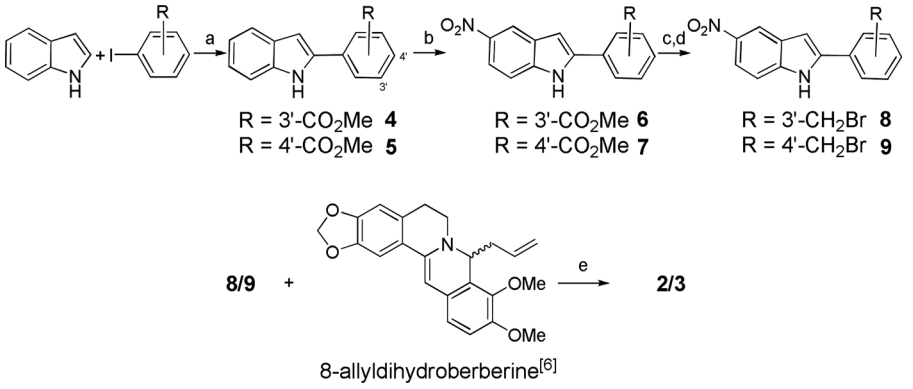


Figure 1. Structures of dual action antibacterial hybrids **1**–**3** and the NorA MDR pump inhibitor INF55.

Scheme 1) would provide access to **2** and **3**, respectively. Our route to 2′-bromomethyl-5-nitro-2-phenylindole[7] was not suitable for accessing **8** and **9** since it only provided 2-arylindoles bearing *ortho* substituents in the phenyl ring for later conversion to bromomethyl groups. An alternative strategy was thus implemented for the preparation of the key bromide precursors **8** and **9**.

Numerous methods are known for preparing 2-arylindoles[8,9] but the recently reported Rh(III)-catalyzed direct C2-arylation of unprotected NH-indoles with functionalized iodobenzenes[10] appeared as the most direct route towards 2-arylindoles with appropriate functionality in place for elaboration to **8** and **9**. Accordingly, methyl esters of 3-iodo- and 4-iodobenzoic acid were reacted with indole (Scheme 1) using the optimized catalytic conditions reported by Sames and colleagues[10] for related reactions. These reactions consistently provided only modest



Scheme 1. Reagents and conditions: (a) $[Rh(coe)_2Cl]_2$, CsOPiv, $(p\text{-}CF_3\text{-}C_6H_4)_3P$, 1,4-dioxane, reflux 3 days, **4** 14–18%, **5** 22–28%; (b) $NaNO_3$, H_2SO_4 (conc.), −20 °C, slow addition, **6** 81%, **7** 96%; (c) $LiBH_4$, THF, RT, 24 h, quant., (d) PPh_3, CBr_4, THF-Et_2O, 1 h, **8** 79%, **9** 94% (over 2 steps), (e) (i) CH_3CN, 60 °C, 24 h, (ii) 100–110 °C, 2–3 days (sealed vial), Br^-/Cl^- exchange, 40–45%.

yields (14–28%) of the 2-arylated indole esters **4** and **5**. Unreacted indole was the only other species isolated from the reactions. Optimization attempts met with limited success although it was observed that carrying the reactions out in 1,4-dioxane at reflux under N_2 for 3 days afforded similar or better yields compared to the reported method of stirring in a sealed tube at 120 °C. This finding made the reactions more practical on a multigram scale and allowed production of usable quantities of **4** and **5** despite the low yields. Evidence that arylation had occurred at the indole C2-positions was unambiguously confirmed from *g*-DQFCOSY and ROESY NMR experiments.

Esters **4** and **5** underwent nitration at low temperature at their respective indole 5-positions to afford the 5-nitro-2-phenylindole methyl esters **6** and **7** in excellent yields (81% and 96%, respectively).[11] Regioselective nitration at the indole 5-positions was confirmed from *g*-DQFCOSY and ROESY NMR experiments. The nitration procedure was adapted from the method reported by Noland et al. for the nitration of 2-phenylindole.[12] The modified procedure involved dissolving a stoichiometric quantity of $NaNO_3$ in conc. H_2SO_4 and adding this solution *via* syringe pump over 2 h to a dilute solution of the indoles **4** and **5** in conc. H_2SO_4 at −20 °C. Reactions were quenched immediately after the addition was complete by pouring onto a large quantity of crushed ice. Filtration followed by trituration with 4:1 MeOH:H_2O afforded pure **6** and **7**. It was found that nitration had to be carried out after Rh(III)-catalyzed C2-arylation as 5-nitroindole did not undergo coupling with 3′- and 4′-methyl iodobenzoates. This may have been due to the increased acidity of the indolic NH group containing a strongly electron-withdrawing nitro group at C5 resulting in indolide anion formation in the presence of cesium pivalate.

Selective reduction of the methyl esters **6** and **7** with $LiBH_4$ in THF to their corresponding benzylic alcohols[11] was quantitative as observed by TLC (SiO_2; 1:1 Petroleum Spirit:EtOAc). The crude alcohols were taken on directly to the key benzylic bromide precursors **8** and **9** by reaction with CBr_4 and PPh_3 under standard conditions. Bromides **8** and **9** were obtained in yields of 79% and 94%, respectively, (over two steps) and were used immediately in the final coupling step to minimize decomposition. Reactions of **8** and **9** with 8-allyldihydroberberine[6] proceeded as expected and produced 40–45% yields of the target hybrids **2** and **3** after preparative RP-HPLC. It has been proposed that these reactions proceed *via* a three-step cascade comprising an enamine alkylation, [3,3]-sigmatropic rearrangement and final retro-ene reaction.[6] Careful monitoring of the reactions of **8** and **9** with 8-allyldihydroberberine by analytical RP-HPLC and mass spectrometry confirmed that the enamine alkylation step with **8** and **9** was clean and quantitative after stirring overnight at 60 °C, and that increased heating to at least 100 °C was required to effect the subsequent electrocyclic reactions. Reactions were stopped when the RP-HPLC peak corresponding to the alkylated enamine had disappeared from the HPLC trace (typically after 3 days). Preparative RP-HPLC of **2** and **3** was carried out in the presence of HCl to initially provide mixed Cl^-/Br^- salts which were subsequently converted to pure Cl^- salts by stirring with a quaternary ammonium chloride anion exchange resin in CH_3OH at room temperature.

In summary, a compact 5-step synthesis of two new berberine-containing antibacterial hybrids **2** and **3** has been developed. A feature of the synthesis was accessing the required indolic benzylic bromide precursors **8** and **9** using an initial

Rh(III)-catalyzed direct C2-arylation of indole without NH group protection or C2 functionalization and subsequent selective functional group manipulations.

EXPERIMENTAL

A summary of the preparation of compounds **4**–**7** was provided as part of our earlier communication.[11] Full experimental details are now provided here.

General

Indole (Sigma Aldrich) was recrystallized from hot petroleum spirit immediately prior to use. $[Rh(coe)_2Cl]_2$ and $(p\text{-}CF_3\text{-}C_6H_4)_3P$ were purchased from Strem Chemicals Inc. (MA, USA) and used without further purification. Cesium pivalate (CsOPiv) was freshly prepared and dried according to the method of Campo and Larock.[13] Methyl 3-iodobenzoate (CAS Registry Number 618-91-7) and methyl 4-iodobenzoate (CAS Registry Number 619-44-3) were prepared in 95+% yields from the corresponding iodobenzoic acids (Sigma Aldrich) by refluxing in CH_3OH containing a catalytic quantity of conc. H_2SO_4 for 2 days. Anhydrous 1,4-dioxane was purchased from Sigma Aldrich. Anhydrous tetrahydrofuran (THF) and diethyl ether were freshly distilled from sodium benzophenone ketyl. Acetonitrile was freshly distilled from CaH_2. All other solvents were of analytical reagent (AR) grade and used without further purification. The term petroleum spirit refers to petroleum spirit within the boiling range 40–60 °C. Column chromatography was performed using silica gel 60 (230–400 mesh, Merck). Reaction monitoring by thin layer chromatography (TLC) was carried out using Merck Silica Gel 60 F_{254} (0.2 mm) plates. Compounds were visualized by examination under UV light and by staining with cerium ammonium molybdate. Reaction monitoring by RP-HPLC was carried out using a Waters 600 chromatography system fitted with a Waters 486 UV-Vis detector. The separations were performed using gradient elutions (30% solvent B to 100% solvent B over 30 mins) with solvents A (100% H_2O, 0.1% HCl) and B (10% H_2O, 90% CH_3CN, 0.1% HCl) on a Phenomenex C_{18} 4.6×150 mm (5 μm) column run at $1.0\,mL \cdot min^{-1}$. Preparative RP-HPLC was carried out using a Waters LC-150 chromatography system fitted with a Waters 2489 dual wavelength detector. The separations were performed using gradient elutions (30% solvent B to 100% solvent B over 30 mins) on a Waters RadPak C18 150×40 mm column at a flow rate of $40\,mL \cdot min^{-1}$ with detection at 254 nm. High resolution EIMS (for M^+) were recorded using a VG Autospec spectrometer operating at 70 eV and a source temperature of 250 °C with PFK reference. High resolution ESI-TOF-MS (for M^+) were recorded using a factory modified Waters QToF Ultima™ Mass Spectrometer (Wyntheshawe, UK). ^1H and ^{13}C NMR spectra were recorded on either a Varian Unity-300 (299.92 and 75.42 MHz, respectively) or a Varian-Inova-500 (499.91 and 125.71 MHz, respectively) spectrometer. NMR spectra recorded in CD_3COCD_3 were referenced to the acetone ^1H methyl signal at 2.09 ppm and ^{13}C methyl signal at 30.60 ppm. Spectra recorded in $CDCl_3$ were referenced to the $CHCl_3$ ^1H signal at 7.26 ppm and ^{13}C signal at 77.0 ppm. Spectra recorded in DMSO-*d6* were referenced to the DMSO ^1H methyl signal at 2.49 ppm and ^{13}C methyl signal at 39.5 ppm. Spectra recorded in DMF-*d7* were referenced to the DMF ^1H formyl signal at 8.01 ppm

and ^{13}C carbonyl signal at 167.7 ppm. Melting points were determined using a Reichert melting point apparatus and are uncorrected. The representative synthetic procedures described below can be used interchangeably for accessing the 3′- and 4′-isomers of **2** and **3** and their respective intermediates.

Methyl 4-(1*H*-Indol-2-yl)benzoate (5)[11]

Indole (8.0 g, 68.3 mmol, 1.0 eq), methyl 4-iodobenzoate (21.56 g, 81.95 mmol, 1.2 eq), $[Rh(coe)_2Cl]_2$ (990 mg, 1.38 mmol, 0.02 eq), (p-CF_3-$C_6H_5)_3P$ (1.83 g, 4.1 mmol, 0.06 eq) and cesium pivalate (22.37 g, 95.6 mmol, 1.4 eq; freshly prepared and dried according to the method of Campo and Larock[13]) were weighed into separate vials and dried under high vacuum in a desiccator for 24 h. The powders were added successively to a dry 3-neck 250 mL round bottom flask fitted with a thermometer and a condenser and placed under an atmosphere of dry N_2. Anhydrous 1,4-dioxane (60 mL; pre-purged with N_2 for 1 h immediately prior to use) was added to the flask and the reaction brought to reflux. The reaction was monitored by TLC (SiO_2; petroleum spirit:EtOAc 5:1) and stopped after refluxing for 3 days when no further progress was observed. The dark mixture was filtered through a plug of silica and the filter cake rinsed thoroughly with EtOAc. After evaporating the solvent, the crude residue was triturated with 50:1 petroleum spirit:Et_2O (3 × 250 mL) and recrystallized from EtOAc/petroleum spirit to yield 4.74 g of **5** (28%) as small grey crystals: mp 204–206 °C; ^1H NMR (CD_3COCD_3, 500 MHz) δ 10.82 (br.s, 1H), 8.07 (d, 2H, $J = 8.5$ Hz), 7.98 (d, 2H, $J = 8.5$ Hz); 7.61 (d, 1H, $J = 8$ Hz), 7.45 (d, 1H, $J = 7.5$ Hz), 7.16 (t, 1H, $J = 7$ Hz), 7.07–7.04 (m, 2H), 3.90 (s, 3H); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 166.9, 138.8, 137.8, 137.4, 130.8, 130.0, 129.5, 125.6, 123.5, 121.5, 120.8, 112.2, 102.0, 52.5; HRMS [found: (EI+) M^+ m/z 251.0942; $C_{16}H_{13}NO_2$ requires 251.0946].

Methyl 3-(1*H*-Indol-2-yl)benzoate (4)[11]

Method of preparation as for **5**: Yield = 14 −18%, mp 148–150 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.59 (br.s, 1H), 8.34 (s, 1H), 7.98 (d, 1H, $J = 8.1$ Hz); 7.87 (d, 1H, $J = 8.1$ Hz), 7.66 (d, 1H, $J = 8.1$ Hz), 7.50 (t, 1H, $J = 7.5$ Hz), 7.42 (d, 1H, $J = 8.4$ Hz), 7.23 (t, 1H, $J = 7.2$ Hz), 7.15 (t, 1H, $J = 6.9$ Hz), 6.91 (s, 1H), 3.91 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.9, 137.0, 136.6, 132.7, 130.8, 129.5, 129.1, 128.8, 128.5, 125.8, 122.7, 120.8, 120.4, 111.0, 100.7, 52.3; HRMS [found: (EI+) M^+ m/z 251.0946; $C_{16}H_{13}NO_2$ requires 251.0946].

Methyl 4-(5-Nitro-1*H*-indol-2-yl)benzoate (7)[11]

Indole **5** (2.5 g, 9.95 mmol) was dissolved in conc. H_2SO_4 (170 mL) in a 500 mL round bottom flask and cooled to −20 °C. A solution of $NaNO_3$ (896 mg, 10.55 mmol, 1.06 eq) in conc. H_2SO_4 (60 mL) was then added dropwise to the cooled solution (syringe pump) over 2 h. TLC analysis (SiO_2; petroleum spirit:EtOAc 1:1) immediately after the addition was finished confirmed complete consumption of **5**. The reaction was poured rapidly onto a large amount of crushed ice in a 2 L beaker (**Careful!** large amount of heat is given off due to exothermic dissolution of H_2SO_4) and stirred

until all the ice had melted. The crude product was filtered and recrystallized from $MeOH/H_2O$ to yield 2.82 g of **7** (96%) as a bright yellow solid: mp 280–286 °C; ^{1}H NMR (CD_3COCD_3, 500 MHz) δ 11.58 (br.s, 1H), 8.61 (s, 1H), 8.12–8.04 (m, 5H), 7.64 (d, 1H, $J = 10$ Hz), 7.36 (s, 1H), 3.93 (s, 3H); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 166.7, 142.9, 141.6, 141.0, 136.52, 131.0, 130.6, 129.2, 126.3, 118.5, 118.3, 112.6, 103.72, 52.4; HRMS [found: (EI+) M^+ m/z 296.0792; $C_{16}H_{12}N_2O_4$ requires 296.0797].

Methyl 3-(5-Nitro-1*H*-indol-2-yl)benzoate (6)[11]

Method of preparation as for **7**: Yield = 81%, mp 216–220 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.59 (br.s, 1H), 8.34 (s, 1H), 7.96 (d, 1H, $J = 7.8$ Hz), 7.87 (d, 1H, $J = 8.7$ Hz), 7.66 (d, 1H, $J = 8.1$ Hz), 7.50 (t, 1H, $J = 7.8$ Hz), 7.42 (d, 1H, $J = 8.4$ Hz), 7.23 (t, 1H, $J = 7.2$ Hz), 7.15 (t, 1H, $J = 8.1$ Hz), 6.91 (s, 1H), 3.96 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.9, 137.0, 136.6, 132.7, 130.9, 129.5, 129.1, 128.8, 128.5, 125.8, 122.7, 120.8, 120.4, 111.0, 100.7, 52.3; HRMS [found: (EI+) M^+ m/z 296.0792; $C_{16}H_{12}N_2O_4$ requires 296.0797].

2-(4-Bromomethyl-phenyl)-5-nitro-1*H*-indole (9)

A solution of the methyl ester **7** (82 mg, 0.28 mmol) in dry THF (5.0 mL) was treated with $LiBH_4$ (31 mg, 1.4 mmol, 5.0 eq). The reaction was monitored by TLC (SiO_2; petroleum spirit:EtOAc 1:1) and upon completion (typically 24 h) was slowly quenched by the dropwise addition of saturated aqueous NH_4Cl. After bubbling had ceased, the mixture was diluted with H_2O (50 mL) and extracted with EtOAc (4×50 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated to yield the benzylic alcohol as a bright yellow powder. mp 215–218 °C; ^{1}H NMR (CD_3COCD_3, 500 MHz) δ 11.39 (br.s, 1H), 8.56 (s, 1H), 8.04 (d, 1H, $J = 8.5$ Hz), 7.89 (d, 2H, $J = 8.5$ Hz), 7.59 (d, 1H, $J = 8.5$ Hz), 7.51 (d, 2H, $J = 8.5$ Hz), 7.17 (s, 1H), 4.72 (s, 2H), 4.35 (s, 1H); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 144.0, 142.7, 141.3, 130.8, 129.5, 128.0, 126.2, 117.8, 117.7, 112.2, 110.6, 101.5, 64.3. The crude alcohol (27 mg, 0.1 mmol, 1.0 eq) and CBr_4 (100 mg, 0.3 mmol, 3.0 eq) were added to a dry flask under Ar and 5.0 mL of anhydrous Et_2O/THF (1:1) was added. After stirring for 10 min, PPh_3 (79 mg, 0.3 mmol, 3.0 eq) was added to the stirring mixture in one portion. The reaction was stirred at rt for 1 h (monitored by TLC on SiO_2; petroleum spirit:EtOAc 1:2) before concentrating *in vacuo*. The crude residue was purified by column chromatography (SiO_2; petroleum spirit/EtOAc 4:1→3:1) and the product triturated with petroleum spirit (to remove traces of CBr_4 and PPh_3) to yield the benzylic bromide **9** (31 mg; 94%) as a yellow powder that was used immediately to prepare **3**. ^{1}H NMR (CD_3COCD_3, 500 MHz) δ 11.40 (br.s, 1H), 8.57 (s, 1H), 8.05 (d, 1H, $J = 8.5$ Hz), 7.91 (d, 2H, $J = 7.5$ Hz), 7.61–7.57 (m, 3H), 7.22 (s, 1H), 4.71 (s, 2H).

2-(3-Bromomethyl-phenyl)-5-nitro-1*H*-indole (8)

Method of preparation as for **9**: Yield = 79%, ^{1}H NMR (CD_3COCD_3, 500 MHz) δ 11.45 (br.s, 1H), 8.58 (d, 1H, $J = 2$ Hz), 8.06 (dd, 1H, $J = 9.25$, 2 Hz),

8.02 (s, 1H), 7.86–7.88 (m, 1H), 7.61 (d, 1H, $J = 9.25$ Hz), 7.51–7.53 (m, 2H), 7.25 (s, 1H), 4.75 (s, 2H).

9,10-Dimethoxy-13-[3-(5-nitro-1*H*-indol-2-yl)benzyl]-5,6-dihydrobenzo[*g*]-1,3-benzodioxolo[5,6-*a*]quinolizinium Chloride (2)

8-Allyldihydroberberine[6] (39 mg, 0.10 mmol, 1.1. eq) and **8** (31 mg, 0.094 mmol, 1.0 eq) were added to a dry 5 mL screw-cap vial under Ar. Anhydrous CH_3CN (freshly distilled from CaH_2) (2.5 mL) was then added and the vial capped and stirred at 60 °C. After 24 h, an aliquot was removed from the reaction and RP-HPLC analysis showed clean enamine alkylation. The vial was then heated to 100 °C and the reaction monitored by RP-HPLC for 2 to 3 days until all of the alkylated enamine intermediate ($t_R = 28.0$ mins) had been consumed. The crude reaction was purified by preparative RP-HPLC to yield the mixed Cl^-/Br^- salt of **2** ($t_R = 22.5$ mins) after lyophilisation. The mixed salt was redissolved in MeOH and stirred at rt for 2 h with excess Amberlite IRA-904 quaternary ammonium Cl^- anion exchange resin. The resin was filtered and solvent removed *in vacuo* to yield 37 mg of **2** (45%) as a yellow amorphous solid: mp 220 °C (decomp.); ^{1}H NMR (DMSO-*d6*, 500 MHz) δ 12.79 (s, 1H), 10.08 (s, 1H), 8.48 (s, 1H), 8.06 (d, 1H, $J = 7.3$ Hz), 7.94 (d, 1H, $J = 7$ Hz), 7.88–7.79 (m, 3H), 7.53 (d, 1H, $J = 7$ Hz), 7.47 (m, 1H), 7.21–7.08 (m, 4H), 6.07 (s, 2H), 4.93 (s, 2H), 4.82 (s, 2H), 4.12 (s, 3H), 4.00 (s, 3H), 3.20 (s, 2H) ; ^{13}C NMR (DMSO-*d6*, 125 MHz) δ 150.1, 149.2, 146.3, 145.8, 144.2, 141.0, 140.3, 140.2, 137.5, 134.0, 132.7, 131.8, 129.9, 129.7, 128.0, 127.7, 127.6, 126.1, 125.0, 124.0, 121.8, 121.7, 120.2, 117.0, 116.9, 111.9, 108.7, 108.3, 101.9, 101.0, 61.8, 57.4, 57.3, 35.8, 27.4; ESI-TOF-MS m/z 586.1998 ($C_{35}H_{28}N_3O_6$ requires 586.1978).

9,10-Dimethoxy-13-[4-(5-nitro-1*H*-indol-2-yl)benzyl]-5,6-dihydrobenzo[*g*]-1,3-benzodioxolo[5,6-*a*]quinolizinium Chloride (3)

Method of preparation as for **2**: Yield = 40%, mp 230 °C (decomp.); ^{1}H NMR (DMF-*d7*, 500 MHz) δ 13.23 (br.s, 1H), 10.28 (s, 1H), 8.54 (s, 1H), 8.16 (d, 2H, $J = 7.5$ Hz), 7.96–8.00 (m, 2H), 7.70 (d, 2H, $J = 9$ Hz), 7.38 (d, 2H, $J = 7.5$ Hz), 7.25 (s, 1H), 7.20 (s, 1H), 7.13 (s, 1H), 6.15 (s, 2H), 5.13 (s, 2H), 4.92 (s, 2H), 4.19 (s, 3H), 4.11 (s, 3H), 3.34 (s, 2H); ^{13}C NMR (DMF-*d7*, 125 MHz) δ 156.3, 155.2, 152.6, 150.7, 147.5, 147.1, 146.7, 145.3, 143.6, 140.0, 138.9, 136.2, 134.7, 134.4, 134.1, 133.7, 132.2, 131.8, 127.6, 127.3, 126.3, 122.3, 122.2, 117.3, 114, 113.9, 107.9, 106.4, 67.3, 62.9, 62.2, 41.0, 33.1; ESI-TOF-MS m/z 586.1998 ($C_{35}H_{28}N_3O_6$ requires 586.1978).

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REFERENCES

1. Ball, A. R.; Casadei, G.; Samosorn, S.; Bremner, J. B.; Ausubel, F. M.; Moy, T. I.; Lewis, K. Conjugating berberine to a multidrug resistance pump inhibitor creates an effective antimicrobial. *ACS Chem. Biol.* **2006**, *1*, 594–600.
2. German, N.; Wei, P.; Kaatz, G. W.; Kerns, R. J. Synthesis and evaluation of fluoroquinolone derivatives as substrate based inhibitors of bacterial efflux pumps. *Eur. J. Med. Chem.* **2008**, *43*, 2453–2463.
3. Barbachyn, M. R. Recent advances in the discovery of hybrid antibacterial agents: *Annu. Rep. Med. Chem.* Macor, J. E. (Ed.), Elsevier Academic Press, San Diego, **2008**, *43*, 281–290.
4. Charifson, P. S.; Grillot, A. L.; Grossman, T. H.; Parsons, J. D.; Badia, M.; Bellon, S.; Deininger, D. D.; Drumm, J. E.; Gross, C. H.; LeTiran, A.; Liao, Y.; Mani, N.; Nicolau, D. P.; Perola, E.; Ronkin, S.; Shannon, D.; Swenson, L. L.; Tang, Q.; Tessier, P. R.; Tian, S. K.; Trudeau, M.; Wang, T.; Wei, Y.; Zhang, H.; Stamos, D. Novel dual-targeting benzimidazole urea inhibitors of DNA gyrase and topoisomerase IV possessing potent antibacterial activity: Intelligent design and evolution through the judicious use of structure-guided design and structure-activity relationships. *J. Med. Chem.* **2008**, *51*, 5243–5263.
5. Tomkiewicz, D.; Casadei, G.; Larkins-Ford, J.; Moy, T. I.; Garner, J.; Bremner, J. B.; Ausubel, F. M.; Lewis, K.; Kelso, M. J. Berberine-INF55 (5-nitro-2-phenylindole) hybrid antimicrobials: Effects of varying the relative orientation of the berberine and INF55 components. *Antimicrob. Agents Chemother.* **2010**, *54*, 3219–3224.
6. Bremner, J. B.; Samosorn, S. 8-Allyldihydroberberine as an alternative precursor for the synthesis of 13-substituted berberine derivatives. *Aust. J. Chem.* **2003**, *56*, 871–873.
7. Samosorn, S.; Bremner, J. B.; Ball, A.; Lewis, K. Synthesis of functionalized 2-aryl-5-nitro-1*H*-indoles and their activity as bacterial NorA effux pump inhibitors. *Bioorg. Med. Chem.* **2006**, *14*, 857–865.
8. Cacchi, S.; Fabrazi, G. Synthesis and functionalization of indoles through palladium-catalyzed reactions. *Chem. Rev.* **2005**, *105*, 2873–2920.
9. Leboho, T. C.; Michael, J. P.; van Otterloo, W. A. L.; van Vuuren, S. F.; de Koning, C. B. The synthesis of 2- and 3-aryl indoles and 1,3,4,5-tetrahydropyrano[4,3-*b*]indoles and their antibacterial and antifungal activity. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4948–4951.
10. Wang, X.; Lane, B. S.; Sames, D. Direct C-arylation of free (NH)-indoles and pyrroles catalyzed by Ar-Rh(III) complexes assembled in situ. *J. Am. Chem. Soc.* **2005**, *127*, 4996–4997.
11. Ambrus, J. I.; Kelso, M. J.; Bremner, J. B.; Ball, A. R.; Casadei, G.; Lewis, K. Structure-activity relationships of 2-aryl-1*H*-indole inhibitors of the NorA efflux pump in *Staphylococcus aureus*. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4294–4297.
12. Noland, W. E.; Rush, K. R.; Smith, L. R. Nitration of indoles. IV. The nitration of 2-phenylindole. *J. Org. Chem.* **1966**, *31*, 65–69.
13. Campo, M. A.; Larock, R. C. Synthesis of fluoren-9-ones via palladium-catalyzed cyclocarbonylation of *o*-halobiaryls. *Org. Lett.* **2000**, *2*, 3675–3677.