Synthesis of a Calothrixin B Isomer with a Novel 7*H*-Indolo[2,3-*j*]phenanthridine-7,13(8*H*)-dione Structure

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Abstract: Synthesis of an N-protected 2-bromo-1*H*-carbazole-1,4(9*H*)-dione is reported for the first time. We took full advantage of the high polarization and electrophilic properties of this new compound to engage it in a regioselective hetero-Diels–Alder reaction. The resultant cycloadduct was next transformed to an isomer of the naturally occurring calothrixin B displaying a new 7*H*-indolo[2,3-*j*]phenanthridine-7,13(*8H*)-dione structure.

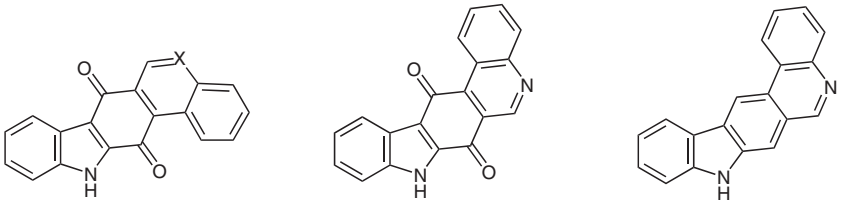
Key words: 2-bromo-1*H*-carbazole-1,4(9*H*)-dione, hetero-Diels–Alder reaction, 7*H*-indolo[2,3-*j*]phenanthridine-7,13(8*H*)-dione, calothrixin B isomer

Calothrixins A and B (**1a**, **1b**; Figure 1) were first reported in 1999 as antiproliferative agents from the strains of *Calothrix cyanobacterium* (a blue-green alga) and shown to have an indolo[3,2-*j*]phenanthridine core structure on the basis of their spectroscopic data.[1] Owing to their biological properties and their unusual structure, there has been much interest in these alkaloids and several total syntheses have recently appeared.[2] As part of an ongoing research project in the quest for novel analogues to improve the spectrum of activity of these alkaloids, we became interested in the synthesis of the calothrixin B isomer **2** which features a 7*H*-indolo[2,3-*j*]phenanthridine-7,13(8*H*)-dione structure. To the best of our knowledge such a structure has not yet been synthesized; however, two examples of the preparation of the 12*H*-indolo[3,2-*j*]phenanthridine ring **3** have been already reported, one based on an electrocyclization reaction,[3] the other on a Diels–Alder cycloaddition–lactamization sequence.[4] It may be added that the same motif can be found embedded in a structurally more complex orange dye.[5]

The most salient feature of our calothrixin B synthesis[2d,g] was a fully regioselective hetero-Diels–Alder cycloaddition between a 3-bromo-1*H*-carbazole-1,4(9*H*)-dione **4** and the 'push–pull' diene **5**. By analogy, we thus thought that a similar strategy based on the cycloaddition reaction of a 2-bromo-1*H*-carbazole-1,4(9*H*)-dione **6** with diene **5** would be well suited to reach the targeted calothrixin isomer **2**. Herein, we describe the synthesis of the yet unknown dienophiles **6a** and **6b** and the transformation of **6b** to **2** following the retrosynthetic plan shown in Scheme 1.

Our initial approach to **2** is outlined in Scheme 2, where a benzyl protecting group had been chosen to temporarily mask the indole nitrogen atom. As shown in Scheme 2, compound **6a** was easily prepared from the 9-benzyl-9*H*-carbazol-4-ol (**8**), itself prepared in a three-step sequence from the commercially available carbazole (**7**).[2d] Its reaction with diene **5** led to the expected addition product **10** in 73% yield. Although the transformation of this adduct to compound **13** could be accomplished uneventfully following a route, which, at the same time, proved successful to prepare the *N*-benzyl-protected calothrixine B,[2d] efforts to deprotect the indole nitrogen atom to give **2** in practical yield remained unfruitful.

Because of this failure, we were led to reconsider the choice of the indole nitrogen protecting group and we



1a calothrixin A (X = NO)
1b calothrixin B (X = N)

2 **3**

Figure 1

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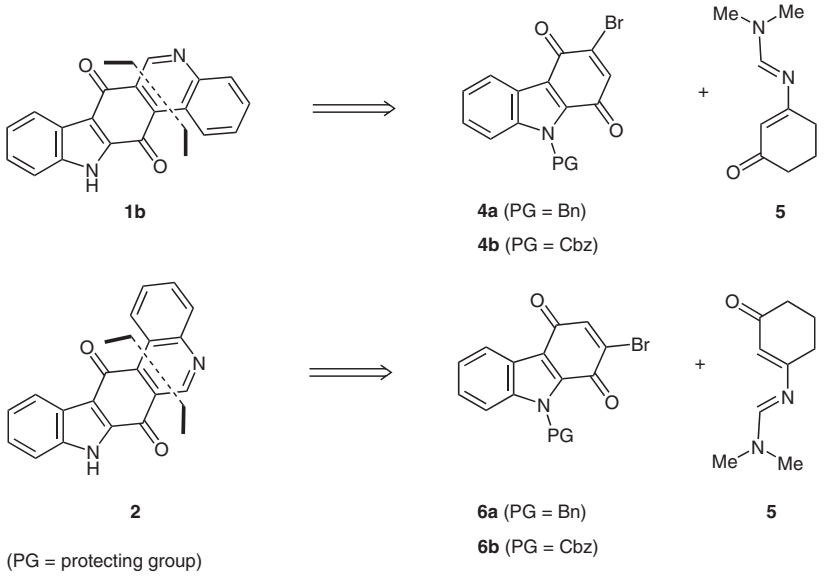
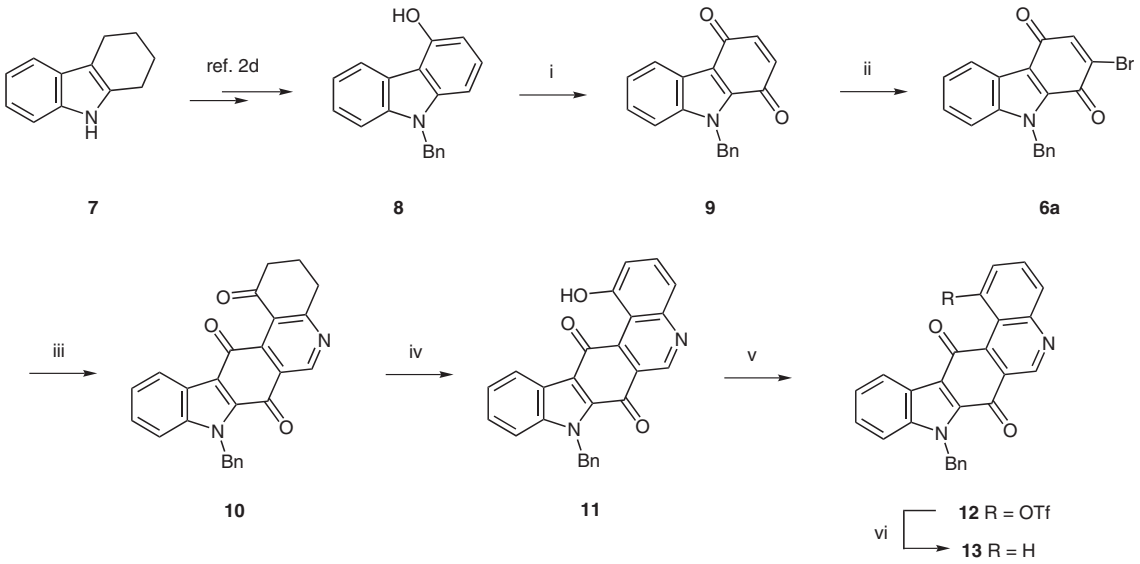


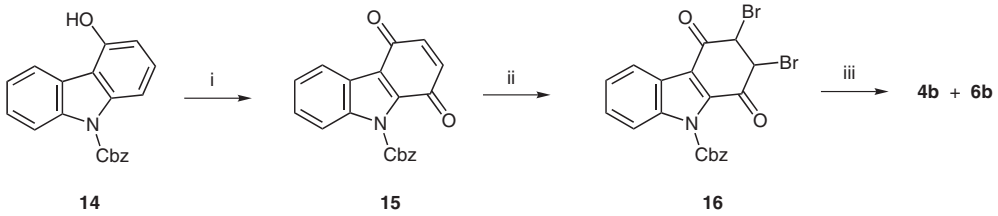
Scheme 1



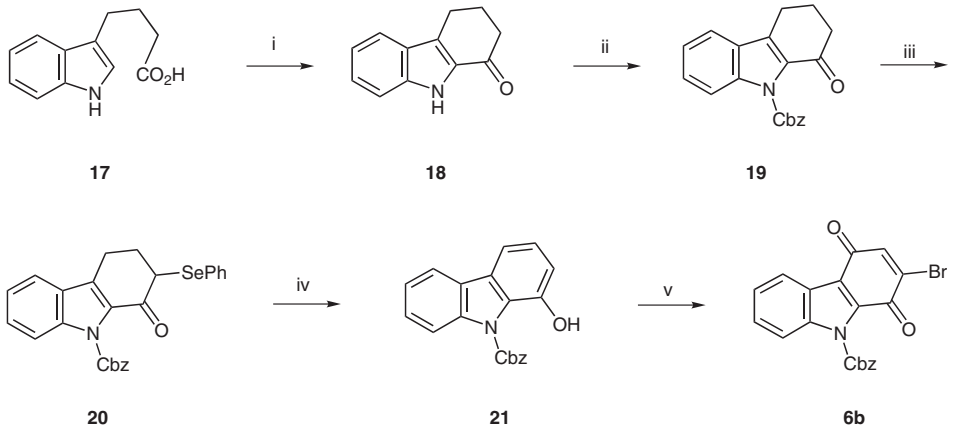
Scheme 2 *Reagents and conditions*: (i) $PhI(OCOCF_3)_2$ (3 equiv), MeCN–H_2O, 2 h, 40 °C, 75%; (ii) Br_2, AcOH, 20 min, 20 °C then ice-water; crude solid taken up in EtOH, 1 h, reflux, 70%; (iii) **6a** + **5** (1 equiv each), MeCN, 40 °C, 48 h, 73%; (iv) 10% Pd/C (0.25 equiv), PhOPh, 1 h, reflux, 73%; (v) TfOTf (1.2 equiv), Et_3N (5 equiv), CH_2Cl_2, 1 h, –78 °C; (vi) $Pd(PPh_3)_4$ (0.04 equiv), Et_3N (4 equiv), HCO_2H (2.7 equiv), dioxane, reflux, 30 min, 81% (two steps).

thought that a more labile Cbz group could be a useful alternative to the initially chosen benzyl group. The synthesis of the new key intermediate **6b** was first envisaged following a reaction sequence similar to that we used for the preparation of **6a** (Scheme 3). Thus, the benzyl 4-hydroxy-9*H*-carbazole-9-carboxylate (**14**), prepared from carbazole (**7**),[2g] was first oxidized to the benzyl 1,4-dioxo-1,4-dihydro-9*H*-carbazole-9-carboxylate (**15**) by exposure to phenyliodoso trifluoroacetate in aqueous acetonitrile. Bromination of **15** led to the expected formation of the dibromo adduct **16**. However, and in sharp contrast with its *N*-benzyl analogue, subsequent efforts to achieve a regioselective β-elimination of HBr met with little success. Indeed, irrespective of the conditions used to effect this transformation, all attempts led to mixtures of **4b** and **6b** in ratios ranging from 1:1 to ca. 1:2.[6]

Consequently, we turned our attention to an alternative strategy involving bromination of the *N*-Cbz-protected 9*H*-carbazol-1-ol **21**. Scheme 4 outlines our successful approach to this compound. First of all, *N*-Cbz protection of the 2,3,4,9-tetrahydro-1*H*-carbazol-1-one (**18**), efficiently prepared from 4-(1*H*-indol-3-yl)butanoic acid (**17**),[7] afforded compound **19** which was next fully aromatized following a two-step procedure to give the required **21** via the seleno intermediate **20**. Finally, when submitted to the Grunwell reaction conditions,[8] **21** led to the required **6b**[9] (33% overall yield) whose structure was fully ascertained by a single crystal X-ray analysis[10] (Figure 2).



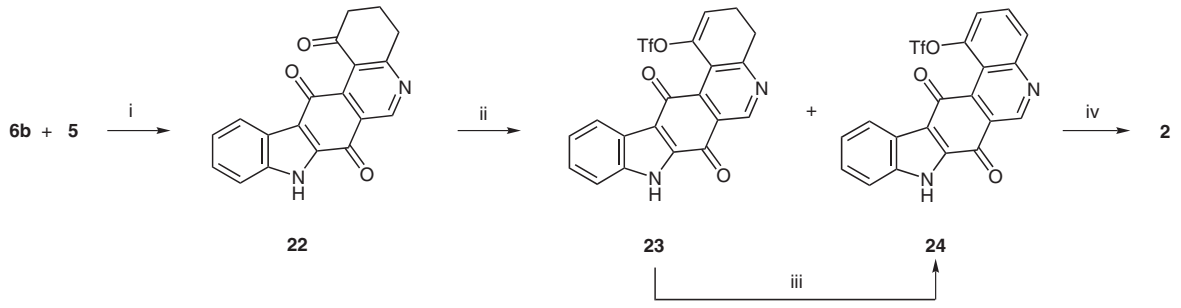
Scheme 3 *Reagents and conditions*: (i) $PhI(OCOCF_3)_2$ (3 equiv), $MeCN–H_2O$, 1 h, 20 °C, 84%; (ii) Br_2 (1 equiv), AcOH, 20 min, 20 °C then ice-water and filtration of the precipitate (→ **16**), quant.; (iii) see ref. 6.



Scheme 4 *Reagents and conditions*: (i) PPA (15 equiv), toluene, reflux, 5 h, 98%; (ii) **18**, NaH (1.3 equiv), DMF, 0 °C, 1 h then $ClCO_2Bn$, 1 h, 20 °C, 90%; (iii) **19**, THF, –78 °C then LiHMDS (1.3 equiv), 15 min, then PhSeCl (1.2 equiv) in THF, –78 °C; (iv) crude **20**, CH_2Cl_2, 0 °C then H_2O_2 (3 equiv), 30 min, 65% (two steps); (v) NBS (5 equiv), $AcOH–H_2O$ (1:2), 70 °C, then **21** in AcOH, 70 °C, 1.5 h, 60%.

Proceeding on to reach the targeted **2** we next reacted **6b** with diene **5**. After heating in chloroform at 50 °C for 18 hours, the 3,4-dihydro-2*H*-indolo[2,3-*j*]phenanthridine-1,7,13(8*H*)-trione (**22**)[11–14] could be isolated in 70% yield (Scheme 5). It is noteworthy that cleavage of the *N*-Cbz protecting group occurred in the course of the reaction.[15] The final steps of the synthesis were accomplished as shown in Scheme 5. Chemoselective O-triflation provided enol triflate **23** along with phenol triflate **24**. Treatment of this mixture with DDQ afforded **24** in 55% overall yield. Finally, reduction of triflate **24**, following the procedure reported by Cacchi et al.,[16] provided the calothrixin isomer **2**.[17]

In conclusion, a synthesis of a calothrixin B isomer, featuring a new 7*H*-indolo[2,3-*j*]phenanthridine-7,13(8*H*)-dione structure, has been achieved in 11 steps and in ca. 5.5% global yield. The most salient features of this synthesis include a hetero-Diels–Alder reaction to assemble the five-ring framework of the molecule and the preparation and use of a new 2-bromo-1*H*-carbazole-1,4(9*H*)-dione dienophile protected by an easily removed *N*-Cbz group. Preliminary evaluation of in vitro cytotoxic effects against MCF-7 and KB human cancer cell lines were realized for compounds **10** and **13**. It is interesting to note that compound **13** displays an activity of the same order as that showed by its *N*-benzyl calothrixin B isomer ($IC_{50} \approx 0.1$ μM) and that both **10** and **13** display a similar activity. Moreover, compound **10** and its N-debenzylated analogue are equally cytotoxic, the same being true for the corresponding calothrixin B synthetic intermediates. It thus appears that deprotection of the indole nitrogen and full aromatization of the Diels–Alder adducts are not key transformations to get significant cytotoxicities.



Scheme 5 *Reagents and conditions*: (i) **5** (1.5 equiv), $CHCl_3$, 20 °C, then **6b** in $CHCl_3$, 50 °C, 18 h, 70%; (ii) **22**, THF–HMPA (2.5:1), –78 °C, LiHMDS (2.5 equiv), 1 h, then $PhNTf_2$ (1.5 equiv) in THF, 1 h, –78 °C; (iii) **23** + **24**, DDQ (2 equiv), dioxane, reflux, 2 h, 55% (two steps); (iv) **24**, dioxane, 20 °C, then Et_3N (4 equiv), $Pd(PPh_3)_4$ (0.04 equiv), HCO_2H (2.7 equiv), reflux, 4 h, 50%.

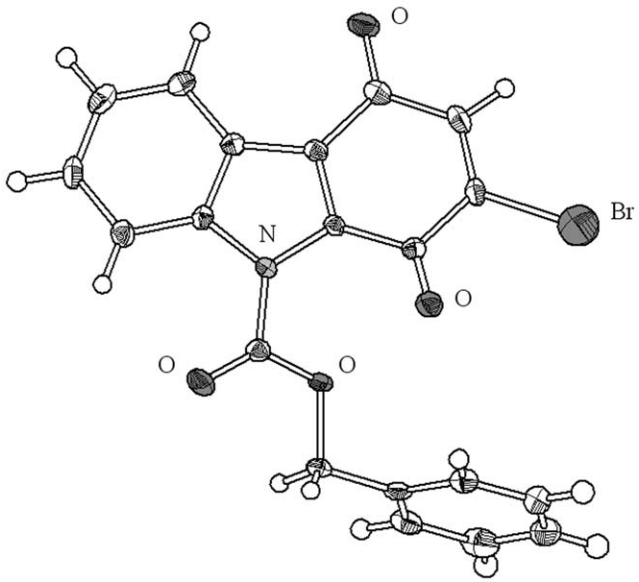


Figure 2

Acknowledgment

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References and Notes

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(6) Conditions tested from **16** for HBr β-elimination: EtOH, reflux, 1.5 h (**4b**:**6b** = 1.5:1); $CHCl_3$, $MgBr_2$ (1 equiv), 40 °C, 4 h (**4b**:**6b** = 2:1); SiO_2 (**4b**:**6b** = 1:1); EtOH, $BF_3{\cdot}Et_2O$, 55 °C, 2 h (**4b**:**6b** = 1:1.5).

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(9) **Benzyl 2-Bromo-1,4-dioxo-1*H*-carbazole-9(4*H*)-carboxylate (6b)**: orange solid; mp 148 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.53 (s, 2 H, OCH_2Ph), 7.23 (s, 1 H, H-3), 7.30–7.70 (m, 7 H, H_{Ar}), 7.99 (d, *J* = 8.4 Hz, 1 H, H-8), 8.28 (d, *J* = 7.5 Hz, 1 H, H-5). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 71.0 (OCH_2Ph), 114.5 (C-3), 122.8 (quaternary C), 123.2 (quaternary C), 123.4 (CH_{Ar}), 126.1 (CH_{Ar}), 128.8 (2 × CH_{Ar}), 129.2 (3 × CH_{Ar}), 129.7 (CH_{Ar}), 133.6 (quaternary C), 133.8 (quaternary C), 137.6 (quaternary C + CH_{Ar}), 138.8 (quaternary C), 149.9 (NCO), 170.2 (C-1), 181.2 (C-4). FT-IR (KBr): 3300, 3054, 1755, 1682, 1654 cm^{-1}. MS (CI, NH_3): *m*/*z* (%) = 427 [M + NH_4^+, ^{79}Br], 410 [M + H^+, ^{79}Br], 366, 332, 145, 91. HRMS (EI): *m*/*z* calcd for $C_{20}H_{12}BrNO_4$: 408.9950; found: 408.9954.

(10) $C_{20}H_{12}BrNO_4$: The data set was collected on a Nonius-Bruker Kappa CCD diffractometer, using the Mo–$KL_{2,3}$ radiation. $C_{20}H_{12}BrNO_4$ (M = 410.2): triclinic, space group $P\bar{1}$, D_c = 1.660 g cm^{-3}, a = 7.1218(3), b = 9.6798(4), c = 13.2182(7) Å, α = 72.048(3)°, β = 84.294(6)°, γ = 71.184(5)°, V = 820.54(7) $Å^3$, Z = 2, λ = 0.71069 Å, μ = 2.532 mm^{-1}, T = 150 K, $R(F^2)$ = 0.0547 for 3460 observed reflections [I > 2 σ(I)] and $R_w(F^2)$ = 0.1183 for all 4692 reflections. The data have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 654172.

(11) The orientation of addition of **6b** to **5** is governed by the bromo substituent. We have already observed such an effect in previous works.[12] First observations were reported by Cameron et al.[13]

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(14) **3,4-Dihydro-2*H*-indolo[2,3-*j*]phenanthridine-1,7,13 (8*H*)-trione (22)**: To a stirred solution of diene **5** (250 mg, 1.47 mmol) in anhydrous $CHCl_3$ (5 mL) at r.t was added a solution of dienophile **6b** (400 mg, 0.98 mmol) in anhydrous $CHCl_3$ (8 mL). After the mixture was heated at 50 °C for 18 h, it was cooled to r.t. and concentrated in vacuo. The residue was purified by column chromatography on silica gel (elution with EtOAc–hexanes, 1:1) to give the cycloadduct **22** (215 mg, 0.68 mmol, 70%) as an orange-red solid: mp >300 °C ($CHCl_3$–hexanes) (dec.). ^{1}H NMR (300 MHz, DMSO): δ = 2.20 (quint, *J* = 6.3 Hz, 2 H, H-3), 2.91 (t, *J* = 6.6 Hz, 2 H, H-2), 3.12 (t, *J* = 6.0 Hz, 2 H, H-4), 7.42 (t, *J* = 7.5 Hz, 1 H, H-10 or H-11), 7.52 (t, *J* = 7.5 Hz, 1 H, H-11 or H-10), 7.65 (d, *J* = 8.4 Hz, 1 H, H-9), 8.12 (d, *J* = 7.8 Hz, 1 H, H-12), 9.20 (s, 1 H, H-6), 13.27 (br s, 1 H, NH). ^{13}C NMR (75 MHz, DMSO): δ = 21.5 (C-3), 33.0 (C-4), 39.4 (C-2), 114.6 (C-9), 118.7 (quaternary C), 122.8 (CH_{Ar}), 124.2 (quaternary C), 124.8 (CH_{Ar}), 126.7 (quaternary C), 128.0 (CH_{Ar}), 129.8 (quaternary C), 136.7 (quaternary C), 139.0 (quaternary C), 141.4 (quaternary C), 149.3 (C-6), 169.2 (C-7), 176.7 (C-4a), 179.0 (C-13), 199.0 (C-1). FT-IR (KBr): 3287, 2954, 1685, 1655, 1653 cm^{-1}. MS (EI, 70 eV): *m*/*z* (%) = 316 (60) [M^+], 288 (100), 260 (21), 232 (11), 203 (24), 177 (24), 115 (24). HRMS (EI): *m*/*z* calcd for $C_{19}H_{12}N_2O_3$: 316.0848; found: 316.0849.
Compound **22** and its regioisomer, arising from the cycloaddition of **4b** to diene **5** (a precursor of calothrixin B),[2g] display distinguishable NMR signals (Δδ = 0.06 ppm).

(15) Isolation of benzyl dimethylcarbamate, next to **22**, demonstrates that the N–Cbz bond cleavage resulted from the attack of $Me_2NH{\cdot}HBr$ generated in the course of the Diels–Alder primary adduct aromatization process.

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(17) **8*H*-Indolo[2,3-*j*]phenanthridine-7,13-dione (2)**: red solid; mp >350 °C. ^{1}H NMR (300 MHz, DMSO): δ = 7.35 (t, *J* = 7.2 Hz, 1 H, H_{Ar}), 7.43 (t, *J* = 7.2 Hz, 1 H, H_{Ar}), 7.56 (d, *J* = 8.4 Hz, 1 H, H_{Ar}), 7.81 (t, *J* = 7.6 Hz, 1 H, H_{Ar}), 7.91 (t, *J* = 7.6 Hz, 1 H, H_{Ar}), 8.10 (d, *J* = 8.4 Hz, 1 H, H_{Ar}), 8.20 (d, *J* = 8.0 Hz, 1 H, H_{Ar}), 9.50 (s, 1 H, H-6), 9.65 (d, *J* = 8.4 Hz, 1 H, H_{Ar}), 13.10 (br s, 1 H, NH). ^{13}C NMR (75 MHz, DMSO): δ = 114.0 (CH_{Ar}), 118.5 (quaternary C), 122.3 (CH_{Ar}), 123.0 (quaternary C), 124.0 (2 × quaternary C), 124.3 (CH_{Ar}), 127.1 (CH_{Ar}), 127.9 (CH_{Ar}), 129.7 (CH_{Ar}), 129.9 (CH_{Ar}), 131.8 (CH_{Ar}), 134.1 (quaternary C), 135.4 (quaternary C), 138.4 (quaternary C), 146.9 (CH_{Ar}), 151.8 (quaternary C), 177.6 (C-7), 186.7 (C-13). FT-IR (KBr): 3444, 1659, 1535 cm^{-1}. MS (EI, 70 eV): *m*/*z* (%) = 298 (100) [M$^+$], 270 (55), 242 (26), 241 (26), 214 (38). HRMS (EI): *m*/*z* calcd for $C_{19}H_{10}N_2O_2$: 298.0742; found: 298.0743.