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Synthesis of hybrids between the alkaloids rutaecarpine and luotonins A, B

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ABSTRACT

The synthesis of 7,12-dihydroindolo[2′,3′:3,4]pyrrolo[2,1-*b*]quinazolin-5-one, a hybrid compound containing common structural features of the natural alkaloids rutaecarpine (*Evodia rutaecarpa*) and luotonin A (*Peganum nigellastrum*), was performed by active methylene group transformations of deoxyvasicinone. The synthesis of 7-hydroxy-8-norrutaecarpine was accomplished via the first total synthesis of bouchardatine (*Bouchardatia neurococca*) and its acid-catalyzed ring closure. The synthesized alkaloid analogues are the first representatives of a new heterocyclic ring system. Preliminary testing of the synthesized compounds showed cytotoxic activities against HeLa cells and apoptosis inducing effects at a concentration comparable to that of the control drug, etoposide.

In search of new polyheterocyclic systems of potential pharmacological value, we prepared new pentacyclic compounds by fusing pyrroloquinazolone and indole rings. Compounds **1** (Fig. 1), described in this Letter, are structurally close to natural alkaloids such as rutaecarpine **2** and the luotonins **3** which have shown antitumour activities.[1–3]

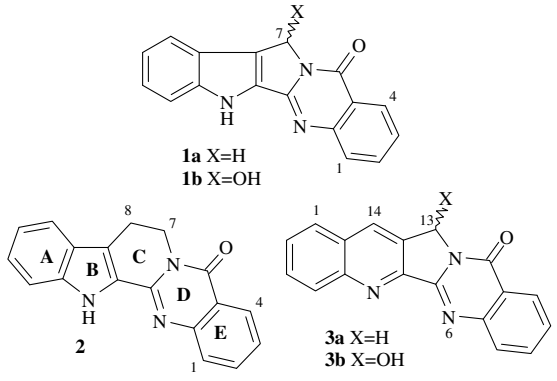

Figure 1. Structure of 8-norrutaecarpine **1a**, 7-hydroxy-8-norrutaecarpine **1b**, rutaecarpine **2**, luotonin A **3a** and luotonin B **3b**.

Rutaecarpine-type alkaloids constitute an important class of indolopyridoquinazolinone heterocycles, which belong to the subgroup of quinazoline-type alkaloids isolated from both the heartwood and the fruit of numerous plants and trees of the *Rutaceae* family.[3] Their extracts have long been used as important remedies in Chinese traditional medicine for the treatment of various diseases.[4] The Chinese herbal drug, Wu-Chu-Yu, the dried unripe fruit of *Evodia rutaecarpa*, showed remarkable activities against headache, cholera, dysentery, worm infestations and post partum disturbances.[5] Rutaecarpine **2** is a major quinazolinocarboline alkaloid isolated from *E. rutaecarpa* and shows a variety of pharmacological activities including antithrombotic, vasorelaxant and cyclooxygenase (COX-2) inhibitory effects.[6] In 2004, rutaecarpine was described as an antihypertensive drug which activates the vanilloid receptor (VR-1).[7] Various total syntheses of **2** have been reported[8] including our original synthetic approaches.[9]

Luotonins A and B, **3a,b**, are recently isolated members of a new alkaloid family extracted from the aerial parts of *Peganum nigellastrum* which contain a pyrroloquinazolinoquinoline skeleton.[10] The plant extracts are used in traditional Chinese medicine and are reported to exhibit activity against a range of ailments including rheumatism, inflammation, influenza, hepatitis and leukaemia.[11] Luotonin A seems to be very promising as a campthotecin-like antitumour agent selectively inhibiting human DNA topoisomerase I.[12] In the past decade, luotonin A has become an important synthetic target and a variety of preparative methods have been published.[13] The structural similarity between rutaecarpine **2** and luotonins **3a,b** is remarkable and both alkaloids exhibit

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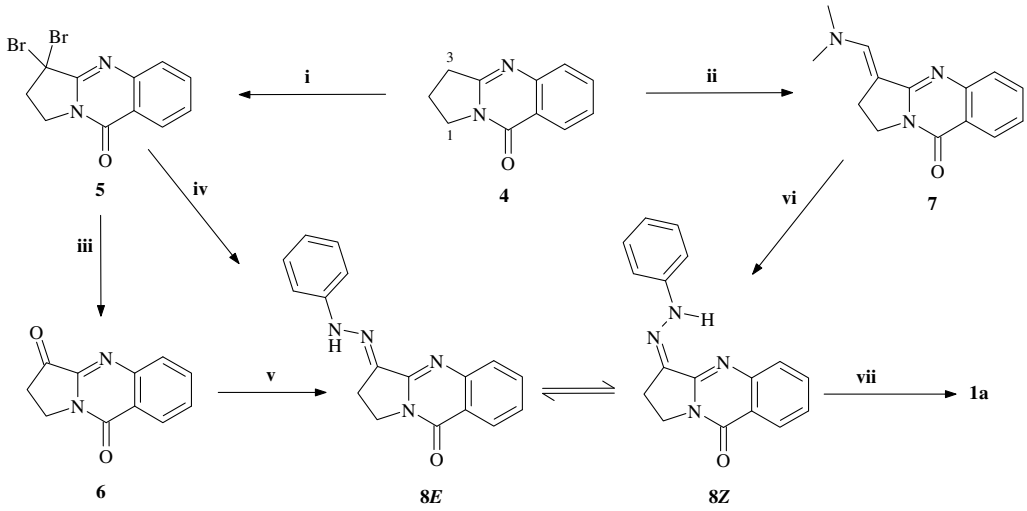
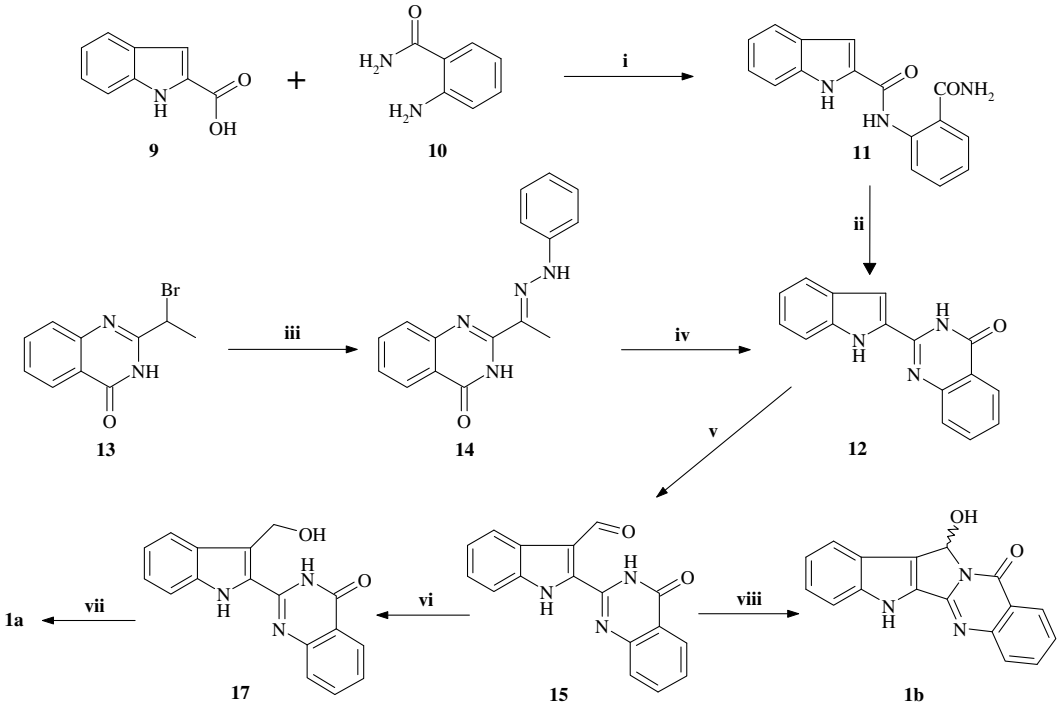
topoisomerase inhibitory activity. An obvious difference between the two compounds is the flexibility of the six-membered C-ring of rutaecarpine compared to the rigidity of the five-membered C-ring of luotonin, which was postulated to be essential for biological activity.[2]

Deoxyvasicinone **4**, a natural alkaloid of *Peganum harmala*, is readily available by cyclocondensation of 2-methoxy-3,4-dihydro-2*H*-pyrrole and anthranilic acid.[14] Compound **4** is weakly active towards electrophilic reagents at the C3 carbon atom. Bromination of **4** was carried out with bromine in the presence of sodium acetate in 75% acetic acid solution at 60 °C, producing the 3,3-dibromo derivative **5** in 82% yield (Scheme 1). Substitution of **5** with excess of 25% aqueous hydrazine hydrate solution in ethanol (60 °C, 2 h) led to the 3-oxo derivative **6** in 79% yield. A small-scale method was described to produce **6** directly from **4** via SeO_2 oxidation in inert solution in moderate yield.[15] Nucleophilic substi-



Scheme 1. Reagents and conditions: (i) 2 equiv Br_2, 75% AcOH, CH_3COONa, 60 °C (82% yield); (ii) dimethylformamide, 2 equiv $POCl_3$, rt, 1 h, 60 °C 3 h (94% yield); (iii) 25% $NH_2NH_2{\cdot}H_2O$, 60 °C, 2 h (79% yield); (iv) Ph-$NHNH_2$, EtOH, 4 h reflux (89% yield); (v) Ph-$NHNH_2$, EtOH, 6 h reflux (95% yield); (vi) Ph-$N_2^+Cl^-$, AcOH/H_2O, 0 °C 3 h (96% yield); (vii) polyphosphoric acid, 180 °C, 20 min (67% yield).



Scheme 2. Reagents and conditions: (i) DCCI, THF, 20 °C, 12 h (78% yield); (ii) Na, ethylene glycol, 180 °C, 30 min (93% yield); (iii) 3 equiv $PhNHNH_2$, EtOH, 80 °C, 3 h (92% yield); (iv) polyphosphoric acid, 180 °C, 20 min (84% yield); (v) DMF, $POCl_3$, 0 °C, 24 h (89% yield); (vi) $NaBH_4$, MeOH, 20 °C, 2 h (95% yield); (vii) 30% H_2SO_4, EtOH, reflux, 3 h (67% yield); (viii) 30% H_2SO_4, EtOH, reflux, 1 h (72% yield).

tution of **5** or condensation of 3-ketone **6** with excess phenyl hydrazine in ethanol afforded 3-phenylhydrazone **8** in excellent yield (89% and 95%, respectively).

Vilsmeier–Haack formylation of **4** using 2 equiv of phosphoryl chloride in dimethylformamide at 60 °C for 3 h gave the 3-dimethylaminomethylene derivative **7** in 94% yield. Japp-Klingemann reaction of **7** with phenyldiazonium chloride[16] in acetic acid solution (0 °C, 3 h) resulted in phenylhydrazone derivative **8** in 96% yield. Compound **8** exhibits solvent dependent *E–Z* geometric isomerism, as indicated by the NMR spectra. In DMSO-d_6 the sterically more favoured *E* form is dominant, due to an intermolecular hydrogen bond between the amino group and the solvent molecules. In DMSO-d_6 the *E–Z* ratio is 55:45 at rt.[17] These facts indicate a low activation energy for isomerization of the exocyclic C(3)=N double bond.[18]

Fischer indolization of compound **8** in polyphosphoric acid gave 8-norrutaecarpine **1a**. The crude product was purified by flash chromatography[19] on silica gel to give **1a** in good yield. The resulting compound, 7,12-dihydroindolo[2′,3′:3,4]pyrrolo[2,1-*b*]quinazolin-5-one **1a**, which can also be named as 8-norrutaecarpine or 14-norluotonin A constitutes a new pentacyclic ring system, which contains common structural features of the two bioactive natural alkaloids.

An alternative synthetic approach was elaborated for the preparation of the pentacyclic ring system starting from anthranylamide **10** (Scheme 2). Condensation of **10** with indole-2-carboxylic acid **9** using DCCI in tetrahydrofuran solution and subsequent base-catalyzed cyclocondensation provided the indolylquinazolone derivative **12**. The same product **12** was also available by reaction of 2-bromoethylquinazolone **13** with 3 equiv of phenylhydrazine and indolization of the obtained phenylhydrazone **14** in polyphosphoric acid at 180 °C. Vilsmeier–Haack formylation of **12** using a slight excess of phosphoryl chloride in dimethylformamide under mild conditions (0 °C, 24 h) provided the 3-formylindole derivative **15** in almost quantitative yield.

The prepared compound **15** is identical with bouchardatine, the recently isolated natural alkaloid, from the aerial parts of *Bouchardatia neurococca* (*Rutaceae*).[20] This procedure is also the first total synthesis of the indolylquinazolone alkaloid **15**.[21] Conversion of the tetracyclic compound **15** into the pentacyclic system was performed using an analogous literature method to that applied in the synthesis of luotonin.[22] Reduction of the formyl group in **15** with sodium borohydride in methanol and acid-catalyzed ring closure of the obtained 3-hydroxymethyl indole derivative **17** with 30% sulfuric acid provided 8-norrutaecarpine **1a**. Similarly, acid-catalyzed ring closure of **15** led to 7-hydroxy-8-norrutaecarpine **1b**.

Preliminary apoptotic studies were performed as follows: To test the effects of 8-norrutaecarpine **1a**, bouchardatine **15** and rutaecarpine **2**, HeLa cells were incubated with 10^{-6} mol/l of the compounds for 72 h and nucleosomal DNA fragmentation, a marker of apoptotic cell death, was analyzed by flow cytometry. The percent of apoptotic cells corresponding to the sub-G_1 phase was found to be 38.6 ± 3.3 with bouchardatine **15**, 24.1 ± 3.8 with 8-norrutaecarpine **1a** and 14.5 ± 2.8 with rutaecarpine **2**. Cells were incubated with etoposide as a positive control, and 15.4 ± 1.9 percent were observed to undergo apoptosis.

In conclusion, we have synthesized a new pentacyclic indolopyrroloquinazoline ring system. Facile alternative preparative procedures have been carried out from readily available starting materials. In all these methods, the synthetic strategy involved construction of the BC ring and/or to build the connection between the B and D rings from substituted quinazoline derivatives.

Unfortunately, the low solubility of hybrid compounds **1** is a limitation preventing correct estimation of their biological activity. The preparation of various substituted derivatives that may exhibit improved solubility (which will then allow their biological evaluation) is in progress and will be described later.

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17. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.00 (t, *J* = 7.21 Hz, 2H), 4.19 (t, *J* = 7.21 Hz, 2H), 6.90 (t, *J* = 6.63 Hz, 1H), 7.31 (m, 2H, overlap), 7.32 (m, 2H, overlap), 7.48 (t, *J* = 7.80 Hz, 1H), 7.75 (d, *J* = 7.82 Hz, 1H), 7.80 (td, *J* = 1.24, 7.81 Hz, 1*H*), 8.14, (d, *J* = 7.82 Hz, 1H), (δ N*H* 10.03 and 11.96), *E*:*Z* ratio: 55:45.
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19. Preparation of 8-norrutaecarpine **1a**: 3.3 mmol of **8** was added to polyphosphoric acid (10.0 g, Fluka) at 180 °C and the reaction mixture was stirred for 30 min. Cold water (100 ml) was added and the aqueous mixture was stirred for 1 h. The precipitated crystals were filtered off, treated with 10% NaOH solution and water. The resulting dried dark-green crude solid was chromatographed on Kieselgel 60 (Merck) (230–400 mesh ASTM) solid phase, eluting with CH_2Cl_2/EtOAc (4:1). The middle fractions gave a pale yellow solid in 67% yield, mp: 305 °C. UV: in EtOH λ_{max} (logε): 349.2 (4.444), 333.2 (4.538), 319.6 (4.490), 239.2 (4.442), 226 (4.453), 202.8 (4.481) nm. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.12 (s, 2H), 7.19 (ddd, *J* = 0.80, 7.13, 7.92 Hz, 1H), 7.34 (ddd, *J* = 1.07, 7.03, 8.17 Hz, 1H), 7.52 (td, *J* = 0.72, 8.26 Hz, 2H), 7.74 (d, *J* = 7.64 Hz, 1H), 7.80 (d, *J* = 7.99 Hz, 1H), 7.85 (ddd, *J* = 1.55, 7.15, 8.28 Hz, 1H), 8.24 (dd, *J* = 1.31, 7.80 Hz, 1H), 12.41 (s, 1H, N*H*). ^{13}C NMR (150 MHz, DMSO-d_6) δ 45.7, 113.3, 119.7, 120.4, 120.5, 121.5, 124.9, 125.0, 125.8, 126.0, 126.7, 133.7, 134.2, 142.4, 149.0, 149.1, 159.6. HRMS (ESI): calcd for $(M+H)^+$ ($C_{17}H_{12}N_3O$) requires *m*/*z* 274.2967, found 274.2959.

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21. Preparation of bouchardatine **15**: 1.71 ml (18 mmol) of phosphoryl chloride (Aldrich) was dissolved in 9 ml of anhydrous dimethyl formamide at 0 °C. 0.522 g (2 mmol) of 2-(2-indolyl)-quinazolone **12** was dissolved in 13 ml of dimethyl formamide and added dropwise to the first solution. The mixture was stirred at 0 °C for 24 h, then the solution was added dropwise to 15 ml of saturated sodium hydrogencarbonate solution. Ten millilitres of 10% sodium hydroxide solution were poured onto the mixture, and the precipitated solid was filtered off and washed with water. Recrystallization from ethanol provided a beige solid in 89% yield, mp: over 260 °C. UV: in EtOH λ_{max} ($\log\varepsilon$): 391.5 (4.035), 371.0 (4.179), 262.5 (4.105), 231.5 (4.073) nm. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.35 (ddd, J = 1.10, 7.10, 8.18 Hz, 1H), 7.43 (ddd, J = 1.08, 7.02, 8.18 Hz, 1H), 7.61 (ddd, J = 1.53, 7.11, 8.47 Hz, 1H), 7.69 (d, J = 8.19 Hz, 1H), 7.86 (d, J = 7.65 Hz, 1H), 7.92 (ddd, J = 1.53, 7.18, 8.39 Hz, 1H), 8.22 (dd, J = 1.24, 7.61 Hz, 1H), 8.27 (d, J = 8.14 Hz, 1H), 10.43 (s, 1H), 13.12 (s, 1H, indole N*H*), 13.63 (s, 1H, amide N*H*). ^{13}C NMR (150 MHz, DMSO-d_6) δ 113.3, 115.1, 120.2, 121.8, 123.3, 125.4, 125.5, 126.1, 127.4, 127.6, 134.9, 135.8, 135.8, 145.3, 148.3, 161.1, 187.5. HRMS (ESI): calcd for $(M+H)^+$ ($C_{17}H_{12}N_3O_2$) requires m/z 290.2961, found 290.2948.
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