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Antifolate chemistry: synthesis of 4-{*N*-[(6*RS*)-2-methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoic acid *via* a (propargyl)$Co_2(CO)_6^+$ complex

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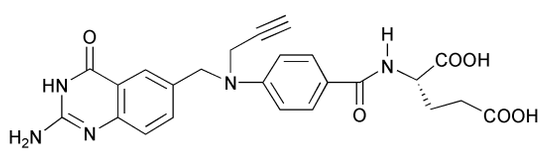
A new route to compound **3** (4-{*N*-[(6*RS*)-2-methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoic acid), a crucial intermediate for the synthesis of potent inhibitors of thymidylate synthase (TS), is described. In this sequence the C^6–N^{10} bond was constructed first, by the reductive amination of 5-acetamido-6-bromoindan-1-one **6** with *tert*-butyl 4-aminobenzoate, then the cyclopenta[*g*]quinazolinone ring was formed and the propargyl group was introduced on the N^{10}-position using the (propargyl)$Co_2(CO)_6^+$ complex as the electrophilic propargyl reagent.

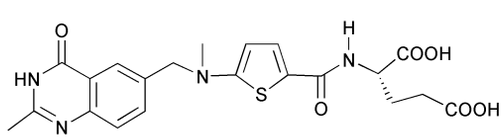
Introduction

Thymidylate synthase (TS) catalyses the conversion of 2′-deoxyuridine 5′-monophosphate (dUMP) to thymidine 5′-monophosphate (TMP) and this enzyme proved to be an attractive target in anticancer drug design.[1] The prototype folate-based inhibitor of TS is CB3717 **1**, a compound that was first synthesised at the Institute of Cancer Research, UK and showed activity in clinical trials.[2,3] Over the last two decades a large amount of research in the antifolate area has been directed towards the development of inhibitors of thymidylate synthase (TS).[4] As a result a number of molecules have reached the stage of clinical trials, and Raltitrexed **2**[5,6] has been approved in various countries for the treatment of colorectal cancer.

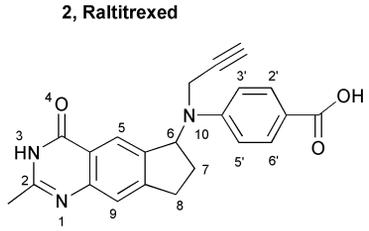
We have recently reported the synthesis of cyclopenta[*g*]-quinazoline-based antifolates, a novel class of TS inhibitor.[7] 4-{*N*-[(6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta-[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoic acid **3**, the key intermediate for the synthesis of this class of compounds, was prepared from *N*-(4-{*N*-[(6*RS*)-2-methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)-amino}benzoyl)-L-glutamic acid by the enzymatic cleavage of its glutamyl residue.[7,8] We now report an improved route to this key intermediate **3** in which the propargyl group was introduced in the penultimate step using the (propargyl)$Co_2(CO)_6^+$ complex as the electrophilic propargyl reagent.



1, CB3717



2, Raltitrexed



3

Results and discussion

The route to compound **3** is outlined in Scheme 1. This methodology differs significantly from the published route to **3**.[7,8]

The oxo-functionality, required for the formation of the C^6–N^{10} bond *via* a reductive amination reaction, was introduced in the initial steps of the synthesis whereas the cyclopenta[*g*]quinazoline ring was constructed after the formation of the C^6–N^{10} bond making this route simpler and more flexible. Most significantly, the propargyl group was introduced in the penultimate step under mild conditions utilising the (propargyl)$Co_2(CO)_6^+$ complex as the electrophilic propargyl synthon.

In this synthetic sequence (Scheme 1) 5-acetamido-6-bromoindane **4** was used as the starting material. Oxidation of **4** with CrO_3 in acetic acid[9] gave a mixture of two regioisomers **5** and **6**; the desired ketone **6** was isolated by column chromatography in 43% yield. Reductive amination of the ketone **6** with *tert*-butyl 4-aminobenzoate under classical conditions (*i.e.* TsOH, DME, reflux/$NaBH_3CN$, MeOH, AcOH, room temperature) resulted in the formation of **7** in 35% yield. This yield was increased to 81% when the reductive amination was performed using decaborane in methanol.[10] Cyanation of the aryl bromide **7** was effected utilising the Rosenmund–von Braun reaction[11] by heating **7** with copper(I) cyanide in 1-methyl-2-pyrrolidinone (NMP) at 140 °C. In the next step, the indane derivative **8** was cyclised to the cyclopenta[*g*]quinazolin-4-one **9** in 83% yield by treatment with H_2O_2, NaOH in EtOH–H_2O. It was envisaged that the introduction of the N^{10}-propargyl substituent could be achieved by using a cobalt-complexed propargyl cation as the electrophilic propargyl synthon. It is known that cobalt-complexed propargyl cations react with a variety of nucleophiles[12] including primary or secondary amines.[13] Indeed the tetrafluoroborate salt **12**, prepared by treatment of the dicobalt hexacarbonyl complex **11** with propionic acid and HBF_4[14] at −20 °C, reacted smoothly with **9** at room temperature to afford **13** in high yield (76%) without the need to protect the N^3–H. Decomplexation of **13** with $Fe(NO_3)_3$ in EtOH afforded the N^{10}-propargylated derivative **14**. In the final step the *tert*-butyl group was removed with TFA to afford the desired product **3** as

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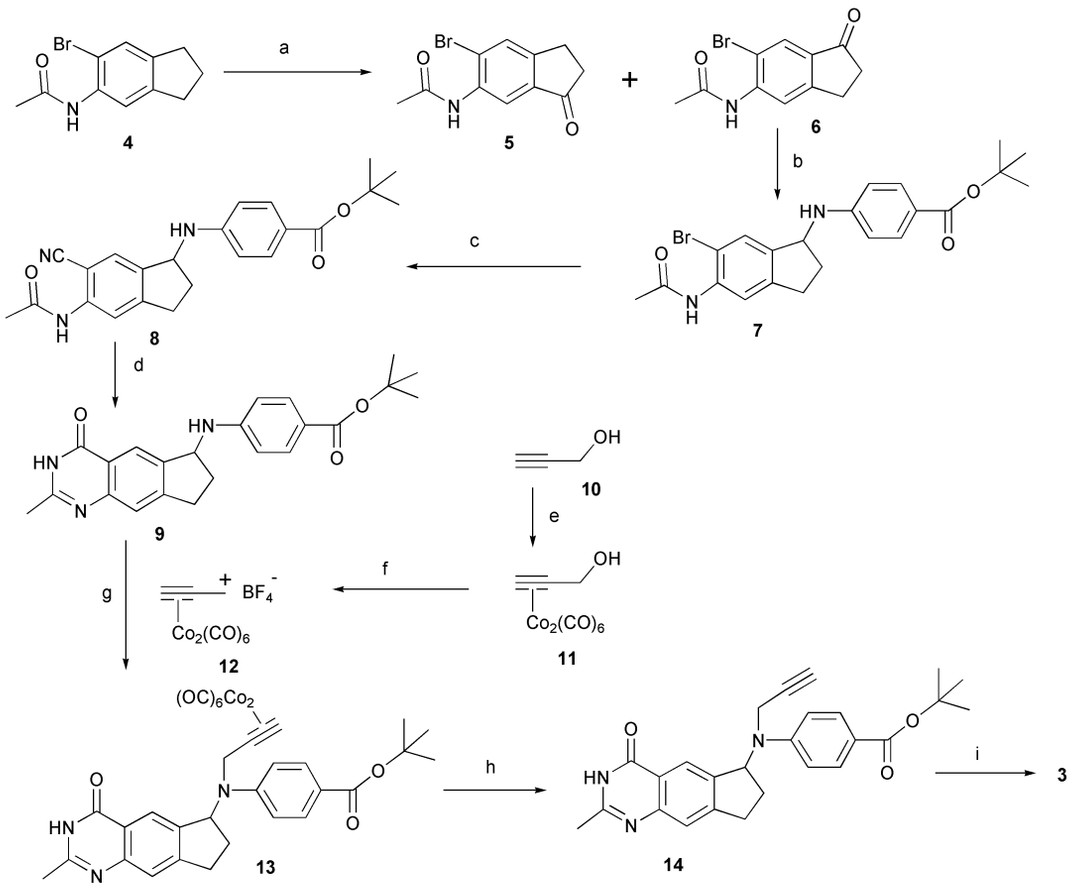

Scheme 1 *Reagents and conditions:* (a), CrO_3 AcOH, 55 °C; (b), *tert*-butyl 4-aminobenzoate, TsOH, DME, molecular sieves, reflux/$NaBH_3CN$, AcOH, MeOH, room temp. or *tert*-butyl 4-aminobenzoate, decaborane, MeOH, room temp.; (c) CuCN, NMP, 140 °C; (d), H_2O_2, NaOH, EtOH–H_2O, 55 °C; (e), $Co_2(CO)_8$, CH_2Cl_2, room temp.; (f), propionic acid, HBF_4, −20 °C; (g), DIEA, CH_2Cl_2, room temp.; (h), $Fe(NO_3)_3$, EtOH, room temp.; (i), CH_2Cl_2/TFA.

a racemic mixture. Chiral HPLC (column: Astec cyclobond BETA; mobile phase: 83% 25 mM Na_2HPO_4/25 mM NaH_2PO_4–17% CH_3CN; flow 1 ml min^{-1}; $\lambda = 230$ nM) indicated two peaks for compound **3** in a ratio of 1 : 1.

In summary, a new, simpler, and more flexible route to compound **3**, a crucial intermediate for the synthesis of novel inhibitors of TS, has been developed. In this route the N^{10}-propargyl substituent was introduced under mild conditions utilising the (propargyl)$Co_2(CO)_6^+$ complex as the electrophilic propargyl synthon. It should be noted that an N^{10} propargyl substituent provides optimum TS binding in a variety of classical quinazoline-based inhibitors of TS. Therefore, this methodology may be applied for the introduction of an N^{10}-propargyl substituent in other antifolates.

Experimental

Thin layer chromatography (TLC) was performed on precoated sheets of silica $60F_{254}$ (Merck Art 5735) visualised under UV light. Merck silica 60 (Art 15111) was used in low-pressure column chromatography. Petrol refers to light petroleum (bp 60–80 °C). Electrospray ionisation (ESI) mass spectra were recorded using a TSQ 700 triple quadrupole mass spectrometer (Finnigan MAT) fitted with an electrospray ionisation source (Analytica). Proton NMR spectra were recorded using a Bruker AC250 spectrometer at 250 MHz. Field strengths are expressed in units of δ (ppm) relative to tetramethylsilane, and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; dm, doublet of multiplets; t, triplet; q, quartet, br s, broad singlet, m, multiplet. Melting points were determined on a Kofler block and are uncorrected. Elemental analyses were determined by C.H.N. Analysis Ltd., Leicester, UK.

Synthesis

5-Acetamido-6-bromoindan-1-one 6. This compound was prepared as described in ref. 9: Purification of the crude product by column chromatography using a gradient of ethyl acetate in dichloromethane (10 to 20%) afforded in order of elution:

a. 5-Acetamido-6-bromoindan-1-one as a white solid which was further purified by trituration with ethyl acetate–hexane (1 : 4, v/v): 2.60 g (43%), mp 162–164 °C (Found: C, 49.18; H, 3.65; N, 5.13; Br, 29.89; $C_{11}H_{10}BrNO_2$ requires C, 49.28; H, 3.76; N, 5.22; Br, 29.80%); ν_{max} (film)/cm^{-1} 3234 (w), 1702 (s), 1662 (s), 1526 (s); δ_H ($CDCl_3$) 2.30 (1 H, s, Me), 2.71 (2 H, m, 2-H), 3.11 (2 H, t, *J* 5.6, 3-H), 7.93 (1 H, br s, CONH), 7.94, 8.60 (each 1 H, s, 4-H, 7-H); *m*/*z* (ESI) 268, 270 {$(M + H)^+$, 100%, 95% respectively, bromine isotopic pattern}, 226 (25).

b. 5-Acetamido-6-bromoindan-3-one as a white solid which was further purified by trituration with ethyl acetate–hexane (1 : 4, v/v): 0.45 g, (8%), mp 219–220 °C (Found: C, 49.24; H, 3.67; N, 5.12; Br, 29.73; $C_{11}H_{10}BrNO_2$ requires C, 49.28; H, 3.76; N, 5.22; Br, 29.80%); ν_{max} (film)/cm^{-1} 3276 (w), 1700 (s), 1663 (s), 1605 (s); δ_H ($CDCl_3$) 2.26 (3 H, s, Me), 2.71 (2 H, m, 2-H), 3.10 (2 H, t, *J* 5.6, 1-H), 7.60 (1 H, br s, CONH), 7.72, 8.62 (each 1 H, s, 4-H, 7-H); *m*/*z* (ESI) 268, 270 {$(M + H)^+$, 100%, 95% respectively, bromine isotopic pattern}, 188 (90).

***Tert*-butyl 4-[*N*-(5-acetamido-6-bromoindan-1-yl)amino]-benzoate 7.** *Method A.* To a flask containing 5-acetamido-6-bromoindan-1-one **6** (0.900 g, 3.36 mmol), 4-toluenesulfonic acid monohydrate (0.045 g), and *tert*-butyl 4-aminobenzoate (0.972 g, 5.04 mmol) was added 1,2-dimethoxyethane (dried by distillation over CaH_2; 48 cm^3). An azeotropic distillation apparatus (Aldrich)[15] containing molecular sieves (3 Å) was fitted to the reaction flask that was placed in an oil bath

preheated to 60 °C. The temperature was raised to 110 °C and stirring was continued at this temperature for 7 h under argon. The reaction mixture was then allowed to cool to room temperature, then a solution of sodium cyanoborohydride (0.336 g) in anhydrous methanol (11 cm^3) was added followed immediately by acetic acid (0.6 cm^3). The black reaction mixture was stirred at room temperature for 24 h under argon; then partitioned between ethyl acetate (170 cm^3) and saturated aqueous sodium bicarbonate (100 cm^3). The aqueous layer was extracted with more ethyl acetate (2 × 100 cm^3); the organic extracts were combined, dried ($MgSO_4$) and concentrated *in vacuo* to leave a dark oily residue. Purification by column chromatography on elution with ethyl acetate–hexane (1 : 1, v/v) afforded the title compound **7** as a white solid: 0.520 g (35%).

Method B. To a nearly clear solution of 5-acetamido-6-bromoindan-1-one **6** (0.964 g, 3.60 mmol) in anhydrous methanol (70 cm^3) was added *tert*-butyl 4-aminobenzoate (0.733 g, 3. 8 mmol) followed by decaborane (0.130 g, 1.08 mmol); a clear solution was obtained after stirring for approximately 0.5 h. The reaction mixture was stirred at room temperature overnight before being concentrated *in vacuo*. Purification by column chromatography and elution with a gradient of ethyl acetate in hexane (35 to 40%), afforded a gummy residue which was further purified by trituration with dichloromethane–hexane (1 : 4, v/v). The title compound **7** was obtained as a white solid: 1.32 g (81%) mp 153 °C (Found: C, 59.36; H, 5.62; N, 6.31; Br, 17.96; $C_{22}H_{25}BrN_2O_3$ requires C, 59.33; H, 5.66; N, 6.29; Br, 17.94%); ν_{max} (film)/cm^{-1} 1700 (s), 1684 (s), 1654 (s), 1604 (s); δ_H ($CDCl_3$) 1.57 (9 H, s, $C(CH_3)_3$), 2.25 (3 H, s, Me), 1.91, 2.63 (each 1 H, m, 2-H), 2.95 (2 H, m, 3-H), 5.03 (1 H, t, *J* 6.60, 1-H), 6.64 (2 H, d, *J* 8.75, 3′,5′-H), 7.60 (1 H, br s, CONH), 7.49, 8.25 (each 1 H, s, 4-H, 7-H), 7.85 (2 H, d, *J* 8.75, 2′,6′-H); *m*/*z* (ESI) 467, 469 {$(M + Na)^+$, 100%, 95% respectively, bromine isotopic pattern}, 252, 254 (35).

***Tert*-butyl 4-[*N*-(5-acetamido-6-cyanoindan-1-yl)amino]-benzoate 8.** To a solution of **7** (1.170 g, 2.62 mmol) in 1-methyl-2-pyrrolidinone (NMP) (13 cm^3) was added copper(I) cyanide (0.400 g, 4.70 mmol). The reaction mixture was placed in an oil-bath preheated to 140 °C and stirred at this temperature for 1 h and 40 min. The reaction mixture was allowed to cool to room temperature, then poured into a mixture of aqueous ammonia (d = 0.88 g cm^{-3}, 12 cm^3) and ice (~34 cm^3) and the resulting brown mixture was stirred at room temperature for ~10 min. The brown solid was collected by filtration washed with plenty of water, then dissolved in dichloromethane (100 cm^3). The mixture was stirred at room temperature for 10 min, dried ($MgSO_4$), and concentrated *in vacuo*. Purification by column chromatography and elution with 35% ethyl acetate in hexane, afforded a crispy solid that was reprecipitated from ethyl acetate–hexane. The title compound **8** was obtained as a white solid: 0.714 g, (70%) mp 173–174 °C (Found: C, 70.35; H, 6.44; N, 10.62; $C_{23}H_{25}N_3O_3$ requires C, 70.57; H, 6.44; N, 10.73%); ν_{max} (film)/cm^{-1} 2224 (w), 1700 (s), 1684 (s), 1653 (s), 1604 (s); δ_H ($CDCl_3$) 1.58 (9 H, s, $C(CH_3)_3$), 2.28 (3 H, s, Me), 1.96, 2.67 (each 1 H, m, 2-H), 3.04 (2 H, m, 3-H), 4.23 (1 H, d, *J* 8.31, N–H), 5.06 (1 H, q, *J* 7.50, 1-H), 6.65 (2 H, d, *J* 8.75, 3′,5′-H), 7.60 (1 H, br s, CONH), 7.54, 8.32 (each 1 H, s, 4-H, 7-H), 7.87 (2 H, d, *J* 8.75, 2′,6′-H); *m*/*z* (ESI) 783 {$(2M + H)^+$, 100%,}, 414 {$(M + Na)^+$, 55%}, 199 (15).

***Tert*-butyl 4-{*N*-[(6*RS*)-2-methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]amino}benzoate 9.** A mixture of **8** (1.33 g, 3.40 mmol), ethanol (15 cm^3), and water (3.1 cm^3) was cooled in an ice-bath, then 30% aqueous H_2O_2 solution (2.8 cm^3) was added followed by granulated sodium hydroxide pellets (0.230 g, 5.78 mmol). The reaction mixture was stirred at ~0 °C for 10 min, then it was placed in an oil bath preheated to 55 °C and stirred at this temperature for 40 min. The reaction mixture was allowed to cool to room temperature, then the solvents were removed *in vacuo* and the residue was suspended in water (~40 cm^3). The pH of this mixture was adjusted to ~5 with 1 N hydrochloric acid. The white precipitate was collected by filtration, washed with water, dried *in vacuo* over P_2O_5, then it was triturated with ether, collected by filtration and dried *in vacuo*. The title compound **9** was obtained as a white solid 1.11 g (83%), mp 277–281 °C (it melts with decomposition), (Found: C, 70.22; H, 6.43; N, 10.65; $C_{23}H_{25}N_3O_3$ requires C, 70.57; H, 6.44; N, 10.73%); δ_H (DMSO-d_6) 1.50 (9 H, s, $C(CH_3)_3$), 2.31 (3 H, s, Me), 1.87, 2.55 (each 1 H, m, 7-H), 2.97 (2 H, m, 8-H), 5.15 (1 H, m, 6-H), 6.77 (2 H, d, *J* 8.6, 3′,5′-H), 6.91 (1 H, d, *J* 8.70, N^{10}–H), 7.44, 7.87 (each 1 H, s, 5-H, 9-H), 7.66 (2 H, d, *J* 8.75, 2′,6′-H); *m*/*z* (ESI) 783 {$(2M + H)^+$, 100%}, 392 {$(M + H)^+$, 30%}, 199 (90).

Dicobalt hexacarbonyl propargyl alcohol complex 11. This is a known compound [13] and in this study was prepared according to Nicholas' methodology: [14] To a round bottom flask charged with $Co_2(CO)_8$ (5.12 g, 15.0 mmol) under argon in a well ventilated hood was added anhydrous dichloromethane (170 cm^3) followed by a solution of propargyl alcohol (0.840 g, 15.0 mmol) in anhydrous dichloromethane (20 cm^3). The deep red reaction mixture was stirred at room temperature for 7 h under argon, then it was filtered through a thin layer of neutral alumina. The filtrate was concentrated *in vacuo* to give a red residue. Purification by column chromatography, on elution with 40% diethyl ether in hexane, afforded the title compound **11** as a red solid 4.10 g (80%); δ_H ($CDCl_3$) 1.83 (1 H, t, *J* 6.0, OH, exchanges with D_2O), 4.80 (2 H, d, *J* 6.0, CH_2), 6.08 (1 H, s, C–H).

(Propargyl)$Co_2(CO)_6^+BF_4^-$ 12. This is a known compound [13] and in this study was prepared according to Nicholas' methodology: [14] To a round bottom flask charged with the dicobalt hexacarbonyl complex **11** (1.60 g, 4.7 mmol) under argon was added (syringed *via* a septum) propionic acid (2.2 cm^3). The reaction mixture was cooled to −20 °C and then a solution of HBF_4 in diethyl ether (54% w/w, 2.05 cm^3) was slowly syringed into the reaction mixture *via* a septum. The reaction mixture was stirred at −20 °C for 40 min, then pre-cooled diethyl ether (50 cm^3) was added. Trituration afforded a red precipitate that was collected by filtration, washed with plenty of dry diethyl ether and dried *in vacuo* over P_2O_5: 1.71 g (90%). This was immediately used in the next reaction without any further purification.

Dicobalt hexacarbonyl complex 13. To a round bottom flask containing the tetrafluoroborate salt **12** (1.44 g, 3.5 mmol) was added anhydrous dichloromethane (dried by distillation over P_2O_5; 100 cm^3). The nearly clear red dark solution was stirred at room temperature for a few minutes under argon, then **9** (1.04 g, 2.66 mmol) was added in one portion; a clear solution was obtained after approximately 2 min. Stirring was continued at this temperature for 5 min then diisopropylethylamine (0.92 cm^3, 5.32 mmol) was added and the reaction mixture was stirred at room temperature for 45 min under argon. The reaction mixture was partitioned between ethyl acetate (300 cm^3) and brine (120 cm^3). The organic layer was washed with 10% aqueous citric acid (100 cm^3), brine (100 cm^3), dried (Na_2SO_4), and concentrated *in vacuo*. Purification by column chromatography and elution with ethyl acetate in dichloromethane (60 to 70%) gave **13** as a red solid 1.45 g (76%), mp >150 °C (with decomposition, red crystals turn black); δ_H ($CDCl_3$) 1.58 (9 H, s, $C(CH_3)_3$), 2.53 (3 H, s, 2-Me), 2.32, 2.60 (each 1 H, m, 7-H), 3.05, 3.20 (each 1 H, m, 8-H), 4.55 (2 H, AB system, *J* 17.2, N^{10}–CH_2), 5.63 (1 H, t, *J* 8.6, 6-H), 5.96 (1 H, s, propargyl complex C–H), 6.93 (2 H, d, *J* 8.9, 3′,5′-H), 7.58, 8.00 (each 1 H, s, 5-H, 9-H), 7.90 (2 H, d, *J* 8.9, 2′,6′-H), 10.58 (1 H, s, N^3–H); *m*/*z* (ESI) 716 {$(M + H)^+$, 80%}, 231 (100), 199 (70).

***Tert*-Butyl 4-{*N*-[(6*RS*)-2-methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoate 14.** To a solution of the complex **13** (1.40 g, 1.96 mmol) in ethanol (200 cm^3) was added $Fe(NO_3)_3$ (26 g). The clear solution was stirred at room temperature for 10 min then a second portion of $Fe(NO_3)_3$ (~15 g) was added. The reaction mixture was stirred at room temperature for a further 10 min then a final portion of $Fe(NO_3)_3$ (~10 g) was added; the nearly clear solution turned dark red. Stirring was continued at room temperature for an extra 15 min, the reaction mixture was then partitioned between ethyl acetate (700 cm^3) and dilute brine (200 cm^3). The organic (not clear) layer was washed with more brine (3 × 150 cm^3), dried (Na_2SO_4), and concentrated *in vacuo* to leave a brown solid. Purification by column chromatography and elution with 5% methanol in chloroform, afforded a solid. Trituration with dichloromethane–hexane gave the title compound **14** as an off white solid, 0.565 g (67%), mp 244–246 °C; (Found: C, 72.60; H, 6.37; N, 9.67; $C_{26}H_{27}N_3O_3$ requires C, 72.71; H, 6.34; N, 9.78%); δ_H (DMSO-d_6) 1.50 (9 H, s, $C(CH_3)_3$), 2.32 (3 H, s, 2-Me), 2.18, 2.55 (obscured by the DMSO peak) (each 1 H, m, 7-H), 2.97 (1 H, m, 8-H), 3.14 (2 H, m, C≡CH, 8-H), 3.96 (2 H, AB system, *J* 18.1, $CH_2C{\equiv}C$), 5.75 (1 H, t, *J* 8.2, 6-H), 6.98 (2 H, d, *J* 9.5, 3′,5′-H), 7.48, 7.76 (each 1 H, s, 5-H, 9-H), 7.74 (2 H, d, *J* 8.7, 2′,6′-H), 12.11 (1 H, s, N^3–H); *m*/*z* (ESI) 430 {$(M + H)^+$, 90%}, 374 (55%), 231 (100), 199 (60).

***N*-[(6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta-[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoic acid 3.** A solution of **14** (0.245 g, 0.57 mmol) in dichloromethane (2 cm^3) and trifluoroacetic acid (10 cm^3) was stirred at room temperature for 1.5 hours, then the solvents were removed *in vacuo*. The residue was triturated with diethyl ether and the precipitate was collected by filtration, washed with diethyl ether and dried *in vacuo* over P_2O_5 to afford the title compound as the trifluoracetate salt (0.255 g). Part of this material (0.067 g) was suspended in water (6 cm^3), and the pH was adjusted to ~12 with 1 N aqueous sodium hydroxide. The pH of the clear solution was adjusted to ~4 with 1 N aqueous hydrochloric acid. The white precipitate was collected by filtration, washed with water and dried *in vacuo* over P_2O_5 to afford the title compound **3** as a white solid (0.040 g) mp 305–307 °C (ref. 7: >325 °C); (Found: C, 68.65; H, 4.97; N, 10.87; $C_{22}H_{19}N_3O_3{\cdot}0.6H_2O$ requires C, 68.78; H, 5.29; N, 10.93%); δ_H (DMSO-d_6) 2.33 (3 H, s, 2-Me), 2.24, 2.50 (obscured by the DMSO peak) (each 1 H, m, 7-H), 2.97 (1 H, m, 8-H), 3.20 (1 H, m (obscured by H_2O peak), 8-H), 3.13 (1 H, s, C≡CH), 3.96 (2 H, AB system, *J* 19.0, $CH_2C{\equiv}C$), 5.75 (1 H, t, *J* 8.3, 6-H), 7.01 (2 H, d, *J* 9.0, 3′,5′-H), 7.48, 7.78 (each 1 H, s, 5-H, 9-H), 7.80 (2 H, d, *J* 9.4, 2′,6′-H), 12.11 (1 H, s, N^3–H); *m*/*z* (ESI) 747 {$(2M + H)^+$, 100%}, 374 {$(M + H)^+$, 70%}, 199 (20%). Chiral HPLC (column: Astec cyclobond BETA; mobile phase: 83% 25mM Na_2HPO_4–25 mM NaH_2PO_4–17% CH_3CN; flow 1 ml min^{-1}; λ = 230 nm): two peaks in a ratio of approximately 1 : 1; retention times: 621, 680 seconds.

Acknowledgements

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