

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
13 March 2003 (13.03.2003)

PCT

(10) International Publication Number  
WO 03/020748 A1

- (51) International Patent Classification<sup>7</sup>: C07K 5/02, C07D 239/90, 403/12, A61K 38/05, 31/517, A61P 35/00
- (21) International Application Number: PCT/GB02/03979
- (22) International Filing Date: 30 August 2002 (30.08.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
0121151.5 31 August 2001 (31.08.2001) GB  
0129387.7 7 December 2001 (07.12.2001) GB  
60/340,253 18 December 2001 (18.12.2001) US
- (71) Applicant (for all designated States except US): BTG INTERNATIONAL LIMITED [GB/GB]; 10 Fleet Place, Limeburner Lane, London EC4M 7SB (GB).

(74) Agent: ENGLAND, Christopher, David; BTG International Limited, 10 Fleet Place, Limeburner Lane, London EC4M 7SB (GB).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

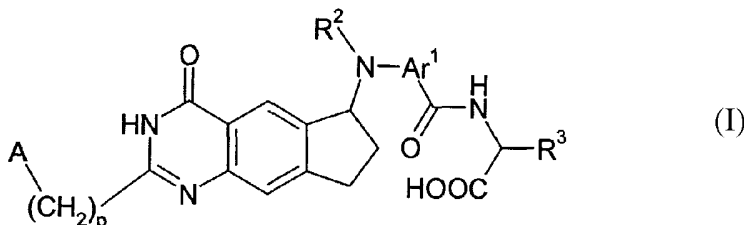
(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

- (72) Inventors; and  
(75) Inventors/Applicants (for US only): BAVETSIAS, Vasilios [GR/GB]; 3 Chiddingstone Close, Sutton, Surrey SM2 6NS (GB). JACKMAN, Ann, Lesley [GB/GB]; 17 Foxholes, Rudgwick, Horsham, West Sussex, RH12 3DX (GB).

Published:  
— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ANTI-CANCER CYCLOPENTA[G]QUINAZOLINE COMPOUNDS



(57) Abstract: Cyclopenta[g]quinazolines of the formula (I):- wherein: A is a group OR or NR<sup>0</sup>R<sup>1</sup> wherein R<sup>0</sup> and R<sup>1</sup> are each independently hydrogen C<sub>1-4</sub> alkyl, C<sub>3-4</sub> alkenyl, C<sub>3-4</sub> alkynyl, C<sub>2-4</sub> hydroxyalkyl, C<sub>2-4</sub> halogenoalkyl or C<sub>1-4</sub> cyanoalkyl, or R<sup>0</sup> and R<sup>1</sup> together with the intermediate N form a five- or six-membered heterocyclic ring; p is an integer in the range 1 to 4; R<sup>2</sup> is hydrogen, C<sub>1-4</sub> alkyl, C<sub>3-4</sub> alkenyl, C<sub>3-4</sub> alkynyl, C<sub>2-4</sub> hydroxyalkyl, C<sub>2-4</sub> halogenoalkyl or C<sub>1-4</sub> cyanoalkyl; Ar<sup>1</sup> is phenylene, thiophenediyl, thiazolediyl, pyridinediyl or pyrimidinediyl which may optionally bear one or two substituents selected from halogeno, hydroxy, amino, nitro, cyano, trifluoromethyl, C<sub>1-4</sub> alkyl and C<sub>1-4</sub> alkoxy; and R<sup>3</sup> is a group of one of the following formulae: -A<sup>1</sup>-Ar<sup>2</sup>-A<sup>2</sup>-Y<sup>1</sup>-A<sup>5</sup>-CON(R)CH(Y<sup>4</sup>)Y<sup>5</sup>-A<sup>8</sup>-X-Ar<sup>4</sup> and pharmaceutically acceptable salts or esters thereof are of therapeutic value particularly in the treatment of cancer.

WO 03/020748 A1

## ANTI-CANCER CYCLOPENTA [G] QUINAZOLINE COMPOUNDS

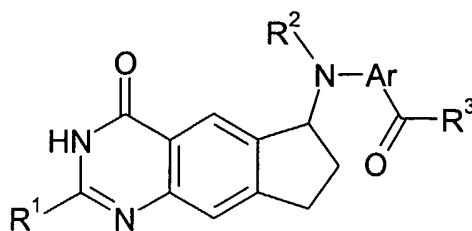
This invention relates to novel anti-cancer agents and more particularly it relates to cyclopenta[g]quinazoline derivatives which possess antiproliferative activity.

5 One group of anti-cancer agents comprises antimetabolites having antifolate activity, such as the dihydrofolate reductase inhibitor, methotrexate and the thymidylate synthase (TS) inhibitors CB3717, raltitrexed and ZD9331. CB3717 is described and claimed in EP-B-0031237, raltitrexed in EP-B-0239362 and ZD9331 in EP-B-0562734. All of these TS inhibitors have demonstrable clinical activity in a  
10 range of solid tumours (see *Cancer Treatment Reports*, 1986, 70, 1335 and Beale *et al.*, "Tomudex: Clinical Development" in *Antifolate Drugs in Cancer Therapy* (ed. Jackman), Humana Press, Totowa, New Jersey, USA, pp. 177–181, 1999). Side-effects of raltitrexed and ZD9331 are predominantly related to inhibition of TS in gut and bone-marrow.

15 TS catalyses the methylation of deoxyuridine monophosphate to produce thymidine monophosphate which is required for DNA synthesis. The anticancer activity of these agents may be assessed *in vitro* by determining their inhibitory effect on that enzyme, and in cell cultures by their inhibitory effect on a range of mouse and human cancer cell lines (see Boyle *et al.*, "ZD9331: Preclinical and clinical studies"  
20 in *Antifolate Drugs in Cancer Therapy* (ed. Jackman), Humana Press, Totowa, New Jersey, USA, pp. 243–260, 1999 and Hughes *et al.*, "Raltitrexed (Tomudex), a highly polyglutamatable antifolate thymidylate synthase inhibitor: design and preclinical activity" in *Antifolate Drugs in Cancer Therapy* (ed. Jackman), Humana Press, Totowa, New Jersey, USA, pp. 147–165, 1999).

25 More recently, cyclopenta[g]quinazoline derivatives showing a good level of activity both as regards their ability to inhibit TS and also as regards their anticancer activity against various cell lines have been developed.

WO-A-94/11354 (British Technology Group Limited) discloses tricyclic compound of formula:



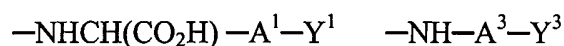
wherein R<sup>1</sup> is hydrogen, amino, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> hydroxyalkyl or C<sub>1-4</sub> fluoroalkyl;

R<sup>2</sup> is hydrogen, C<sub>1-4</sub> alkyl, C<sub>3-4</sub> alkenyl, C<sub>3-4</sub> alkynyl, C<sub>2-4</sub> hydroxyalkyl C<sub>2-4</sub> halogenoalkyl or C<sub>1-4</sub> cyanoalkyl;

Ar is phenylene, thiophenediyl, thiazolediyl, pyridinediyl or pyrimidinediyl which may optionally bear one or two substituents selected from halogeno, hydroxy, amino, nitro, cyano, trifluoromethyl, C<sub>1-4</sub> alkyl and C<sub>1-4</sub> alkoxy; and

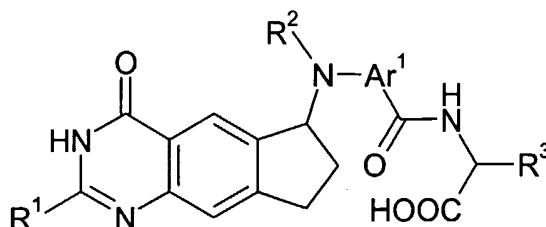
R<sup>3</sup> is a group of one of the following formulae:

10



or R<sup>3</sup> is a *N*-linked naturally-occurring amino acid selected from the group consisting of L-alanine, L-leucine, L-isoleucine, L-valine and L-phenylalanine. Among the compounds disclosed is the L-Glu- $\gamma$ -D-Glu compound CB300638, also mentioned in *Clinical Cancer Research*, **5**, November 1999 (Supplement) at #566 (Theti *et al.*) and *Proceedings of the American Association for Cancer Research*, **41**, March 2000 at #33 (Jackman *et al.*), as well as in *J. Med. Chem.*, 2000, **43**, 1910–1926, where it is disclosed on page 1923 as compound **7b**.

20 WO-A-95/30673 (British Technology Group Limited) discloses cyclopenta-[g]quinazolines of formula:



wherein R<sup>1</sup> is hydrogen, amino, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> hydroxyalkyl or C<sub>1-4</sub> fluoroalkyl;

25

$R^2$  is hydrogen,  $C_{1-4}$  alkyl,  $C_{3-4}$  alkenyl,  $C_{3-4}$  alkynyl,  $C_{2-4}$  hydroxyalkyl,  $C_{2-4}$  halogenoalkyl or  $C_{1-4}$  cyanoalkyl;

$Ar^1$  is phenylene, thiophenediyl, thiazolediyl, pyridinediyl or pyrimidinediyl which may optionally bear one or two substituents selected from halogeno, hydroxy, amino, nitro, cyano, trifluoromethyl,  $C_{1-4}$  alkyl and  $C_{1-4}$  alkoxy; and

$R^3$  is a group of one of the following formulae:



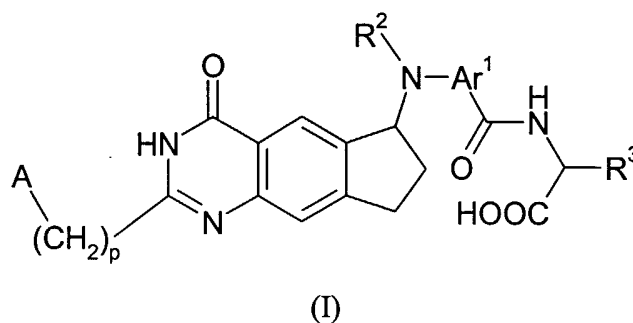
The  $\alpha$ -isoform of the folate receptor ( $\alpha$ -FR; membrane-associated folate-binding protein) is a glycosylphosphatidylinositol anchored cell membrane protein that has very high affinity for folic acid and the more biologically relevant reduced-folates ( $K_d \sim 0.1$  nM). The mechanism of folate internalisation is receptor-mediated endocytosis. The  $\alpha$ -FR is overexpressed in many carcinomas, particularly those of ovarian origin where it is overexpressed highly and homogeneously in 90% of cases; see *Cancer Res.* **51**, 5329–5338, 1991 (Campbell *et al.*, 1991). Furthermore, high  $\alpha$ -FR expression has been linked to aggressive, platinum resistant disease and poor prognosis—see *Int. J. Cancer* **74**, 193–198, 1997 and *Int. J. Cancer* **79**, 121–126, 1998 (both Toffoli *et al.*). The  $\beta$ -isoform is widely expressed in tumours of epithelial and non-epithelial origin with expression levels being generally low/moderate and high, respectively, reviewed in *Critical Rev. Therap. in Drug Carrier Systems* **15**, 587–627, 1998 (Reddy and Low).

Folate receptors ( $\alpha$  and  $\beta$ ) are expressed in some adult normal tissues (low to moderate expression). Significant expression of the  $\alpha$ -FR is largely restricted to kidney proximal tubules and choroid plexus although it is suggested that it is localised to the apical membrane surface in these organs and therefore may not play a significant role in folate uptake from blood (Reddy and Low, *ibid.*). There may be a specialised function of the  $\alpha$ -FR in the proximal tubules of the kidney to salvage folates that escape in the filtrate.

The  $\alpha$ -FR is hypothesised to be involved in cell signalling pathways. For example, in IGROV-1 ovarian carcinoma cells, immunoprecipitation experiments have shown that the  $\alpha$ -FR is associated in membranes with the G protein  $G_{\alpha 1-3}$ , and the non-receptor kinase lyn.

High FR expression in some tumours relative to normal tissues is being exploited in several areas of cancer medicine, including the selective tumour delivery of conjugates of folic acid and toxins, liposomes, imaging or cytotoxic agents (Reddy and Low, *ibid.*). For example, folic acid-deferrioxamine-<sup>111</sup>In conjugates are detected only in FR-expressing tumours and not normal tissues of mice, with the exception of kidney epithelial cells. The high selectivity of this approach resides in the very low and high affinities of folic acid (not a major component of plasma) for the RFC (reduced-folate carrier) and FR respectively. Thus antifolate drugs with similarly low and high affinity for the RFC and  $\alpha$ -FR respectively could be highly selective for  $\alpha$ -FR over-expressing tumours relative to normal tissues. In contrast with the folic acid conjugates they would not require intracellular cleavage to be active.

We have now discovered that certain compounds within the general class of cyclopenta[g]quinazolines have an unexpectedly high level of selectivity for  $\alpha$ -folate receptor expressing human tumour cell lines. Accordingly the present invention comprises a cyclopenta[g]quinazoline of formula (I):



wherein:

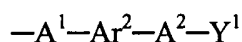
A is a group OR<sup>0</sup> or NR<sup>0</sup>R<sup>1</sup> wherein R<sup>0</sup> and R<sup>1</sup> are each independently hydrogen, C<sub>1-4</sub> alkyl, C<sub>3-4</sub> alkenyl, C<sub>3-4</sub> alkynyl, C<sub>2-4</sub> hydroxyalkyl, C<sub>2-4</sub> halogenoalkyl or C<sub>1-4</sub> cyanoalkyl, or R<sup>0</sup> and R<sup>1</sup> together with the intermediate N form a five- or six-membered heterocyclic ring;

p is an integer in the range 1 to 4;

R<sup>2</sup> is hydrogen, C<sub>1-4</sub> alkyl, C<sub>3-4</sub> alkenyl, C<sub>3-4</sub> alkynyl, C<sub>2-4</sub> hydroxyalkyl, C<sub>2-4</sub> halogenoalkyl or C<sub>1-4</sub> cyanoalkyl;

Ar<sup>1</sup> is phenylene, thiophenediyl, thiazolediyl, pyridinediyl or pyrimidinediyl which may optionally bear one or two substituents selected from halogeno, hydroxy, amino, nitro, cyano, trifluoromethyl, C<sub>1-4</sub> alkyl and C<sub>1-4</sub> alkoxy; and

R<sup>3</sup> is a group of the formula:



in which  $A^1$  is a bond between the  $\alpha$ -carbon atom of the group  
 5  $-CO-NH-CH(CO_2H)-$  and  $Ar^2$  or is a  $C_{1-2}$  alkylene group;

$Ar^2$  is phenylene, tetrazolediyl, thiophenediyl, thiazolediyl, pyridinediyl or  
 pyrimidinediyl which in the case of phenylene may optionally bear one or two  
 substituents on the ring selected from halogeno, nitro,  $C_{1-4}$  alkyl and  $C_{1-4}$  alkoxy;

$A^2$  is a  $C_{1-3}$  alkylene or  $C_{2-3}$  alkenylene group; and

10  $Y^1$  is carboxyl, tetrazol-5-yl,  $N-(C_{1-4}$  alkylsulfonyl)carbamoyl,  $N$ -(phenyl-  
 sulfonyl)carbamoyl which may optionally bear one or two substituents on the phenyl  
 ring selected from the group consisting of halogeno, nitro,  $C_{1-4}$  alkyl and  $C_{1-4}$  alkoxy,  
 tetrazol-5-ylthio, tetrazol-5-ylsulfinyl or tetrazol-5-ylsulfonyl; or

$Y^1$  is a group of the formula:

15



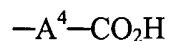
in which R is hydrogen,  $C_{1-4}$  alkyl,  $C_{3-4}$  alkenyl or  $C_{3-4}$  alkynyl;

20  $Y^2$  is carboxy, tetrazol-5-yl,  $N-(C_{1-4}$  alkylsulfonyl)carbamoyl,  $N$ -(phenyl-  
 sulfonyl)carbamoyl which may optionally bear one or two substituents on the phenyl  
 ring selected from the group consisting of halogeno, nitro,  $C_{1-4}$  alkyl and  $C_{1-4}$  alkoxy,  
 tetrazol-5-ylthio, tetrazol-5-ylsulfinyl or tetrazol-5-ylsulfonyl; and

$Y^3$  is the residue of a naturally occurring amino acid  $NH_2CH(CO_2H)Y^3$ ; or

$Y^3$  is a group of the formula:

25



in which  $A^4$  is a  $C_{2-6}$  alkylene group;

$R^3$  is a group of the formula:

30

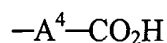


in which  $A^5$  is a  $C_{1-6}$  alkylene group and R is as defined above;

$Y^4$  is carboxy, tetrazol-5-yl, *N*-( $C_{1-4}$  alkylsulfonyl)carbamoyl, *N*-(phenylsulfonyl)carbamoyl which may optionally bear one or two substituents on the phenyl ring selected from the group consisting of halogeno, nitro,  $C_{1-4}$  alkyl and  $C_{1-4}$  alkoxy, tetrazol-5-ylthio, tetrazol-5-ylsulfinyl or tetrazol-5-ylsulfonyl; and

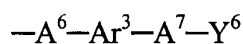
5  $Y^5$  is the residue of a naturally occurring amino acid  $NH_2CH(CO_2H)Y^5$ ; or

$Y^5$  is a group of the formula:



10 in which  $A^4$  is as defined above; or

$Y^5$  is a group of the formula:



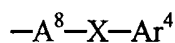
15 in which  $A^6$  is a bond between the  $\alpha$ -carbon atom of the group  $-A^5-CON(R)CH(Y^4)-$  and  $Ar^3$  or is a  $C_{1-2}$  alkylene group;

$Ar^3$  is phenylene, tetrazolediyl, thiophenediyl, thiazolediyl, pyridinediyl or pyrimidinediyl which in the case of phenylene may optionally bear one or two substituents on the ring selected from halogeno, nitro,  $C_{1-4}$  alkyl and  $C_{1-4}$  alkoxy;

20  $A^7$  is a  $C_{1-3}$  alkylene or  $C_{2-3}$  alkenylene group; and

$Y^6$  is carboxy, tetrazol-5-yl, *N*-( $C_{1-4}$  alkylsulfonyl)carbamoyl, *N*-(phenylsulfonyl)carbamoyl which may optionally bear one or two substituents on the phenyl ring selected from the group consisting of halogeno, nitro,  $C_{1-4}$  alkyl and  $C_{1-4}$  alkoxy, tetrazol-5-ylthio, tetrazol-5-ylsulfinyl or tetrazol-5-ylsulfonyl; or

25  $R^3$  is a group of the formula:



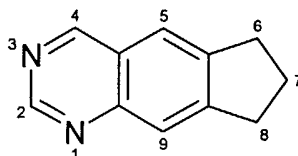
in which  $A^8$  is a  $C_{1-4}$  alkylene group;

30  $X$  is sulfinyl, sulfonyl or methylene; and

$Ar^4$  is 1,2,3-triazol-4-yl, 1,2,4-triazol-3-yl or, except when  $X$  is methylene, tetrazol-5-yl;

the compound (I) optionally being in the form of a pharmaceutically acceptable salt or ester.

In this specification the terms alkyl, alkenyl, alkynyl and alkylene include both straight and branched chain groups but references to individual alkyl or alkylene groups, such as "propyl", are specific for the straight chain group only. An analogous convention applies to other generic terms. Moreover, the numbering system used for  
 5 the cyclopenta[g]quinazoline nucleus is the conventional one as shown below:



Amino-acid residues are designated herein in the standard manner (*Pure and Applied Chemistry*, 1974, **40**, 317 and *European Journal of Biochemistry*, 1984, **138**, 9). Thus, for example,  $\gamma$ -glutamyl denotes the radical  $\text{H}_2\text{NCH}(\text{CO}_2\text{H})\text{CH}_2\text{CH}_2\text{CO}-$  or  
 10  $-\text{NHCH}(\text{CO}_2\text{H})\text{CH}_2\text{CH}_2\text{CO}-$  according to the context, the carbon atoms in these radicals being numbered from the carbon atom of the  $\alpha$ -carboxy group as position 1.

It will be observed that a cyclopenta[g]quinazoline of the invention contains at least two asymmetric carbon atoms [present at the point of attachment of the group  $-\text{N}(\text{R}^2)-$  to the tricyclic ring system and at the  $\alpha$ -carbon atom of the group  
 15  $-\text{CONHCH}(\text{CO}_2\text{H})-$ ] and can therefore exist in racemic and optically active forms. It is to be understood that this invention encompasses both racemic and optically active forms of the physiologically active cyclopenta[g]quinazolines, it being a matter of common general knowledge how such optically active forms may be obtained by stereospecific synthesis or by separation of a mixture of isomeric compounds. It will  
 20 be appreciated that one isomer may be of more interest than another due to the nature of the activity which it exhibits or due to superior physical properties, for example aqueous solubility.

It is also to be understood that a cyclopenta[g]quinazoline of the formula (I) may exhibit the phenomenon of tautomerism and that the formulae shown in this  
 25 specification represent only one of the possible tautomeric forms. Moreover, it will be appreciated that when, for example,  $\text{Y}^1$ ,  $\text{Y}^2$ ,  $\text{Y}^4$  or  $\text{Y}^6$  is a tetrazol-5-yl group, that group may be in the form of a 1*H*-tetrazol-5-yl group or a 2*H*-tetrazol-5-yl group. It is to be understood therefore that the invention is not limited merely to any one tautomeric form which is illustrated.

It is also to be understood that certain cyclopenta[g]quinazolines of the formula (I) can exist in solvated as well as unsolvated forms such as, for example, hydrated forms.

A suitable value for  $R^0$ ,  $R^1$  or  $R^2$  when it is  $C_{1-4}$  alkyl, or for a  $C_{1-4}$  alkyl substituent which may be present on  $Ar^1$ ,  $Ar^2$  or  $Ar^3$  or on a phenyl group-containing group  $Y^1$ ,  $Y^2$ ,  $Y^4$  or  $Y^6$  present in  $R^3$ , or for a group  $R$  present in  $R^3$  when it is  $C_{1-4}$  alkyl, is, for example, methyl, ethyl, propyl or isopropyl.

A suitable value for a  $C_{1-4}$  alkoxy substituent which may be present on  $Ar^1$ ,  $Ar^2$  or  $Ar^3$  or on a phenyl-containing group  $Y^1$ ,  $Y^2$ ,  $Y^4$  or  $Y^6$  is, for example, methoxy, ethoxy, propoxy, isopropoxy or butoxy.

A suitable value for a halogeno substituent which may be present on  $Ar^1$ ,  $Ar^2$  or  $Ar^3$  or on a phenyl-containing group  $Y^1$ ,  $Y^2$ ,  $Y^4$  or  $Y^6$  is, for example, fluoro, chloro or bromo.

A suitable value for  $R^0$ ,  $R^1$  and  $R^2$  when it is  $C_{3-4}$  alkenyl or for a group  $R$  present in  $R^3$  when it is alkenyl, is, for example, prop-2-enyl, but-2-enyl, but-3-enyl or 2-methylprop-2-enyl; and when it is  $C_{3-4}$  alkynyl is, for example, prop-2-ynyl or but-3-ynyl.

A suitable value for  $R^0$ ,  $R^1$  and  $R^2$  when it is  $C_{2-4}$  hydroxyalkyl is, for example, 2-hydroxyethyl or 3-hydroxypropyl; when it is  $C_{2-4}$  halogenoalkyl is, for example, 2-fluoroethyl, 2-chloroethyl, 2-bromoethyl, 3-fluoropropyl, 3-chloropropyl or 3-bromopropyl; and when it is  $C_{1-4}$  cyanoalkyl is, for example, cyanomethyl, 2-cyanoethyl or 3-cyanopropyl.

When  $R^0$  and  $R^1$  together with the intermediate N form a five- or six-membered heterocyclic ring, this may bear substituents, but the ring is preferably an unsubstituted saturated ring such as pyrrolidine or piperidine.

A suitable value for  $Ar^1$ ,  $Ar^2$  or  $Ar^3$  when it is phenylene is, for example, 1,3- or 1,4-phenylene, especially 1,4-phenylene.

A suitable value for  $Ar^1$ ,  $Ar^2$  or  $Ar^3$  when it is thiophenediyl is, for example, thiophene-2,4-diyl or thiophene-2,5-diyl; when it is thiazolediyl is, for example thiazole-2,4-diyl or thiazole-2,5-diyl; when it is pyridinediyl is, for example, pyridine-2,4-diyl, pyridine-2,5-diyl, pyridine-2,6-diyl or pyridine-3,5-diyl; and when it is pyrimidinediyl is, for example, pyrimidine-2,4-diyl, pyrimidine-2,5-diyl or pyrimidine-4,6-diyl.

As indicated, Ar<sup>1</sup> and a phenylene group Ar<sup>2</sup> or Ar<sup>3</sup> or a phenyl group in Y<sup>1</sup> may carry one or two substituents. A preferred level of substitution in Ar<sup>1</sup>, where substitution is present, is either two substituents or especially one substituent; and the one or two substituents may conveniently be at positions adjacent to the atom bonded to the group —CONHCH(CO<sub>2</sub>H)—R<sup>3</sup>, halogeno substituents such as fluoro being preferred. A preferred level of substitution on a phenylene group Ar<sup>2</sup> or Ar<sup>3</sup> or on a phenyl group in Y<sup>1</sup>, where substitution is present, is one substituent.

When R<sup>3</sup> is a group of the formula:



a suitable value for A<sup>1</sup> when it is a C<sub>1-2</sub> alkylene group is, for example methylene or ethylene and for A<sup>2</sup> when it is a C<sub>1-3</sub> alkylene group is, for example, methylene, ethylene or trimethylene. A suitable value for A<sup>2</sup> when it is a C<sub>2-3</sub> alkenylene group is, for example, vinylene or especially propenylene (—CH<sub>2</sub>CH=CH— or —CH=CH—CH<sub>2</sub>—). A preferred value for both A<sup>1</sup>, when it is not a bond, and for A<sup>2</sup> is methylene or ethylene. Suitable values for Ar<sup>2</sup> include those which have been discussed hereinbefore, such as thiophenediyl or most especially phenylene, or additionally tetrazole-1,5-diyl or tetrazole-2,5-diyl. A suitable value for Y<sup>1</sup> or for Y<sup>2</sup> in a group Y<sup>1</sup> of formula —CON(R)CH(Y<sup>2</sup>)Y<sup>3</sup> when it is *N*-(C<sub>1-4</sub> alkylsulfonyl)carbamoyl is, for example, *N*-methylsulfonylcarbamoyl, *N*-ethylsulfonylcarbamoyl or *N*-propylsulfonylcarbamoyl.

In a group Y<sup>1</sup> of formula —CON(R)CH(Y<sup>2</sup>)Y<sup>3</sup> suitable values for C<sub>1-4</sub> alkyl, C<sub>3-4</sub> alkenyl and C<sub>3-4</sub> alkynyl groups R have been discussed hereinbefore but R is preferably either methyl or especially hydrogen; a suitable value for Y<sup>3</sup> when it is the residue of a naturally occurring amino acid is the residue of alanine (Y<sup>3</sup> = CH<sub>3</sub>), arginine (Y<sup>3</sup> = (CH<sub>2</sub>)<sub>3</sub>NHC(NH<sub>2</sub>)=NH), aspartic acid (Y<sup>3</sup> = CH<sub>2</sub>CO<sub>2</sub>H), cysteine (Y<sup>3</sup> = CH<sub>2</sub>SH), isoleucine (Y<sup>3</sup> = CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>), leucine (Y<sup>3</sup> = CH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>3</sub>), ornithine (Y<sup>3</sup> = (CH<sub>2</sub>)<sub>3</sub>NH<sub>2</sub>), phenylalanine (Y<sup>3</sup> = CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), serine (Y<sup>3</sup> = CH<sub>2</sub>OH) and valine (Y<sup>3</sup> = CH(CH<sub>3</sub>)<sub>2</sub>) and especially glutamic acid (Y<sup>3</sup> = CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>H). A suitable value for A<sup>4</sup> when Y<sup>3</sup> is a group of the formula —A<sup>4</sup>—CO<sub>2</sub>H is trimethylene, pentamethylene or hexamethylene, A<sup>4</sup> preferably being a C<sub>3-6</sub> alkylene group with especially suitable values for Y<sup>3</sup> being —(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H where n is 3, 4 or 5.

A preferred value for Y<sup>1</sup> or for Y<sup>2</sup>, Y<sup>4</sup> and Y<sup>6</sup>, is tetrazol-5-yl or especially carboxy.

When R<sup>3</sup> is a group of the formula:



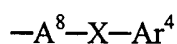
a suitable value for A<sup>5</sup> is, for example, methylene, ethylene, trimethylene, tetramethylene, pentamethylene or hexamethylene and a suitable value for R is as described hereinbefore. A suitable value for Y<sup>4</sup> when it is *N*-(C<sub>1-4</sub> alkylsulfonyl)-carbamoyl is, for example, *N*-methylsulfonylcarbamoyl, *N*-ethylsulfonylcarbamoyl or *N*-propylsulfonylcarbamoyl.

10

A suitable value for Y<sup>5</sup> when it is the residue of a naturally occurring amino acid is alanine (Y<sup>5</sup> = CH<sub>3</sub>), arginine (Y<sup>5</sup> = (CH<sub>2</sub>)<sub>3</sub>NHC(NH<sub>2</sub>)=NH), aspartic acid (Y<sup>5</sup> = CH<sub>2</sub>CO<sub>2</sub>H), cysteine (Y<sup>5</sup> = CH<sub>2</sub>SH), isoleucine (Y<sup>5</sup> = CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>), leucine  
 15 (Y<sup>5</sup> = CH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>3</sub>), ornithine (Y<sup>5</sup> = (CH<sub>2</sub>)<sub>3</sub>NH<sub>2</sub>), phenylalanine (Y<sup>5</sup> = CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), serine (Y<sup>5</sup> = CH<sub>2</sub>OH), valine (Y<sup>5</sup> = CH(CH<sub>3</sub>)<sub>2</sub>), and especially glutamic acid (Y<sup>5</sup> = CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>H). When Y<sup>5</sup> is a group of the formula -A<sup>4</sup>-CO<sub>2</sub>H, suitable values for A<sup>4</sup> and Y<sup>5</sup> are as described hereinbefore in relation to a group Y<sup>3</sup> of the formula -A<sup>4</sup>-CO<sub>2</sub>H.

20 A suitable value for A<sup>6</sup> in a group Y<sup>5</sup> of the formula -A<sup>6</sup>-Ar<sup>3</sup>-A<sup>7</sup>-Y<sup>6</sup> is as described hereinbefore for A<sup>1</sup> and for A<sup>7</sup> is as described for A<sup>2</sup>. A suitable value for Ar<sup>3</sup> is as described hereinbefore for Ar<sup>2</sup>. A suitable value for Y<sup>6</sup> in such a group Y<sup>5</sup> when it is *N*-(C<sub>1-4</sub> alkylsulfonyl)carbamoyl is, for example, *N*-methylsulfonylcarbamoyl, *N*-ethylsulfonylcarbamoyl or *N*-propylsulfonylcarbamoyl.

25 When R<sup>3</sup> is a group of the formula:



a suitable value for A<sup>8</sup> is, for example, methylene, ethylene, trimethylene or  
 30 tetramethylene.

Groups R<sup>3</sup> of particular value have the formula -A<sup>1</sup>-Ar<sup>2</sup>-A<sup>2</sup>-Y<sup>1</sup>, especially when Y<sup>1</sup> is a group not of the formula -CON(R)CH(Y<sup>2</sup>)Y<sup>3</sup>, such as carboxy or tetrazol-5-yl.

Specific examples of such groups  $R^3$  are groups  $-A^1-Ar^2-A^2-Y^1$  in which  $A^1$  is a bond or methylene or ethylene,  $Ar^2$  is phenylene, thiophenediyl or tetrazolediyl,  $A^2$  is methylene, ethylene or trimethylene and  $Y^1$  is carboxy or tetrazol-5-yl.

5 Other groups  $R^3$  of particular value have the formula  $-A^5-CON(R)CH(Y^4)Y^5$ , especially when  $Y^5$  is a group of the formula  $-A^6-Ar^3-A^7-Y^6$  in which  $Ar^3$  is phenylene, thiophenediyl or tetrazolediyl.

Specific examples of such groups  $R^3$  are groups  $-A^5-CON(R)CH(Y^4)-A^6-Ar^3-A^7-Y^6$  in which  $A^5$  is methylene or ethylene,  $R$  is  
 10 hydrogen or methyl,  $Y^4$  is carboxy or tetrazol-5-yl,  $A^6$  is a bond or is methylene or ethylene,  $Ar^3$  is phenylene, thiophenediyl or tetrazolediyl,  $A^7$  is methylene, ethylene or trimethylene and  $Y^6$  is carboxy or tetrazol-5-yl.

A suitable pharmaceutically-acceptable salt form of a cyclopenta[g]-quinazoline of the invention is, for example, an acid addition salt with an inorganic or  
 15 organic acid, for example hydrochloric, hydrobromic, trifluoroacetic or maleic acid; or an alkali metal, for example sodium, an alkaline earth metal, for example calcium, or ammonium, for example tetra(2-hydroxyethyl)ammonium, salt.

A suitable pharmaceutically-acceptable ester form of a cyclopenta[g]-quinazoline of the invention is, for example, an ester with an aliphatic alcohol of up  
 20 to 6 carbon atoms, for example a methyl, ethyl or *tert*-butyl ester.

It is to be understood that  $R^3$  may contain several carboxy groups in addition to the carboxy group in the grouping  $-CONHCH(CO_2H)-$ . When, for example, two carboxy groups are present in the cyclopenta[g]quinazoline, a salt or ester may be  
 25 mono-acid-mono-salt or -ester, di-salt or di-ester and when, for example, three carboxy groups are present a salt or ester may be mono-acid-di-salt or -ester, di-acid-mono-salt or -ester or even tri-salt or -ester.

Particularly preferred values for the various symbols  $R^0$ ,  $R^1$ ,  $R^2$  and  $Ar^1$  individually are as expressed for the preferred cyclopenta[g]quinazolines described hereinafter.

30 A preferred cyclopenta[g]quinazoline of the invention has the formula (I) wherein  $R^0$  and  $R^1$  are each independently hydrogen or  $C_{1-4}$  alkyl, especially methyl; wherein  $R^2$  is ethyl, propyl, prop-2-enyl, prop-2-ynyl, 2-hydroxyethyl, 2-fluoroethyl, 2-bromoethyl or 2-cyanoethyl;

wherein Ar<sup>1</sup> is 1,4-phenylene which may optionally bear one or two substituents selected from the group consisting of chloro and especially fluoro, thiophene-2,5-diyl, thiazole-2,5-diyl or pyridine-2,5-diyl;

wherein R<sup>3</sup> is a group of the formula —A<sup>1</sup>—Ar<sup>2</sup>—A<sup>2</sup>—Y<sup>1</sup> in which A<sup>1</sup> is a bond or is methylene or ethylene, Ar<sup>2</sup> is phenylene, A<sup>2</sup> is methylene, ethylene or trimethylene and Y<sup>1</sup> is a group of the formula:



10 in which R is hydrogen, C<sub>1-4</sub> alkyl, C<sub>3-4</sub> alkenyl or C<sub>3-4</sub> alkynyl;

Y<sup>2</sup> is carboxy, tetrazol-5-yl, N-(C<sub>1-4</sub> alkylsulfonyl)carbamoyl, N-(phenylsulfonyl)carbamoyl which may optionally bear one or two substituents on the phenyl ring selected from the group consisting of halogeno, nitro, C<sub>1-4</sub> alkyl and C<sub>1-4</sub> alkoxy, tetrazol-5-ylthio, tetrazol-5-ylsulfinyl or tetrazol-5-ylsulfonyl; and

15 Y<sup>3</sup> is the residue of a naturally occurring amino acid NH<sub>2</sub>CH(CO<sub>2</sub>H)Y<sup>3</sup>.

Alternatively, a preferred cyclopenta[g]quinazoline of the invention has the formula (I) wherein:

R<sup>0</sup> and R<sup>1</sup> are each independently hydrogen or C<sub>1-4</sub> alkyl;

20 R<sup>2</sup> is ethyl, propyl, prop-2-enyl, prop-2-ynyl, 2-hydroxyethyl, 2-fluoroethyl, 2-bromoethyl or 2-cyanoethyl;

Ar<sup>1</sup> is 1,4-phenylene which may optionally bear one or two substituents selected from the group consisting of chloro, fluoro, thiophene-2,5-diyl, thiazole-2,5-diyl or pyridine-2,5-diyl;

R<sup>3</sup> is a group of the formula:

25



in which A<sup>5</sup> is a C<sub>1-6</sub> alkylene group and R is as defined above;

30 Y<sup>4</sup> is carboxy, tetrazol-5-yl, N-(C<sub>1-4</sub> alkylsulfonyl)carbamoyl, N-(phenylsulfonyl)carbamoyl which may optionally bear one or two substituents on the phenyl ring selected from the group consisting of halogeno, nitro, C<sub>1-4</sub> alkyl and C<sub>1-4</sub> alkoxy, tetrazol-5-ylthio, tetrazol-5-ylsulfinyl or tetrazol-5-ylsulfonyl; and

Y<sup>5</sup> is the residue of a naturally occurring amino acid NH<sub>2</sub>CH(CO<sub>2</sub>H)Y<sup>5</sup>.

A preferred value for p is 1.

A further preferred cyclopenta[g]quinazoline of the invention has the formula (I) wherein A is a group OR<sup>0</sup> in which R<sup>0</sup> is hydrogen or methyl;

wherein R<sup>2</sup> is ethyl or prop-2-ynyl; and

wherein Ar<sup>1</sup> is 1,4-phenylene or 1,4-phenylene having a 2-fluoro substituent as in 2,6-difluoro-1,4-phenylene or especially 2-fluoro-1,4-phenylene or is pyridine 2,5-diyl; and

R<sup>3</sup> is as just described above.

An especially preferred cyclopenta[g]quinazoline of the invention has the formula (I) wherein A is a group OR<sup>0</sup> in which R<sup>0</sup> is hydrogen or methyl;

wherein R<sup>2</sup> is ethyl or preferably prop-2-ynyl;

wherein Ar<sup>1</sup> is 1,4-phenylene or 2-fluoro-1,4-phenylene; and

wherein R<sup>3</sup> is the residue of an acid NH<sub>2</sub>.CH(COOH)R<sup>3</sup> which comprises L-Glu-γ-D-Glu, i.e. *N*-L-γ-glutamyl-D-glutamic acid.

Other quinazolines of the invention of particular interest have the values of R<sup>0</sup>, R<sup>1</sup>, R<sup>2</sup>, and Ar<sup>1</sup> and Ar in combination as indicated above but with R<sup>3</sup> having any value as indicated hereinbefore. However, specific particularly preferred cyclopenta[g]quinazolines of the invention are:

*N*-{*N*-{4-[*N*-((6*RS*)-2-methoxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[g]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}-D-glutamic acid;

*N*-{*N*-{4-[*N*-((6*RS*)-2-hydroxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[g]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}-D-glutamic acid; and

*N*-{*N*-{4-[*N*-((6*RS*)-2-hydroxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[g]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}-*N*-methyl-L-glutamic acid;

or a pharmaceutically acceptable salt or ester thereof.

Although the compounds of the present invention can exist as a mixture of stereoisomers it is preferred that they are resolved into one optically active isomeric form. Such a requirement complicates the synthesis of the compounds and it is preferred therefore that they contain as few asymmetric carbon atoms as possible consistent with achieving the desired activity.

As indicated previously, however, the cyclopenta[g]quinazolines of the present invention contain at least two asymmetric carbon atoms. Of these, that at the 6 position of the ring system preferably has the 6*S* orientation rather than the 6*R* orientation, whilst the alpha carbon atom of the group —CONHCH(CO<sub>2</sub>H)—  
5 preferably has the L rather than the D configuration. The preferred compounds (I) described hereinbefore thus preferably have such a configuration at these two asymmetric carbon atoms or less preferably are a racemic mixture in which one or both of these asymmetric carbon atoms is unresolved.

The asymmetric carbon atom of a residue R<sup>3</sup> of the form  
10 —A<sup>1</sup>—Ar<sup>2</sup>—A<sup>2</sup>—CON(R)CH(Y<sup>2</sup>)Y<sup>3</sup> or —A<sup>5</sup>—CON(R)CH(Y<sup>4</sup>)Y<sup>5</sup> may be of the L- or D-configuration but the amide bond will be stabilised *in vivo* when it is of the D-configuration as it will also be when R is other than hydrogen. When Y<sup>3</sup> or Y<sup>5</sup> is the residue of a naturally occurring amino acid, however, the amino acid intermediate for the synthesis of the cyclopenta[g]quinazoline will of course be more readily  
15 available when this asymmetric carbon atom is of the L-configuration.

A cyclopenta[g]quinazoline of the invention may be prepared by any process known to be applicable to the preparation of chemically-related compounds.

As stated above, cyclopenta[g]quinazolines of the present invention are believed to function as anti-cancer agents at least in part due to their ability to inhibit  
20 the enzyme thymidylate synthase. This anti-cancer activity may be assessed, for example, using one or more of the procedures set out below:

(a) An *in vitro* assay which determines the ability of a test compound to inhibit the enzyme thymidylate synthase. Thymidylate synthase may be obtained in partially purified form from L1210 mouse leukaemia cells and utilised in the assay  
25 using the procedures described by Jackman *et al.* (*Cancer Res.*, 1986, **46**, 2810) and Sikora *et al.* (*Biochem. Pharmacol.*, 1988, **37**, 4047);

(b) An assay which determines the ability of a test compound to bind to the  $\alpha$ -FR relative to that of folic acid, using mouse L1210-FBP cells ( $\alpha$ -FR expression) in the procedure described by Westerhof *et al.* (*Cancer Res.*, 1991, **51**,  
30 5507–5513);

(c) An assay which determines the ability of a test compound to inhibit the growth of human tumour cell lines expressing the  $\alpha$ -FR (A431-FBP vulvular carcinoma transfected with the  $\alpha$ -FR; KB nasopharyngeal carcinoma);

(d) An assay which determines the ability of a test compound to inhibit the growth of human tumour cell lines not expressing the  $\alpha$ -FR (A431 neo-transfected);

(e) An assay confirming or demonstrating that compound-induced growth inhibition is largely attributable to  $\alpha$ -FR mediated uptake into KB or A431-FBP cells.

5 This involves the co-addition of an excess of folic acid (1  $\mu$ M) to compete with the compounds for FR but not RFC binding.

Although the pharmacological properties of the cyclopenta[g]quinazolines of the invention depend on their detailed structure, in general the cyclopenta[g]quinazolines of the invention possess activity in one or more of the above tests (a) to (d)  
10 as indicated below:

Test (a)  $IC_{50}$  in the range, for example, 0.0001–1  $\mu$ M;

Test (b) Inverse relative affinity in the range, for example, 0.05–5 (values greater than 1 implies binding is greater than that of folic acid and values less than 1 implies binding weaker than that of folic acid);  
15

Test (c)  $IC_{50}$  in the range, for example, 0.001–10  $\mu$ M;

Test (d)  $IC_{50}$  in the range, for example, 0.01–100  $\mu$ M;

Test (e)  $IC_{50}$  at least 10-fold higher than that seen in test (b) for the same cell line.

20 A cyclopenta[g]quinazoline of the present invention may itself be active or it may be a pro-drug which is converted *in vivo* to an active compound. A cyclopenta[g]quinazoline of the invention may be administered to a warm-blooded animal, including a human, in the form of a pharmaceutical composition which comprises the cyclopenta[g]quinazoline in association with a pharmaceutically-  
25 acceptable diluent or carrier.

The composition may be in a form suitable for oral use, for example a tablet, capsule, aqueous or oily solution, suspension or emulsion; a form suitable for topical use, for example a cream, ointment, gel or aqueous or oily solution or suspension; a form suitable for nasal use, for example a snuff, nasal spray or nasal drops; a form  
30 suitable for vaginal or rectal use, for example a suppository; a form suitable for administration by inhalation, for example as a finely divided powder such as a dry powder, a microcrystalline form or a liquid aerosol; a form suitable for sub-lingual or buccal use, for example a tablet or capsule; or a form suitable for parenteral use (including intravenous, subcutaneous, intramuscular, intravascular or infusion use),

for example a sterile aqueous or oily solution, emulsion or suspension. In general the above compositions may be prepared in a conventional manner using conventional excipients.

The composition may contain, in addition to the cyclopenta[g]quinazoline of the invention, one or more other anti-cancer substances selected from, for example, 5 other antimetabolites, DNA interacting agents, signal transduction inhibitors or other inhibitors of deregulated pathways in tumours.

The cyclopenta[g]quinazoline will normally be administered to a warm-blooded animal at a dose within a range of 50–25000, particularly 50–5000, mg per 10 square metre body area of the animal, i.e. approximately 1500, particularly 1–100, mg/kg. Where desired, however, dosages outside this range may be employed and, in particular, where the preferred mode of administration involving subcutaneous infusion is used then the dose range may be increased to 1–1000 mg/kg. Preferably a 15 daily dose in the range 10–250 mg/kg is employed, particularly 30–150 mg/kg. However, the daily dose will necessarily be varied depending upon the host treated, the particular route of administration and the severity of the illness being treated. Accordingly, the optimum dosage may be determined by the practitioner who is treating any particular patient.

Accordingly the present invention also includes a method for aiding 20 regression and palliation of cancer in a patient, particularly a warm-blooded animal such as a human, in need of such treatment, which comprises administering to said patient an effective amount of a cyclopenta[g]quinazoline as defined hereinbefore. The invention also provides the use of such a cyclopenta[g]quinazoline in the manufacture of a novel medicament for use in the treatment of cancer.

25 Cyclopenta[g]quinazolines of the present invention are of interest for a wide range of anti-tumour activities particularly the treatment of ovarian cancer.

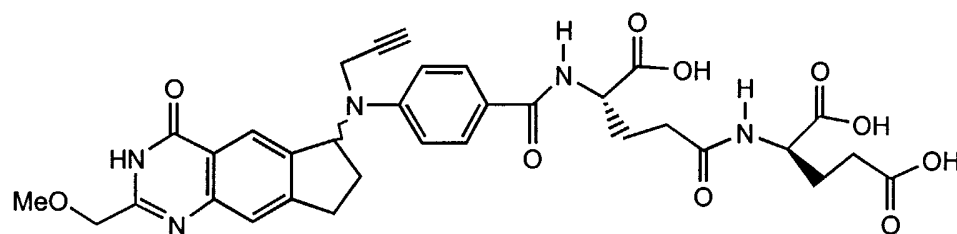
In view of the activity shown by antimetabolites such as aminopterin and methotrexate, which is discussed hereinbefore, the cyclopenta[g]quinazolines of the present invention are also of interest for use in the treatment of other conditions, for 30 example allergic conditions such as psoriasis and inflammatory diseases such as rheumatoid arthritis. In using a cyclopenta[g]quinazoline of the invention for such a purpose the compound will normally be administered at a dose within the range 5–25000, particularly 5–500, mg per square metre body area of the animal, i.e. approximately 0.1–500, particularly 0.1–10, mg/kg. Where desired, however, dosages

outside this range may be employed. In general, for the treatment of an allergic condition such as psoriasis, topical administration of a cyclopenta[g]quinazoline of the invention is preferred. Thus, for example, for topical administration a daily dose in the range, for example, of 0.1 to 10 mg/kg may be used.

5 Compositions containing the quinazolines may be formulated in unit dosage form, i.e. in the form of discrete portions each comprising a unit dose, or a multiple or sub-multiple of a unit dose, for example as a tablet or capsule. Such a unit dosage form may, for example, contain an amount of the cyclopenta[g]quinazoline in the range of 1–250 or 1–500 mg.

10 The invention is illustrated by the following Examples.

**Example 1: synthesis of CB300951 (2-CH<sub>2</sub>OMe derivative of CB300638)**



**CB300951**

15 **5-Methoxyacetamidoindan**

To a solution of 5-aminoindan (4.66 g, 35.0 mmol) in anhydrous DMF (26 ml) was slowly added methoxyacetyl chloride (5.70 g, 52.50 mmol) followed by pyridine (8.5 ml, 105.0 mmol). The red solution was stirred at room temperature for 3.5 hours under argon, then it was partitioned between ethyl acetate (200 ml) and 1N HCl (120 ml). The organic layer was washed with more 1N HCl (120 ml), brine (100 ml), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated *in vacuo*. The residue was triturated with diethyl ether; the white precipitate was collected by filtration, washed with diethyl ether to afford the title compound (5.93 g, 83%), m.p. 104–105 °C; <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>, TMS) 2.06 (m, 2H, 2-CH<sub>2</sub>), 2.87 (m, 4H, 1-CH<sub>2</sub> and 3-CH<sub>2</sub>), 3.50 (s, 3H, OCH<sub>3</sub>), 4.00 (s, 2H, 2-CH<sub>2</sub>OMe), 7.22 (m (overlap with CHCl<sub>3</sub> peak), 2H, 6-H, 7-H), 7.52 (s, 1H, 4-H), 8.18 (s, 1H, CONH); MS (ESI, *m/z*): 432 [(2M+Na)<sup>+</sup>, 30%], 206[(M+H)<sup>+</sup>, 100%]; Found C, 70.10; H, 7.38; N, 6.81; C<sub>12</sub>H<sub>15</sub>NO<sub>2</sub> requires C, 70.22; H, 7.37; N, 6.82%.

20  
25

**5-Methoxyacetamido-6-bromoindan**

A mixture of 5-methoxyacetamidoindan (5.50 g, 0.027 mol) and glacial acetic acid (25 ml) was cooled in an ice-water bath (~10 °C). Bromine (1.5 ml, 0.029 mol) was then dropwise added over a 20 min period while the temperature was kept  
5 between 10-15 °C. The reaction mixture was then stirred for a longer 1 hour and then it was poured into an ice-water bath (100 ml) with the aid of water (70 ml). The precipitate was collected by filtration, washed with plenty of water (150 ml) and dried  
10 *in vacuo* over P<sub>2</sub>O<sub>5</sub> to afford the title compound (6.98 g, 91%), m.p. 84–86 °C; <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>, TMS) 2.09 (m, 2H, 2-CH<sub>2</sub>), 2.88 (m, 4H, 1-CH<sub>2</sub> and  
3-CH<sub>2</sub>), 3.55 (s, 3H, OCH<sub>3</sub>), 4.04 (s, 2H, 2-CH<sub>2</sub>OMe), 7.34, 8.22 (2 x s, 2H, 4-H,  
7-H), 8.83 (s, 1H, CONH);

MS (ESI, *m/z*): 284, 286 [(M+H)<sup>+</sup>, 98%, 100%; Br isotopic pattern]; Found C, 50.62; H, 4.93; N, 4.92; Br, 28.05; C<sub>12</sub>H<sub>14</sub>BrNO<sub>2</sub> requires C, 50.72; H, 4.97; N, 4.93; Br, 28.12%.

15

**5-Methoxyacetamido-6-bromoindan-1-one**

To a solution of 5-methoxyacetamido-6-bromoindan (0.85 g, 3.0 mmol) in glacial acetic acid (7 ml) heated at 55 °C was dropwise added a solution of CrO<sub>3</sub> (1.2 g, 12.0 mmol) in aqueous glacial acetic acid, (7 ml; v/v 1:1) over a 15 min period.  
20 The reaction mixture was then stirred at this temperature for 45 min. The reaction mixture was cooled in an ice-bath, then propan-2-ol (4 ml) was added and the mixture was stirred at this temperature for 10 min before being concentrated *in vacuo*. The black residue was broken up with a spatula with the aid of water and then partitioned  
25 between water (50 ml) and ethyl acetate (150 ml). The aqueous layer was extracted with more ethyl acetate (2 x 40 ml); the combined extracts were dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated *in vacuo* to give an off white residue. Purification by column chromatography on elution with 5% ethyl acetate in dichloromethane afforded in order of elution:

a. **5-methoxyacetamido-6-bromoindan-1-one** as a white solid which was  
30 further purified by trituration with ethyl acetate / hexanes (1:5, v/v): 0.50 g (55%), m.p. 162–163 °C; <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>, TMS) 2.72 (m, 2H, 2-CH<sub>2</sub>), 3.11 (m, 2H, 3-CH<sub>2</sub>), 3.57 (s, 3H, OCH<sub>3</sub>), 4.09 (s, 2H, 2-CH<sub>2</sub>OMe), 7.95 (s, 1H) and 8.65 (s, 1H) (2H, 4-H, 7-H), 9.27 (s, 1H, CONH); MS (ESI, *m/z*) 298, 300 {(M+H)<sup>+</sup>, 100%,

97% respectively, bromine isotopic pattern}; Found: C, 48.13; H, 3.99; N, 4.70; Br, 26.95; C<sub>12</sub>H<sub>12</sub>BrNO<sub>3</sub> requires C, 48.34; H, 4.06; N, 4.70; Br, 26.80%); and

b. **5-acetamido-6-bromoindan-3-one** as a solid which was further purified by trituration with ethyl acetate / hexanes (1:5, v/v): 0.026 g, (3 %), m.p. 149–151 °C  
5 <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>, TMS) 2.71 (m, 2H, 2-CH<sub>2</sub>), 3.01 (m, 2H, 1-CH<sub>2</sub>), 3.56 (s, 3H, OCH<sub>3</sub>), 4.08 (s, 2H, 2-CH<sub>2</sub>OMe), 7.73 (s, 1H) and 8.71 (s, 1H) (2H, 4-H, 7-H), 8.97 (s, 1H, CONH); MS (ESI, *m/z*) 298, 300 {(M+H)<sup>+</sup>, 100%, 98% respectively, bromine isotopic pattern}; Found: C, 47.95; H, 3.96; N, 4.59; Br, 26.63; C<sub>12</sub>H<sub>12</sub>BrNO<sub>3</sub> requires C, 48.34; H, 4.06; N, 4.70; Br, 26.80%).

10

***tert*-Butyl 4-[*N*-(5-methoxyacetamido-6-bromoindan-1-yl)amino]benzoate**

**Method A:** To a flask containing 5-methoxyacetamido-6-bromoindan-1-one (0.357 g, 1.2 mmol), 4-toluenesulfonic acid monohydrate (0.015 g), and *tert*-butyl 4-aminobenzoate (0.289 g, 1.5 mmol) was added 1,2-dimethoxyethane (dried by  
15 distillation over CaH<sub>2</sub>; 15 ml). An Aldrich azeotropic distillation apparatus containing molecular sieves (3A) was fitted to the reaction flask that was placed in an oil bath preheated to 115 °C. The reaction mixture was stirred at this temperature for 3.5 hours under argon; then allowed to cool to room temperature, and a solution of sodium cyanoborohydride in tetrahydrofuran (1M; 1.55 ml, 1.55 mmol) was added  
20 followed immediately by acetic acid (0.044 ml). The black reaction mixture was stirred at room temperature for 1 hour under argon; then it was partitioned between ethyl acetate (150 ml) and saturated aqueous sodium bicarbonate (100 ml). The aqueous layer was extracted with more ethyl acetate (100 ml); the organic extracts were combined, washed with brine (100 ml), dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated *in vacuo* to leave a reddish residue. Purification by column chromatography, on elution  
25 with 35% ethyl acetate in petroleum ether (60–80 °C), afforded the desired product as a white solid: 0.175 g (31 %).

**Method B:** To a nearly clear solution of 5-methoxyacetamido-6-bromoindan-1-one (0.300 g, 1.0 mmol) in anhydrous methanol (40 ml) was added *tert*-butyl  
30 4-aminobenzoate (0.193 g, 1.0 mmol) followed by decaborane (0.044 g). The reaction mixture was stirred at room temperature for 24 hours before being concentrated *in vacuo*. Purification by column chromatography, on elution with 35% ethyl acetate in petroleum ether (60–80 °C), afforded a white solid that was further purified by

reprecipitation from dichloromethane / hexane: 0.340 g, (72%) m.p. 152–153 °C; <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>, TMS) 1.57 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 1.93, 2.63 (2 x m, 2H, indanyl 2-H), 2.97 (m, 2H, indanyl 3-H), 3.55 (s, 3H, OCH<sub>3</sub>), 4.06 (s, 2H, CH<sub>2</sub>OMe), 5.04 (t, J = 6.50 Hz, 1H, 1-H), 6.64 (d, J = 8.78 Hz, 2H, 3,5-H), , 7.51, 8.33 (2 x s, each 1H, indanyl 4-H, 7-H), 7.85 (d, J = 8.75 Hz, 2,6-H), 8.93 (s, 1H, CONH); MS (ESI, *m/z*) 499, 497 {(M+Na)<sup>+</sup>, bromine isotopic pattern}.

***tert*-Butyl 4-[*N*-(5-methoxyacetamido-6-cyanoindan-1-yl)amino]benzoate**

To a solution of *tert*-butyl 4-[*N*-(5-methoxyacetamido-6-bromoindan-1-yl)-amino]benzoate (0.714 g, 1.50 mmol) in NMP (8 ml) [1-methyl-2-pyrrolidone] was added copper (I) cyanide (0.230 g, 2.55 mmol). The reaction mixture was placed in an oil-bath preheated to 140 °C and stirred at this temperature for 2 h. More copper (I) cyanide (0.100 g, 1.10 mmol) was then added and stirring was continued for a longer 3 hours. The reaction mixture was allowed to cool to room temperature, then poured into a mixture of aqueous ammonia (d = 0.88, 7 ml) and ice (~20 ml) and the resulting brown mixture was stirred at room temperature for ~5 min. The brown solid was collected by filtration washed with plenty of water, then suspended in dichloromethane (100 ml). The mixture was stirred at room temperature for 10 min, dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated *in vacuo*. Purification by column chromatography, on elution with 35% ethyl acetate in hexane, afforded an off white solid that was reprecipitated from dichloromethane-ethyl acetate /hexane: 0.328 g, (52%) m.p. 163–164 °C <sup>1</sup>H-NMR (250 MHz, DMSO-d<sub>6</sub>, TMS) 1.50 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 1.85, 2.58 (2 x m, 2H, indanyl 2-H), 2.89 (m, 2H, indanyl 3-H), 3.41 (s, 3H, OCH<sub>3</sub>), 4.05 (s, 2H, CH<sub>2</sub>OMe), 5.06 (m, 1H, indanyl 1-H), 6.73 (d, J = 8.82 Hz, 2H, 3,5-H), 6.82 (d, J = 8.37 Hz, 1H, N<sup>10</sup>-H), 7.59, 7.57 (2 x s, each 1H, indanyl 4-H, 7-H), 7.66 (d, J = 8.77 Hz, 2,6-H), 9.88 (s, 1H, CONH); MS (ESI, *m/z*) 444 {(M+Na)<sup>+</sup>, 100%}; Found: C, 68.21; H, 6.47; N, 9.81; C<sub>24</sub>H<sub>27</sub>N<sub>3</sub>O<sub>4</sub> requires C, 68.39; H, 6.46; N, 9.97%.

***tert*-Butyl 4-{*N*-[(6*RS*)-2-methoxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]amino}benzoate**

A mixture of *tert*-butyl 4-[*N*-(5-methoxyacetamido-6-cyanoindan-1-yl)-amino]benzoate (0.295 g, 0.70 mmol), ethanol (3.2 ml), and water (0.64 ml) was cooled in an ice-bath, then 30% aqueous H<sub>2</sub>O<sub>2</sub> solution (0.60 ml) was added followed

by granulated sodium hydroxide pellets (0.047 g, 1.19 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 30 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 (~15 ml). The pH of this mixture was adjusted to ~12 with 1N NaOH (got a clear solution), then to ~4 with 1N hydrochloric acid. The off white precipitate was collected by filtration, washed with water, dried *in vacuo* over P<sub>2</sub>O<sub>5</sub>: 0.262 g (89%), m.p. >122 °C (softens); <sup>1</sup>H-NMR (250 MHz, DMSO-d<sub>6</sub>, TMS) 1.50 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 1.87, 2.56 (2 x m, each 1H, 7-H), 3.00 (m, 2H, 8-H), 3.34 (s, 3H, OCH<sub>3</sub>), 4.30 (s, 2H, 2-CH<sub>2</sub>), 5.16 (m, 1H, 6-H), 6.78 (d, J = 8.55 Hz, 2H, 3',5'-H), 6.89 (d, J = 8.10, N<sup>10</sup>-H), 7.52, 7.90 (2 x s, each 1H, 5-H, 9-H), 7.67 (d, J = 8.45 Hz, 2',6'-H), 12.12 (s, 1H, N<sup>3</sup>-H); MS (ESI, *m/z*) 444 {(M+Na)<sup>+</sup>, 40%}; Found: C, 67.19; H, 6.31; N, 9.69; C<sub>24</sub>H<sub>27</sub>N<sub>3</sub>O<sub>4</sub> · 0.5H<sub>2</sub>O requires C, 66.96; H, 6.50; N, 9.76%.

#### 15 **Dicobalthexacarbonyl propargyl alcohol complex**

This is a known compound (K.-D. Roth and U. Muller, *Tetrahedron Letters* 1993, **34**, 2919) and in this study was prepared according to Nicholas' methodology (K. L. Salazar and K. M. Nicholas, *Tetrahedron* 2000, **56**, 2211): To a round bottom flask charged with Co<sub>2</sub>(CO)<sub>6</sub> (5.12 g, 15.0 mmol) under argon in a well ventilated hood was added anhydrous dichloromethane (170 ml) followed by a solution of propargyl alcohol (0.840 g, 15.0 mmol) in anhydrous dichloromethane (20 ml). 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 desired product as a red solid 4.10 g (80%); <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>, TMS) 1.83 (t, J = 6.0 Hz, 1H, OH), 4.80 (d, J = 6.0 Hz, 2H, CH<sub>2</sub>), 6.08 (s, 1H, C-H).

#### 30 **(Propargyl)Co<sub>2</sub>(CO)<sub>6</sub><sup>+</sup>BF<sub>4</sub><sup>-</sup>**

This is a known compound (K.-D. Roth and U. Muller, *Tetrahedron Letters* 1993, **34**, 2919) and in this study was prepared according to Nicholas' methodology (K. L. Salazar and K. M. Nicholas, *Tetrahedron* 2000, **56**, 2211): To a round bottom flask charged with dicobalthexacarbonyl propargyl alcohol complex (1.60 g, 4.7

mmol) under argon was added (synged via a septum) propionic acid (2.2 ml). The reaction mixture was cooled to  $-20\text{ }^{\circ}\text{C}$  and then a solution of  $\text{HBF}_4$  in diethyl ether (54% w/w, 2.05 ml) was slowly synged into the reaction mixture via a septum. The reaction mixture was stirred at  $-20\text{ }^{\circ}\text{C}$  for 40 min, then cooled diethyl ether (50 ml) was added. Trituration afforded a red precipitate that was collected by filtration, washed with plenty of dry diethyl ether and dried *in vacuo* over  $\text{P}_2\text{O}_5$ : 1.71 g (90%). This was immediately used in the next reaction without any further purification.

***tert*-Butyl 4-{*N*-[(6*RS*)-2-methoxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoate**

To a round-bottomed flask containing the tetrafluoroborate salt (propargyl) $\text{Co}_2(\text{CO})_6^+\text{BF}_4^-$  (0.271 g, 0.66 mmol) was added anhydrous dichloromethane (dried by distillation over  $\text{P}_2\text{O}_5$ ; 22 ml). The nearly clear red dark solution was stirred at room temperature for few minutes under argon, then *tert*-butyl 4-{*N*-[(6*RS*)-2-methoxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]amino}benzoate (0.215 g, 0.51 mmol) was added in one portion. Stirring was continued at this temperature for 5 min then diisopropylethylamine (0.18 ml, 1.04 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 (150 ml) and brine (60 ml). The aqueous layer was extracted with more ethyl acetate (2 x 50 ml). The combined extracts were washed with 10% aqueous citric acid (50 ml), brine (50 ml), dried ( $\text{Na}_2\text{SO}_4$ ), and concentrated *in vacuo*. Purification by column chromatography, on elution with 40% ethyl acetate in dichloromethane, gave a red solid: 0.285 g (75%). To a solution of this complex (0.267 g, 0.36 mmol) in ethanol (60 ml) was added  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  (~ 8.0 g). The clear solution was stirred at room temperature for 10 min then a second portion of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  (~4.0 g) was added. The reaction mixture was stirred at room temperature for a longer 5 min then a final portion of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  (~5.0 g) was added; the nearly clear solution was turned into a dark red mixture. Stirring was continued at room temperature for an extra 25 min, then the reaction mixture was partitioned between ethyl acetate (150 ml) and dilute brine (700 ml). The aqueous layer was extracted with more ethyl acetate (2 x 70 ml), The combined organics were washed with brine (3 x 70 ml), dried ( $\text{Na}_2\text{SO}_4$ ), and concentrated *in vacuo*. Purification by column chromatography, on elution with 2% methanol in dichloromethane, afforded a white solid which was reprecipitated

from dichloromethane / hexane: 0.122 g (74%), m.p. 191–192 °C; <sup>1</sup>H-NMR (250 MHz, DMSO-d<sub>6</sub>, TMS) 1.51 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 2.23, 2.53 (m-obscured by DMSO peak) (2 x m, each 1H, 7-H), 2.95-3.20 (m, 3H, C≡CH, 8-H), 3.34 (s (obscured by the H<sub>2</sub>O peak), 3H, OCH<sub>3</sub>), 3.96 (ABq, J = 18.0 Hz, 2H, CH<sub>2</sub>C≡C), 4.31 (s, 2H, 2-CH<sub>2</sub>),  
 5 5.79 (t, J = 7.0 Hz, 1H, 6-H), 7.02 (d, J = 9.01 Hz, 2H, 3',5'-H), 7.58, 7.80 ( 2 x s, each 1H, 5-H, 9-H), 7.76 (d, J = 8.45 Hz, 2',6'-H), 12.17 (s, 1H, N<sup>3</sup>-H); MS (ESI, *m/z*) 482 {(M+Na)<sup>+</sup>, 10%}; Found: C, 70.32; H, 6.31; N, 9.09; C<sub>27</sub>H<sub>29</sub>N<sub>3</sub>O<sub>4</sub> requires C, 70.57; H, 6.36; N, 9.14%.

10 **4-{N-[(6RS)-2-Methoxymethyl-4-oxo-3,4,7,8-tetrahydro-6H-cyclopenta-  
[g]quinazolin-6-yl]-N-(prop-2-ynyl)amino}benzoic acid**

A solution of *tert*-butyl 4-{N-[(6RS)-2-methoxymethyl-4-oxo-3,4,7,8-tetrahydro-6H-cyclopenta[g]quinazolin-6-yl]-N-(prop-2-ynyl)amino}benzoate (0.069 g, 0.15 mmol) in dichloromethane (1 ml) and trifluoroacetic acid (3 ml) was stirred at  
 15 room temperature for 1 hour and 10 min, 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<sub>2</sub>O<sub>5</sub> to afford the title compound as the trifluoroacetate salt: 0.061 g, m.p. 225 °C (dec); <sup>1</sup>H-NMR (250 MHz, DMSO-d<sub>6</sub>, TMS) 2.23, 2.53 (obscured by DMSO peak) (2 x m, each 1H, 7-H),  
 20 2.90-3.20 (m, 3H, C≡CH, 8-H), 3.34 (s (obscured by the H<sub>2</sub>O peak), 3H, OCH<sub>3</sub>), 3.97 (ABq, J = 18.0 Hz, 2H, CH<sub>2</sub>C≡C), 4.32 (s, 2H, 2-CH<sub>2</sub>), 5.79 (t, J = 8.1 Hz, 1H, 6-H), 7.03 (d, J = 9.02 Hz, 2H, 3',5'-H), 7.58, (s, 1H, 9-H), 7.81 (m, 3H, 5-H, 2',6'-H), 12.17 (s, 1H, N<sup>3</sup>-H); MS (ESI, *m/z*) 426 {(M+Na)<sup>+</sup>, 25%}, 404 {(M+H)<sup>+</sup>, 70%}.

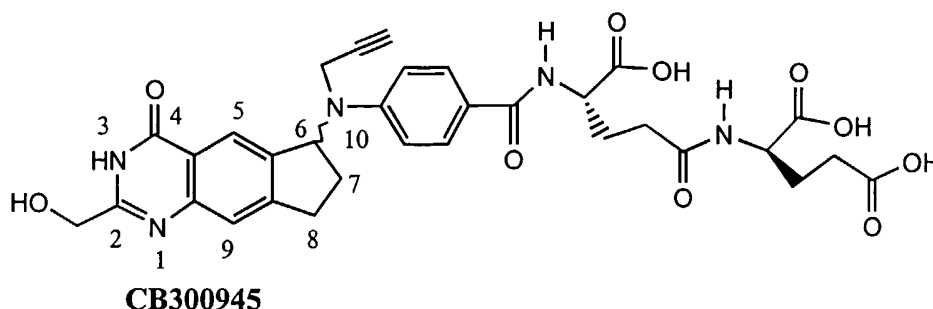
25 **Tri-*tert*-butyl N-{N-[4-[N-((6RS)-2-methoxymethyl-4-oxo-3,4,7,8-tetrahydro-6H-cyclopenta[g]quinazolin-6-yl)-N-(prop-2-ynyl)amino]benzoyl]-  
-L-γ-glutamyl]-D-glutamate**

To a mixture of 4-{N-[(6RS)-2-methoxymethyl-4-oxo-3,4,7,8-tetrahydro-6H-cyclopenta[g]quinazolin-6-yl]-N-(prop-2-ynyl)amino}benzoic acid trifluoroacetate salt (0.056 g, ~0.14 mmol), tri-*tert*-butyl L-γ-glutamyl-D-glutamate (0.090 g, 0.20 mmol) and anhydrous DMF (2.5 ml) was added diethyl cyanophosphonate (0.050 g, 0.31 mmol) followed by triethylamine (0.035 g, 0.35 mmol). The clear solution was stirred at room temperature for 2 hours, then it was partitioned between

ethyl acetate (150 ml) and water (80 ml). The aqueous layer was extracted with ethyl acetate (2 x 70 ml). The combined organics were washed with 10% aqueous citric acid (2 x 40 ml), saturated sodium bicarbonate solution (100 ml) and brine (100 ml), dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated *in vacuo*. Purification by column chromatography, on elution with 1.5% methanol in ethyl acetate, afforded a white solid that was further purified by trituration with hexane with the aid of some dichloromethane: 0.072 g (64 %); m.p. >120 °C; <sup>1</sup>H-NMR (250 MHz, DMSO-d<sub>6</sub>, TMS) 1.38, 1.39, 1.41 (3 x s, 27H, 3 x C(CH<sub>3</sub>)<sub>3</sub>), 1.60-2.35 (m, 9H, 2 x β-CH<sub>2</sub>, 2 x γ-CH<sub>2</sub>, 7-H), 2.52 (m obscured by DMSO peak, 1H, 7-H), 2.90-3.25 (m, 3H, C≡CH, 8-H), 3.97 (ABq, J = 17.0 Hz, 2H, CH<sub>2</sub>C≡C), 4.08, 4.12 (2 x m, 2H, 2 x α-CH), 4.32 (s, 2H, 2-CH<sub>2</sub>), 5.77 (t, J = 7.50 Hz, 1H, 6-H), 7.02 (d, J = 8.85 Hz, 2H, 3',5-H), 7.58 (s, 1H, 9-H), 7.80 (d, J = 9.0 Hz, 2H, 2',6'-H), 7.82 (s, 1H, 5-H), 8.17 (d, J = 8.12 Hz, 1H, CONH), 8.36 (d, J = 7.00 Hz, 1H, CONH), 12.16 (s, 1H, N<sup>3</sup>-H); MS (ESI, *m/z*) 852 {(M+Na)<sup>+</sup>, 20%}, 830 {(M+H)<sup>+</sup>, 100%}; Found C, 64.71; H, 7.21; N, 8.27. C<sub>45</sub>H<sub>59</sub>N<sub>5</sub>O<sub>10</sub> requires C, 65.12; H, 7.17; N, 8.44%.

***N*-{*N*-{4-[*N*-((6*RS*)-2-Methoxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-*L*-γ-glutamyl}-*D*-glutamic acid**

A solution of tri-*tert*-butyl *N*-{*N*-{4-[*N*-((6*RS*)-2-methoxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]-benzoyl}-*L*-γ-glutamyl}-*D*-glutamate (0.056 g, 0.07 mmol) in trifluoroacetic acid (4.5 ml) was stirred at room temperature for 1 hour and 10 min with protection from the light. The solvent was then removed *in vacuo* and the residue was suspended in water (4 ml). The pH was adjusted to ~10 with 1N NaOH, then to ~4 with 1N hydrochloric acid. The white precipitate was collected by filtration and dried *in vacuo* over P<sub>2</sub>O<sub>5</sub>: 0.020 g (45%), m.p. 150–155 °C (softens); <sup>1</sup>H-NMR (250 MHz, DMSO-d<sub>6</sub>, TMS) 1.60-2.30 (m, 9H, 2 x β-CH<sub>2</sub>, 2 x γ-CH<sub>2</sub>, 7-H), 2.52 (m obscured by DMSO peak, 1H, 7-H), 2.90-3.25 (m, 3H, C≡CH, 8-H), 3.36 (s, 3H, OCH<sub>3</sub>), 3.97 (ABq, J = 17.0 Hz, 2H, CH<sub>2</sub>C≡C), 4.20, 4.34 (2 x m, 2H, 2 x α-CH), 4.32 (s, 2H, 2-CH<sub>2</sub>), 5.77 (t, J = 8.02 Hz, 1H, 6-H), 7.02 (d, J = 8.85 Hz, 2H, 3',5-H), 7.58 (s, 1H, 9-H), 7.81 (d, J = 9.0 Hz, 2H, 2',6'-H), 7.83 (s, 1H, 5-H), 8.14 (d, J = 8.12 Hz, 1H, CONH), 8.33 (d, J = 7.78 Hz, 1H, CONH), 12.10 (s, 1H, N<sup>3</sup>-H); MS (ESI, *m/z*) 662 {(M+H)<sup>+</sup>, 100%}.

**Example 2: synthesis of CB300945 (2-CH<sub>2</sub>OH derivative of CB300638)****5            2-Hydroxymethyl-3,4,7,8-tetrahydro-6H-cyclopenta[g]quinazolin-4-one**

A solution of caesium acetate (14.4 g, 75.2 mmol) in dry DMF (40 ml) was heated to 60 °C under argon for 30 min. The mixture was cooled to 40 °C and a suspension of 2-chloromethyl-3,4,7,8-tetrahydro-6H-cyclopenta[g]quinazolin-4-one (L. Skelton, V. Bavetsias, A. Jackman, WO 00/050417-A1; 2.2 g, 9.4 mmol) in dry

10 DMF (60 ml) was added via a cannula. The mixture was heated to 80 °C under argon for 16 h. The mixture was cooled to room temperature and the solvent was removed *in vacuo*. The residue was suspended in water (50 ml) and MeOH (20 ml). The pH was adjusted to 12.5 with 1M sodium hydroxide solution and the brown suspension was stirred for 2 h at room temperature. The insoluble brown solid was removed by

15 filtration and the resulting solution was acidified to pH 5 with 1M hydrochloric acid. The precipitate was collected by filtration, washed with acidified water and dried *in vacuo* over P<sub>2</sub>O<sub>5</sub> to yield the product as a pale yellow solid (1.17g, 58%); m.p. 205–210 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 2.07 (quin, J = 7.4 Hz, 2H, 7-H), 2.98 (q, J = 6.95 Hz, 4H, 6-H and 8-H), 4.38 (s, 2H, 2-CH<sub>2</sub>), 7.46 (s, 1H, 9-H), 7.92 (s, 1H, 5-H); MS

20 (FAB-m/z): Found 217 [(M+H)<sup>+</sup>, 100%]; HRMS: measured 217.0977; calculated for C<sub>12</sub>H<sub>13</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup>: 217.0977; Found C, 64.01; H, 5.23; N, 12.34. C<sub>12</sub>H<sub>13</sub>N<sub>2</sub>O<sub>2</sub>·½H<sub>2</sub>O requires C, 63.93; H, 5.77; N, 12.43%.

**25            2-(2,2-Dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6H-cyclopenta-  
[g]quinazolin-4-one**

2-Hydroxymethyl-3,4,7,8-tetrahydro-6H-cyclopenta[g]quinazolin-4-one (1.0 g, 4.6 mmol), triethylamine (0.77 ml, 5.6 mmol), DMAP (50 mg, 0.4 mmol) and anhydrous CH<sub>2</sub>Cl<sub>2</sub> (50 ml) were mixed in a flask under argon. Pivalic anhydride (1.2

ml, 6.0 mmol) was added dropwise and the suspension stirred at room temperature under argon for 5 h. The solvent was removed *in vacuo* and the residue partitioned between EtOAc (100 ml) and saturated aqueous NaHCO<sub>3</sub> (100 ml). The organic extract was washed with saturated aqueous NaHCO<sub>3</sub> (70 ml), water (70 ml), brine (70 ml), dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent removed *in vacuo*. The residue was triturated with hexane (60 ml) and the product collected by filtration as a yellow solid (1.21 g, 87%); m.p. 185–190°C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 1.22 (s, 9H, CMe<sub>3</sub>), 2.07 (quin, J = 7.4 Hz, 2H, 7-H), 2.98 (q, J = 5.72 Hz, 4H, 6-H and 8-H), 4.94 (s, 2H, 2-CH<sub>2</sub>), 7.42 (s, 1H, 9-H), 7.92 (s, 1H, 5-H), 12.20 (br, 1H, NH); MS (FAB, *m/z*): Found 301 [(M+H)<sup>+</sup>, 100%]; HRMS: measured 301.1539; calculated for C<sub>17</sub>H<sub>21</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup>: 301.1552; Found C, 67.65; H, 6.54; N, 9.54. C<sub>17</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> requires C, 67.98; H, 6.71; N, 9.33%.

**2-(2,2-Dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6H-cyclopenta[g]quinazolin-4,6-dione and 2-(2,2-Dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6H-cyclopenta[g]quinazolin-4,8-dione**

To a stirred solution of (Ph<sub>3</sub>SiO)<sub>2</sub>CrO<sub>2</sub> (L. M. Baker and W. L. Carrick, *J. Org. Chem.* 1970, **35**, 774) (10.6 mg, 0.017 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 ml) was added sequentially aqueous 70% *tert*-butyl hydroperoxide (0.18 ml, 1.3 mmol) and 2-(2,2-dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6H-cyclopenta[g]quinazolin-4-one (0.1 g, 0.33 mmol). The mixture was stirred at room temperature with protection from the light for 24 h. The solvents were removed *in vacuo* and the residue purified by column chromatography (20 g of silica gel) eluting with a gradient of 10–30% EtOAc in CHCl<sub>3</sub> to yield 2-(2,2-dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6H-cyclopenta[g]quinazolin-4,6-dione as a white solid (47 mg, 45%); m.p. 185–190°C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 1.23 (s, 9H, CMe<sub>3</sub>), 2.72 (m, 2H, 7-H), 3.25 (m, 2H, 8-H), 5.00 (s, 2H, 2-CH<sub>2</sub>), 7.70 (s, 1H, 9-H), 8.29 (s, 1H, 5-H), 12.20 (br, 1H, NH); MS (FAB, *m/z*): Found 315 [(M+H)<sup>+</sup>, 100%], 337 [(M+Na)<sup>+</sup>, 75%]; HRMS: measured 315.1360; calculated for C<sub>17</sub>H<sub>19</sub>N<sub>2</sub>O<sub>4</sub> (M+H)<sup>+</sup>: 315.1345; Found C, 64.18; H, 5.72; N, 8.81. C<sub>17</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>.0.2H<sub>2</sub>O requires C, 64.23; H, 5.79; N, 8.82%.

2-(2,2-Dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6H-cyclopenta[g]-quinazolin-4,8-dione; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 1.23 (s, 9H, CMe<sub>3</sub>), 2.76 (m, 2H, 7-H),

3.26 (m, 2H, 8-H), 4.98 (s, 2H, 2-CH<sub>2</sub>), 7.72 (s, 1H, 9-H), 8.29 (s, 1H, 5-H), 12.3 (br, 1H, NH).

**tert-Butyl 4-[N-((6RS)-2-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6H-cyclopenta[g]quinazolin-6-yl)amino]benzoate**

A suspension of 2-(2,2-dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6H-cyclopenta[g]quinazolin-4,6-dione (0.47g, 1.50 mmol) in anhydrous methanol (33 ml) and anhydrous CH<sub>2</sub>Cl<sub>2</sub> (5 ml) was treated with *tert*-butyl 4-aminobenzoate (0.34 g, 1.78 mmol) followed by decaborane (0.07g, 0.58 mmol) and the mixture stirred at room temperature under argon for 18 h. The solvent was removed *in vacuo* and the residue purified by column chromatography (50g of silica gel) eluting with 30% ethyl acetate in CH<sub>2</sub>Cl<sub>2</sub> to yield the desired product as a white solid (0.43g, 58%); m.p. 231 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 1.26 (s, 9H, CMe<sub>3</sub>), 1.58 (s, 9H, CO<sub>2</sub>CMe<sub>3</sub>), 2.00 (m, 1H, 7-H), 2.72 (m, 1H, 7-H), 3.08 (m, 2H, 8-H), 5.10 (s, 2H, 2-CH<sub>2</sub>), 5.15 (m, 1H, 6-H), 6.67 (d, J = 8.8 Hz, 2H, 3'-H, 5'-H), 7.58 (s, 1H, 9-H), 7.87 (d, J = 8.8 Hz, 2H, 2'-H, 6'-H), 8.24 (s, 1H, 5-H); MS (FAB, *m/z*): Found 491 [(M+H)<sup>+</sup>, 25%], 514 [(M+Na)<sup>+</sup>, 100%]; Found C, 68.37; H, 6.86; N, 8.35. C<sub>28</sub>H<sub>33</sub>N<sub>3</sub>O<sub>5</sub> requires C, 68.41; H, 6.77; N, 8.55%.

**tert-Butyl 4-[N-((6RS)-2-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6H-cyclopenta[g]quinazolin-6-yl)-N-(prop-2-ynyl)amino]benzoate**

A suspension of (propargyl)Co<sub>2</sub>(CO)<sub>6</sub><sup>+</sup>BF<sub>4</sub><sup>-</sup> (213 mg, 0.52 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (25 ml) was treated with *tert*-butyl 4-[N-((6RS)-2-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6H-cyclopenta[g]quinazolin-6-yl)-amino]benzoate (200 mg, 0.41 mmol) and the red solution stirred at room temperature under argon for 15 minutes. Diisopropylethylamine (0.15 ml, 0.86 mmol) was added and the mixture stirred at room temperature under argon for 1 h. The mixture was partitioned between ethyl acetate (30 ml) and brine (30 ml). The organic extract was dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent removed *in vacuo*. The residue was purified by column chromatography (20g of silica gel) eluting with a gradient of 0-10% ethyl acetate in CH<sub>2</sub>Cl<sub>2</sub> to yield the complex as a red oil (191 mg, 58%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 1.26 (s, 9H, CMe<sub>3</sub>), 1.59 (s, 9H, CO<sub>2</sub>CMe<sub>3</sub>), 2.31 (m, 1H, 7-H), 2.62 (m, 1H, 7-H), 3.13 (m, 2H, 8-H), 4.57 (AB system, J = 16.9 Hz, 2H, propargyl CH<sub>2</sub>), 5.09 (s, 2H,

2-CH<sub>2</sub>), 5.63 (t, J = 8.3, 1H, 6-H), 5.98 (s, 1H, propargyl CH), 6.91 (d, J = 8.9 Hz, 2H, 3'-H, 5'-H), 7.61 (s, 1H, 9-H), 7.90 (d, J = 8.9 Hz, 2H, 2'-H, 6'-H), 8.14 (s, 1H, 5-H), 10.25 (br s, 1H).

A solution of this complex (186 mg, 0.23 mmol) in ethanol (30 ml) was treated with Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O (1.1 g) and the solution stirred at room temperature for 2 h. The solution was partitioned between ethyl acetate (30 ml) and water (30 ml). The organic extract was washed with brine (30 ml), dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent removed *in vacuo*. The residue was purified by column chromatography (20g of silica gel) eluting with 10% ethyl acetate in CH<sub>2</sub>Cl<sub>2</sub> to yield the desired product as a white solid (94 mg, 78%); m.p. 134 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 1.32 (s, 9H, CMe<sub>3</sub>), 1.61 (s, 9H, CO<sub>2</sub>CMe<sub>3</sub>), 2.23 (s, 1H, propargyl CH), 2.38 (m, 1H, 7-H), 2.62 (m, 1H, 7-H), 3.07 (m, 1H, 8-H), 3.25 (m, 1H, 8-H), 3.94 (AB system, J = 18.6 Hz, 2H, propargyl CH<sub>2</sub>), 5.12 (s, 2H, 2-CH<sub>2</sub>), 5.68 (t, J = 8.2 Hz, 1H, 6-H), 6.99 (d, J = 9.1 Hz, 2H, 3'-H, 5'-H), 7.63 (s, 1H, 9-H), 7.95 (d, J = 9.0 Hz, 2H, 2'-H, 6'-H), 8.16 (s, 1H, 5-H), 9.55 (br s, 1H); MS (ESI, *m/z*) 552 {(M+Na)<sup>+</sup>, 100%}, 530 {(M+H)<sup>+</sup>, 20%}; Found C, 70.14; H, 6.80; N, 7.73. C<sub>31</sub>H<sub>35</sub>N<sub>3</sub>O<sub>5</sub> requires C, 70.30; H, 6.66; N, 7.93%.

**4-[N-((6*RS*)-2-(2,2-Dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-N-(prop-2-ynyl)amino]benzoic acid**

A solution of *tert*-butyl 4-[N-((6*RS*)-2-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-N-(prop-2-ynyl)amino]benzoate (80 mg, 0.15 mmol) in trifluoroacetic acid (5 ml) was stirred at room temperature with protection from the light for 1.5h. The solvent was removed *in vacuo* and the residue triturated with 1:1 diethyl ether and hexane to yield the desired product as a white solid (81 mg, TFA salt); m.p. 133 °C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 1.23 (s, 9H, CO<sub>2</sub>CMe<sub>3</sub>), 2.22 (m, 1H, 7-H), 2.50 (m, 1H, 7-H), 3.03 (m, 2H, 8-H), 3.14 (s, 1H, propargyl CH), 3.97 (AB system, J = 18.8 Hz, 2H, propargyl CH<sub>2</sub>), 4.95 (s, 2H, 2-CH<sub>2</sub>), 5.79 (t, J = 8.6 Hz, 1H, 6-H), 7.03 (d, J = 9.0 Hz, 2H, 3'-H, 5'-H), 7.51 (s, 1H, 9-H), 7.81 (d, J = 6.6 Hz, 2H, 2'-H, 6'-H), 7.83 (s, 1H, 5-H).

**Tri-*tert*-butyl N-{N-[4-[N-((6*RS*)-2-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-N-(prop-2-ynyl)amino]benzoyl]-L-γ-glutamyl]-D-glutamate**

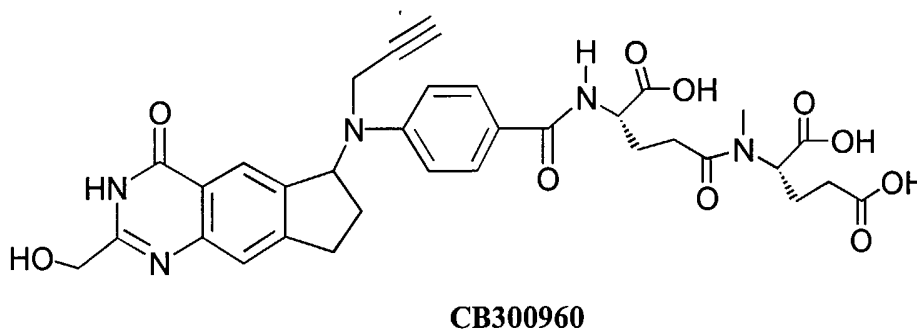
A solution of 4-[*N*-((6*RS*)-2-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoic acid (80 mg, 0.15 mmol) in anhydrous dimethylformamide (7 ml) was treated with tri-*tert* butyl-L- $\gamma$ -glutamyl-D-glutamate (150 mg, 0.33 mmol), diethyl cyanophosphonate (0.06 ml, 0.40 mmol) and triethylamine (0.06 ml, 0.40 mmol). The solution was stirred at room temperature under argon with protection from the light for 2.5 h. The solution was partitioned between ethyl acetate (25 ml) and water (25 ml). The aqueous layer was extracted with ethyl acetate (2 x 20 ml). The combined organic extracts were washed with 10% aqueous citric acid (2 x 30 ml), saturated aqueous NaHCO<sub>3</sub> (30 ml), dilute brine (30 ml), dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent removed *in vacuo*. The residue was purified by column chromatography (30g of silica gel) eluting with 40% ethyl acetate in CH<sub>2</sub>Cl<sub>2</sub> to yield the desired product as a white solid (94 mg, 62%); m.p. 109 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  1.29 (s, 9H, -COOMe<sub>3</sub>), 1.43 (s, 9H, COOMe<sub>3</sub>), 1.47 (s, 9H, COOMe<sub>3</sub>), 1.48 (s, 9H, COOMe<sub>3</sub>), 1.60-2.10 (m, 5H, 2 x glu  $\beta$ -CH<sub>2</sub>, 7-CH), 2.21 (s, 1H, propargyl CH), 2.22-2.50 (m, 4H, 2 x glu  $\gamma$ -CH<sub>2</sub>), 2.59 (m, 1H, 7-H), 3.08 (m, 1H, 8-H), 3.20 (m, 1H, 8-H), 3.92 (AB system, J = 19.0 Hz, 2H, propargyl CH<sub>2</sub>), 4.48, 4.76 (2 x m, 2H, 2 x glu  $\alpha$ -CH), 5.12 (s, 2H, 2-CH<sub>2</sub>), 5.64 (t, J = 8.1 Hz, 1H, 6-H), 6.99 (d, J = 8.8 Hz, 2H, 3'-H, 5'-H), 7.07 (m, 2H, 2 x CONH), 7.64 (s, 1H, 9-H), 7.80 (d, J = 8.8 Hz, 2H, 2'-H, 6'-H), 8.13 (s, 1H, 5-H); MS (ESI, *m/z*) 922 {(M+Na)<sup>+</sup>, 100%}, 900 {(M+H)<sup>+</sup>, 40%}; Found C, 64.85; H, 7.23; N, 7.33. C<sub>49</sub>H<sub>65</sub>N<sub>5</sub>O<sub>11</sub>.0.5H<sub>2</sub>O requires C, 64.76; H, 7.27; N, 7.71%.

***N*-{*N*-{4-[*N*-((6*RS*)-2-Hydroxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L- $\gamma$ -glutamyl}-D-glutamic acid**

Tri-*tert*-butyl *N*-{*N*-{4-[*N*-((6*RS*)-2-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L- $\gamma$ -glutamyl}-D-glutamate (80 mg, 0.09 mmol) was dissolved in trifluoroacetic acid (5 ml) and stirred at room temperature with protection from the light for 1 h. The solvent was removed *in vacuo* and the residue dissolved in methanol (3 ml) and water (3 ml). The pH of the solution was adjusted to pH 12 with 1M sodium hydroxide solution and stirred at room temperature for 6 h. The solution was acidified to pH 4 with 1M hydrochloric acid and cooled to 0°C. The precipitate was collected

by filtration and dried under vacuum over P<sub>2</sub>O<sub>5</sub> to yield the desired product as a pale brown solid (27 mg, 47%); m.p. 172 °C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 1.60-2.10 (m, 5H, 2 x glu β-CH<sub>2</sub>, 7-CH), 2.15-2.40 (m, 5H, 2 x glu γ-CH<sub>2</sub>, 7-H), 2.99 (m, 1H, 8-H), 3.12 (s, 1H, propargyl CH), 3.16 (m, 1H, 8-H), 3.98 (AB system, J = 19.9 Hz, 2H, propargyl CH<sub>2</sub>), 4.18, 4.30 (2 x m, 2H, 2 x glu α-CH), 4.36 (s, 2H, 2-CH<sub>2</sub>), 5.58 (br s, 1H, -OH), 5.77 (t, J = 7.9 Hz, 1H, 6-H), 7.01 (d, J = 8.9 Hz, 2H, 3'-H, 5'-H), 7.54 (s, 1H, 9-H), 7.80 (d, J = 8.5 Hz, 2H, 2'-H, 6'-H), 7.82 (s, 1H, 5-H), 8.15 (d, J = 7.5 Hz, 1H), 8.35 (d, J = 7.2 Hz, 1H) (2 x CONH); MS (ESI, *m/z*) 670 {(M+Na)<sup>+</sup>, 45%}, 648 {(M+H)<sup>+</sup>, 100%}; HRMS: measured 648.2313; calculated for C<sub>32</sub>H<sub>35</sub>N<sub>5</sub>O<sub>10</sub> (M+H)<sup>+</sup>: 648.2306.

**Example 3: synthesis of CB300960 (*N*-methyl derivative of CB300945)**



15

**4-{*N*-[(6*RS*)-2-Hydroxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoic acid**

**Method A:** A solution of *tert*-butyl 4-{*N*-[(6*RS*)-2-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoate (0.150 g, 0.28 mmol) in dichloromethane (2 ml) and trifluoroacetic acid (6 ml) was stirred at room temperature for 1 hour. The solvents were then removed in vacuo, and the residue was suspended in methanol (3 ml) and water (5 ml). The pH was adjusted to ~10 with 1N NaOH (1.1 ml), and the mixture was stirred at room temperature for 4 hours. The reaction mixture was diluted with water (5 ml) and the pH was adjusted to ~5 with 1N HCl. The solid was then collected by filtration, but <sup>1</sup>H-NMR indicated no complete removal of the pivaloyl group. This solid was suspended into the filtrate and then 1N NaOH (0.9 ml, 0.9 mmol) was added (pH ~ 12). The mixture was stirred at room temperature for 3.5 hours, then more 1N NaOH (0.2 ml) was added, and the mixture was stirred at room

25

temperature for a further 0.5 hours. The pH was then adjusted to ~5.0 with 1N HCl. The off-white precipitate was collected by filtration, washed with water, and dried in vacuo over P<sub>2</sub>O<sub>5</sub>: 0.086 g, (79%); <sup>1</sup>H-NMR (250 MHz, DMSO-d<sub>6</sub>, TMS) 2.22 (m, 1H 7-CH), 2.90-3.30 (m, 3H, C≡CH, 8-H), 3.97 (ABq, J = 18.6 Hz, 2H, CH<sub>2</sub>C≡C), 4.37  
5 (d, J = 6.1 Hz, 2H, 2-CH<sub>2</sub>), 5.56 (t, 1H, CH<sub>2</sub>OH), 5.78 (t, J = 7.51 Hz, 1H, 6-H), 7.03 (d, J = 8.9 Hz, 2H, 3', 5'-H), 7.55 (s, 1H, 9-H), 7.82 (m, 3H, 2', 6'-H, 5-H); MS (ESI, m/z) 779 {(2M+H)<sup>+</sup>, 100%}, 390 {(M+H)<sup>+</sup>, 60%}.

**Method B:** A solution of *tert*-butyl 4-{*N*-[(6*RS*)-2-hydroxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}-benzoate (0.050 g, 0.11 mmol) in dichloromethane (1 ml) and trifluoroacetic acid (2.4 ml) was stirred at room temperature for 1 hour. The solvents were then removed in vacuo, and the residue was triturated with diethyl ether. The off-white precipitate was collected by filtration, and washed with ether to obtain the desired product as the trifluoroacetate salt: 0.044 g.

15

**Tri-*tert*-butyl *N*-{*N*-{4-[*N*-[(6*RS*)-2-hydroxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}-*N*-methyl-L-glutamate**

To a mixture of 4-{*N*-[(6*RS*)-2-hydroxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoic acid (0.075 g, ~0.19 mmol), tri-*tert*-butyl L-γ-glutamyl-*N*-methyl-L-glutamate (V. Bavetsias *et al.*, *J. Med. Chem.*, 1997, 40, 1495-1510; 0.110 g, 0.24 mmol), and anhydrous DMF (2.0 ml) was added diethyl cyanophosphonate (0.036 g, 0.22 mmol) with the aid of anhydrous DMF (0.2 ml) followed by triethylamine (0.022 g, 0.22 mmol). The clear solution  
25 was stirred at room temperature for 1.5 hours, then it was partitioned between ethyl acetate (50 ml) and brine (40 ml). The aqueous layer was extracted with more ethyl acetate (2 x 50 ml). The combined organics were washed with 10% aqueous citric acid (40 ml), saturated sodium bicarbonate solution (40 ml), and brine (40 ml), dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated *in vacuo*. Purification by column chromatography, on  
30 elution with a gradient of methanol in dichloromethane (0 to 6%), afforded an off-white solid that was further purified by trituration with hexane/dichloromethane/diethyl ether: 0.062 g (40 %); mp 116-120 °C (softens); <sup>1</sup>H-NMR (250 MHz, DMSO-d<sub>6</sub>, TMS) 1.36, 1.37, 1.38, 1.41 (4 x s, 27H, 3 x C(CH<sub>3</sub>)<sub>3</sub>), 1.70-2.35 (m) and

2.50 (m obscured by DMSO peak) (10H, 2 x  $\beta$ -CH<sub>2</sub>, 2 x  $\gamma$ -CH<sub>2</sub>, 7-CH<sub>2</sub>), 2.63, 2.82 (2 x s, 3H, CONMe), 2.90-3.25 (m, 3H, C $\equiv$ CH, 8-H), 3.97 (ABq, J = 17.6 Hz, 2H, CH<sub>2</sub>C $\equiv$ C), 4.32 (m, 1H, glu  $\alpha$ -CH), 4.38 (d, J = 6.1 Hz, 2H, 2-CH<sub>2</sub>), 4.50, 4.82 (2 x dd, 1H, Meglu  $\alpha$ -CH), 5.56 (t, J = 6.9 Hz, 1H, CH<sub>2</sub>OH), 5.78 (t, J = 7.10 Hz, 1H, 6-H), 7.02 (d, J = 8.6 Hz, 2H, 3',5'-H), 7.55 (s, 1H, 9-H), 7.78 (d, J = 8.9 Hz, 2H, 2',6'-H), 7.82 (s, 1H, 5-H), 8.32 (m, 1H, CONH), 11.81 (s, 1H, N<sup>3</sup>-H); MS (ESI, *m/z*) 830{(M+H)<sup>+</sup>, 100%}.

10 ***N*-{*N*-{4-[*N*-((6*RS*)-2-hydroxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-*L*- $\gamma$ -glutamyl}-*N*-methyl-*L*-glutamic acid**

A solution of tri-*tert*-butyl *N*-{*N*-{4-[*N*-((6*RS*)-2-hydroxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-*L*- $\gamma$ -glutamyl}-*N*-methyl-*L*-glutamate (0.060 g, 0.07 mmol) in trifluoroacetic acid (3.5 ml) was stirred at room temperature for 1 hour and 10 min with protection from the light. The solvent was then removed *in vacuo* and the residue was suspended in water (6 ml). The pH was adjusted to ~10 with 1N NaOH, then to ~4 with 1N hydrochloric acid. The white precipitate was collected by filtration, and dried *in vacuo* over P<sub>2</sub>O<sub>5</sub>: 0.035 g (77%), mp >165 °C (dec); <sup>1</sup>H-NMR (250 MHz, DMSO-d<sub>6</sub>, TMS) 1.80-2.35 (m) and 2.50 (m obscured by DMSO peak) (10H, 2 x  $\beta$ -CH<sub>2</sub>, 2 x  $\gamma$ -CH<sub>2</sub>, 7-CH<sub>2</sub>), 2.66, 2.83 (2 x s, 3H, CONMe), 2.90-3.25 (m, 3H, C $\equiv$ CH, 8-H), 3.97 (ABq, J = 18.4 Hz, 2H, CH<sub>2</sub>C $\equiv$ C), 4.32 (m obscured, 1H, glu  $\alpha$ -CH), 4.38 (d, J = 5.6 Hz, 2H, 2-CH<sub>2</sub>), 4.55, 4.91 (2 x dd, J = 10.0, 4.5 Hz, 1H, Meglu  $\alpha$ -CH), 5.56 (poorly resolved t, 1H, CH<sub>2</sub>OH), 5.77 (t, J = 8.06 Hz, 1H, 6-H), 7.02 (d, J = 7.8 Hz, 2H, 3',5'-H), 7.55 (s, 1H, 9-H), 7.81 (d, J = 10.1 Hz, 3H, 2',6'-H, 5-H), 8.32 (m, 1H, CONH), 11.82 (s, 1H, N<sup>3</sup>-H); MS (ESI, *m/z*) 662 {(M+H)<sup>+</sup>, 100%}; Found: C, 57.52; H, 5.52; N, 10.17; C<sub>33</sub>H<sub>35</sub>N<sub>5</sub>O<sub>10</sub> 1.5 H<sub>2</sub>O requires: C, 57.55; H, 5.56; N, 10.17%.

30 **Example 4: *In vitro* evaluation**

CB300945, the (*RS*)-C2-CH<sub>2</sub>OH analogue of the known cyclopenta[*g*]-quinazoline, CB300638 was synthesised as in Example 1. In addition, the C2-NH<sub>2</sub> was made as a comparative example. The activity of this new series of

cyclopenta[g]quinazolines with C2-CH<sub>3</sub> (*RS*-CB300638), C2-CH<sub>2</sub>OH (*RS*-CB300945) and C2-NH<sub>2</sub> (*RS*-CB300944) substitutions were compared with three other series of quinazoline derivatives in mouse L1210-FBP cells and human tumour cell lines co-expressing the RFC and  $\alpha$ -FR. The human A431-FBP cell line was transfected with the  $\alpha$ -FR and sensitivity compared with the A431 cell line. Human nasopharyngeal KB cells constitutively overexpress this receptor (see Bagnoli *et al. Oncogene*, 19, 4754–4763, 2000).

In the quinazoline series, the C2-CH<sub>2</sub>OH analogues were found to have lower affinities for the  $\alpha$ -FR of L1210-FBP cells (0.21 to 0.29) than the 2-CH<sub>3</sub> (0.37 to 0.54) and particularly the 2-NH<sub>2</sub> (1.3 to 1.7) counterparts. In the cyclopenta[g]quinazoline series the C2-CH<sub>3</sub> analogue (CB300638) had a relative binding affinity of 0.57. However, unexpectedly the C2-CH<sub>2</sub>OH analogue (CB300945) had a higher affinity than predicted (0.71) and the C2-NH<sub>2</sub> analogue (CB300944) had one lower than predicted (0.57). Thus in the cyclopenta[g]quinazoline series C2-substitution did not markedly affect binding to the  $\alpha$ -FR. A similar pattern of binding was noted for  $\alpha$ -FR expressed by A431-FBP cells (data not shown).

A431-FBP and KB cells were highly sensitive to the C2-CH<sub>3</sub> cyclopenta[g]-quinazoline with a L-glu- $\gamma$ -D-glu ligand (*S* or *RS*-CB300638). However, very little activity of the C2-NH<sub>2</sub> analogue (*RS*-CB300944) could be attributed to  $\alpha$ -FR mediated uptake into either of these cell lines (Tables 13). On the other hand the C2-CH<sub>2</sub>OH analogue (*RS*-CB300945) was highly potent in A431-FBP and KB cells and more highly selective for these cells compared with A431 cells, than *RS*-CB300638.

Tables 4 and 5 show the structures of the compounds tested.

### Example 5: Formulation

The following illustrate representative pharmaceutical dosage forms containing a cyclopenta[g]quinazoline of formula (I), particularly in pharmaceutically acceptable salt form, for therapeutic or prophylactic use in humans:

30

|     |                               |                  |
|-----|-------------------------------|------------------|
| (a) | <u>Tablet I</u>               | <u>mg/tablet</u> |
|     | Cyclopenta[g]quinazoline salt | 100              |
|     | Lactose Ph.Eur.               | 182.75           |

|    |     |  |                   |
|----|-----|--|-------------------|
|    |     | Croscarmellose sodium                        | 12.0              |
|    |     | Maize starch paste (5% w/v paste)            | 2.25              |
|    |     | Magnesium stearate                           | 3.0               |
| 5  | (b) | <u>Tablet II</u>                             | <u>mg/tablet</u>  |
|    |     | Cyclopenta[g]quinazoline salt                | 50                |
|    |     | Lactose Ph.Eur.                              | 223.75            |
|    |     | Croscarmellose sodium                        | 6.0               |
|    |     | Maize starch                                 | 15.0              |
| 10 |     | Polyvinylpyrrolidone (5% w/v paste)          | 2.25              |
|    |     | Magnesium stearate                           | 3.0               |
|    | (c) | <u>Tablet III</u>                            | <u>mg/tablet</u>  |
|    |     | Cyclopenta[g]quinazoline salt                | 1.0               |
| 15 |     | Lactose Ph.Eur.                              | 93.25             |
|    |     | Croscarmellose sodium                        | 4.0               |
|    |     | Maize starch paste (5% w/v paste)            | 0.75              |
|    |     | Magnesium stearate                           | 1.0               |
| 20 | (d) | <u>Capsule</u>                               | <u>mg/capsule</u> |
|    |     | Cyclopenta[g]quinazoline salt                | 10.0              |
|    |     | Lactose Ph.Eur.                              | 488.5             |
|    |     | Magnesium stearate                           | 1.5               |
| 25 | (e) | <u>Injection I</u>                           | <u>(50 mg/ml)</u> |
|    |     | Cyclopenta[g]quinazoline salt                | 5.0% w/v          |
|    |     | 1M Sodium hydroxide solution                 | 15.0% v/v         |
|    |     | 0.1M Hydrochloric acid (to adjust pH to 7.6) |                   |
|    |     | Polyethylene glycol 400                      | 4.5% w/v          |
| 30 |     | Water for injection to 100%                  |                   |
|    | (f) | <u>Injection II</u>                          | <u>(10 mg/ml)</u> |
|    |     | Cyclopenta[g]quinazoline salt                | 1.0% w/v          |
|    |     | Sodium phosphate BP                          | 3.6% w/v          |

|     |                                |                                    |
|-----|--------------------------------|------------------------------------|
|     | 0.1M Sodium hydroxide solution | 15.0% v/v                          |
|     | Water for injection to 100%    |                                    |
| (g) | <u>Injection III</u>           | <u>(1 mg/ml, buffered to pH 6)</u> |
| 5   | Cyclopenta[g]quinazoline salt  | 0.1% w/v                           |
|     | Sodium phosphate BP            | 2.26% w/v                          |
|     | Citric acid                    | 0.38% w/v                          |
|     | Polyethylene glycol 400        | 3.5% w/v                           |
|     | Water for injection to 100%    |                                    |

10

The above formulations may be prepared by conventional procedures well known in the pharmaceutical art. The tablets (a) to (c) may be enteric coated by conventional means, for example with a coating of cellulose acetate phthalate.

Table 1: Activity of 2-substituted quinazolines and cyclopenta[g]quinazolines in human A431 and A431-FBP cell lines grown in 20 nM folate (RS LV)

|                | Inhibition of cell growth, IC <sub>50</sub> , μM<br>20 nM LV |   | Inhibition of cell growth, IC <sub>50</sub> , μM<br>20 nM LV   |   |
|----------------|--|---|--|---|
|                | A431   | A431+1μM FA<br>(fold increased IC <sub>50</sub> in<br>presence of folic acid) | A431-FBP<br>(fold increased sensitivity<br>compared with A431) | A431-FBP+1μM FA<br>(fold increased IC <sub>50</sub> in<br>presence of folic acid) |
| CB30195        | 0.4 ± 0.14   | 0.37 ± 0.14<br>(1)  | 0.17 ± 0.065   | 0.23 ± 0.87<br>(1)  |
| CB300612       | 2.2 ± 0.78   | 1.9 ± 0.65<br>(1)   | 1.2 ± 0.17<br>(2)  | 1.3 ± 0.23<br>(1)   |
| CB300604       | 3.7 ± 0.71   | 3.4 ± 0.91<br>(1)   | 0.28 ± 0.057<br>(13)   | 1.2 ± 0.59<br>(4)   |
| ZD9331         | 0.082 ± 0.042  | 0.067 ± 0.029<br>(1)  | 0.018 ± 0.0097<br>(4)  | 0.034 ± 0.0087<br>(2)   |
| CB300512       | -  | -   | -  | -   |
| CB300533       | -  | -   | -  | -   |
| CB300395       | 0.72 ± 0.18  | 0.72 ± 0.12<br>(1)  | 0.47 ± 0.20<br>(2)   | 0.50 ± 0.17<br>(1)  |
| CB300635       | 9.5 ± 5.1  | 9.7 ± 5.4<br>(1)  | 4.1 ± 2.0<br>(2)   | 4.3 ± 2.0<br>(1)  |
| CB300616       | 5.9 ± 1.0  | 6.2 ± 1.6<br>(1)  | 4.6 ± 0.40<br>(1)  | 4.7 ± 1.1<br>(1)  |
| CB300638 (RS)  | 1.4 ± 0.23   | 1.4 ± 0.25<br>(1)   | 0.0065 ± 0.0001<br>(220)                                       | 0.87 ± 0.29<br>(130)  |
| CB300944 (RS)  | 23, 27   | 22, 25<br>(1)   | 8.3 ± 0.64<br>(3)  | 7.1 ± 1.0<br>(1)  |
| CB300945 (RS)  | 9.8 ± 3.4  | 9.3 ± 3.5<br>(1)  | 0.0021 ± 0.0011<br>(4700)                                      | 6.5 ± 0.86<br>(3100)  |
| CB300960 (R,S) | 4.5  | 4.3<br>(1)  | 0.028  | 5.1<br>(180)  |

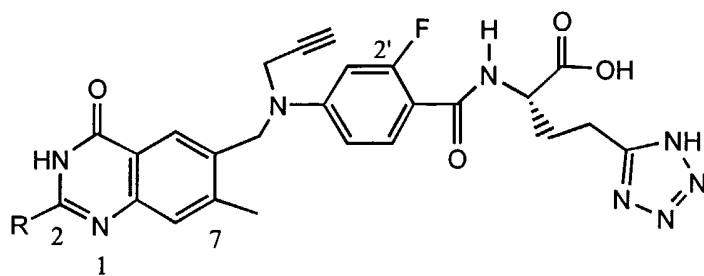
Table 2: Activity of 2-substituted quinazolines and cyclopenta[g]quinazolines in human A431 and A431-FBP cell lines grown in 1 nM folate (RS LV)

|               | Inhibition of cell growth, IC <sub>50</sub> , μM<br>1 nM LV |   | Inhibition of cell growth, IC <sub>50</sub> , μM<br>1 nM LV    |   |
|---------------|---|---|--|---|
|               | A431  | A431+1μM FA<br>(fold increased IC <sub>50</sub> in<br>presence of folic acid) | A431-FBP<br>(fold increased sensitivity<br>compared with A431) | A431-FBP+1μM FA<br>(fold increased IC <sub>50</sub> in<br>presence of folic acid) |
| CB30195       | 0.25 ± 0.13   | 0.21 ± 0.078<br>(1)   | 0.10 ± 0.032<br>(3)  | 0.22 ± 0.085<br>(2)   |
| CB300612      | 1.8 ± 0.91  | 1.5 ± 0.51<br>(1)   | 1.8 ± 0.68<br>(1)  | 2.9 ± 0.55<br>(1)   |
| CB300604      | 1.6 ± 0.36  | 2.0 ± 0.49<br>(1)   | 0.50 ± 0.015<br>(3)  | 1.8 ± 0.058<br>(4)  |
| ZD9331        | 0.061 ± 0.014   | 0.056 ± 0.025<br>(1)  | 0.0088 ± 0.0052<br>(7)   | 0.027 ± 0.0076<br>(3)   |
| CB300512      | -   | -   | -  | -   |
| CB300533      | -   | -   | -  | -   |
| CB300395      | 0.70 ± 0.072  | 0.74 ± 0.053<br>(1)   | 0.43 ± 0.36<br>(2)   | 0.54 ± 0.34<br>(1)  |
| CB300635      | 6.1 ± 1.3   | 6.7 ± 1.9<br>(1)  | 2.7 ± 0.87<br>(2)  | 3.3 ± 1.5<br>(1)  |
| CB300616      | 4.9 ± 2.0   | 5.2 ± 2.1<br>(1)  | 5.5 ± 3.5<br>(1)   | 5.6 ± 3.0<br>(1)  |
| CB300638 (RS) | 1.2 ± 0.21  | 0.98 ± 0.23<br>(1)  | 0.0072 ± 0.001<br>(170)  | 0.61 ± 0.012<br>(85)  |
| CB300944 (RS) | 24 ± 3.6  | 24 ± 2.9<br>(1)   | 9.1 ± 0.83<br>(3)  | 12 ± 2.1<br>(1)   |
| CB300945 (RS) | 7.2 ± 3.6   | 7.3 ± 3.3   | 0.0019 ± 0.0006<br>(3800)                                      | 5.7 ± 1.3<br>(3100)   |

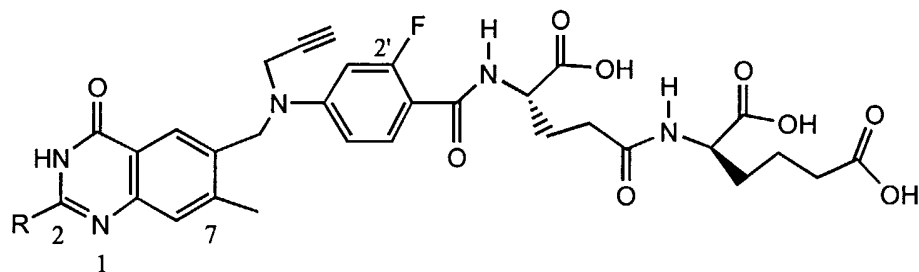
Table 3: Activity of 2-substituted quinazolines and cyclopenta[g]quinazolines in human KB cells grown in 1 and 20 nM folate (RS LV)

|               | Inhibition of cell growth, IC <sub>50</sub> , μM<br>1 nM LV |   | Inhibition of cell growth, IC <sub>50</sub> , μM<br>20 nM LV |   |
|---------------|---|---|--|---|
|               | KB  | KB +1μM FA<br>(fold increase IC <sub>50</sub> in<br>presence of folic acid) | KB   | KB +1μM FA<br>(fold increase IC <sub>50</sub> in<br>presence of folic acid) |
| CB30195       | 0.0053 ± 0.0006   | 0.18 ± 0.012<br>(34)  | 0.027 ± 0.002  | 0.15 ± 0.015<br>(6)   |
| CB300612      | 0.97 ± 0.20   | 2.2 ± 0.35<br>(2)   | 1.7 ± 0.20   | 2.0 ± 0.25<br>(1)   |
| CB300604      | 0.0061 ± 0.00095  | 0.69 ± 0.08<br>(120)  | 0.035 ± 0.005  | 0.65 ± 0.04<br>(20)   |
| ZD9331        | 0.0021 ± 0.0013   | 0.00641 ± 0.0035<br>(3)   | 0.0036 ± 0.0021  | 0.012 ± 0.005<br>(3)  |
| CB300512      | -   | -   | -  | -   |
| CB300533      | -   | -   | -  | -   |
| CB300395      | 0.063 ± 0.081   | 0.23 ± 0.05<br>(4)  | 0.065 ± 0.0092   | 0.17 ± 0.032<br>(3)   |
| CB300635      | 2.8 ± 1.1   | 3.8 ± 0.6<br>(1)  | 3.2 ± 0.6  | 4.4 ± 0.25<br>(1)   |
| CB300616      | 0.0037 ± 0.0018   | 0.58 ± 0.05<br>(160)  | 0.008 ± 0.00072  | 0.59 ± 0.19<br>(74)   |
| CB300638 (RS) | 0.0079 ± 0.001  | 0.61 ± 0.012<br>(76)  | 0.0053 ± 0.0025  | 0.76 ± 0.17<br>(140)  |
| CB300944 (RS) | 19 ± 0  | 19 ± 1.5<br>(1)   | 19 ± 0.58  | 19 ± 1.2<br>(1)   |
| CB300945 (RS) | 0.0034 ± 0.0007   | 7.6 ± 1.3<br>(2200)   | 0.0034 ± 0.0009  | 7.3 ± 2.0<br>(2100)   |

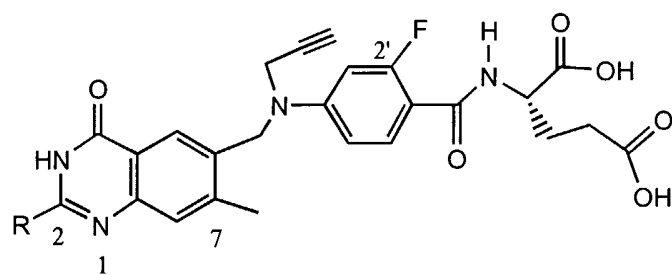
Table 4: Structures of the compounds tested—Comparative compounds



ZD9331, R = Me  
 CB300512, R = NH<sub>2</sub>  
 CB300533, R = CH<sub>2</sub>OH

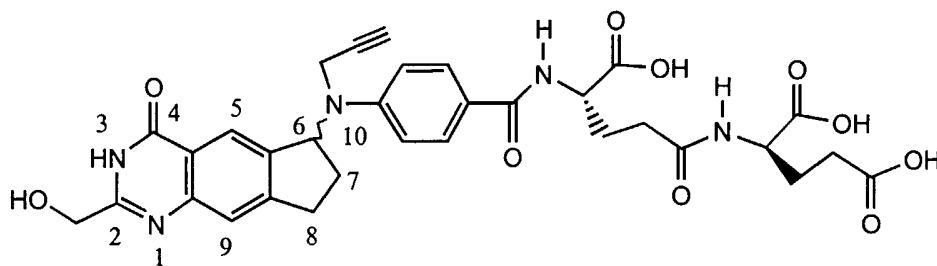


CB300395, R = Me  
 CB300635, R = NH<sub>2</sub>  
 CB300616, R = CH<sub>2</sub>OH

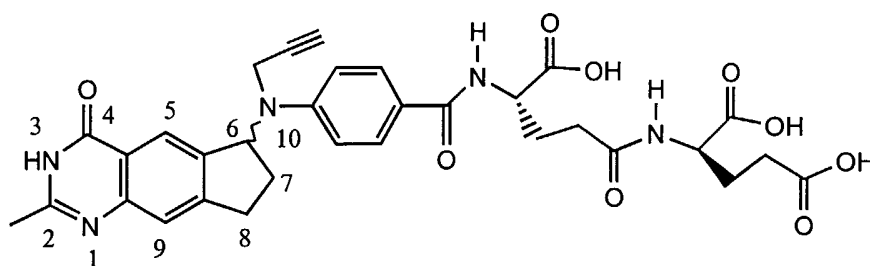


CB30195, R = Me  
 CB300612, R = NH<sub>2</sub>  
 CB300604, R = CH<sub>2</sub>OH

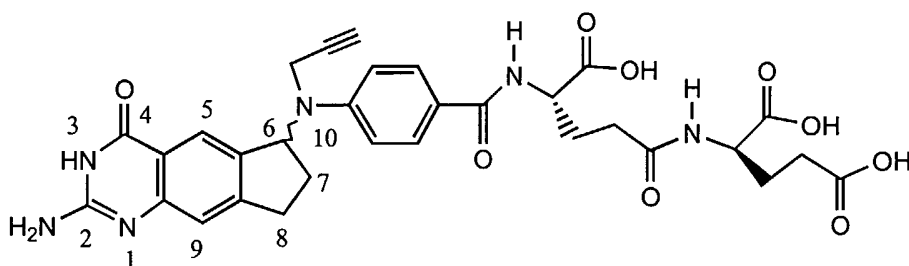
Table 5: Structures of the compounds tested—Compounds of the invention



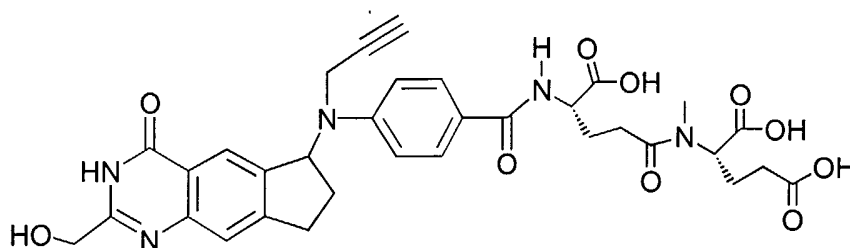
**CB300945**



**CB300638**



**CB300944**

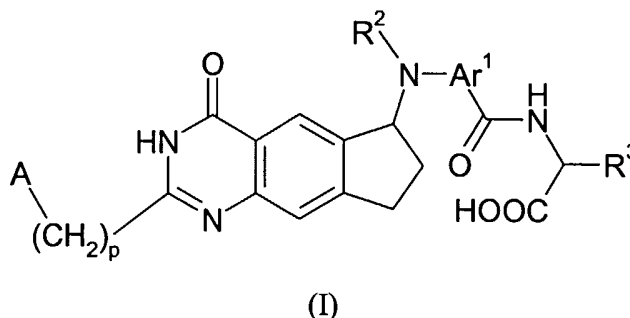


5

**CB300960**

## CLAIMS

1. A cyclopenta[*g*]quinazoline of the formula (I):



5 wherein:

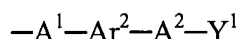
A is a group OR<sup>0</sup> or NR<sup>0</sup>R<sup>1</sup> wherein R<sup>0</sup> and R<sup>1</sup> are each independently hydrogen, C<sub>1-4</sub> alkyl, C<sub>3-4</sub> alkenyl, C<sub>3-4</sub> alkynyl, C<sub>2-4</sub> hydroxyalkyl, C<sub>2-4</sub> halogenoalkyl or C<sub>1-4</sub> cyanoalkyl, or R<sup>0</sup> and R<sup>1</sup> together with the intermediate N form a five- or six-membered heterocyclic ring;

10 p is an integer in the range 1 to 4;

R<sup>2</sup> is hydrogen, C<sub>1-4</sub> alkyl, C<sub>3-4</sub> alkenyl, C<sub>3-4</sub> alkynyl, C<sub>2-4</sub> hydroxyalkyl, C<sub>2-4</sub> halogenoalkyl or C<sub>1-4</sub> cyanoalkyl;

Ar<sup>1</sup> is phenylene, thiophenediyl, thiazolediyl, pyridinediyl or pyrimidinediyl which may optionally bear one or two substituents selected from halogeno, hydroxy, amino, nitro, cyano, trifluoromethyl, C<sub>1-4</sub> alkyl and C<sub>1-4</sub> alkoxy; and

15 R<sup>3</sup> is a group of the formula:



20 in which A<sup>1</sup> is a bond between the α-carbon atom of the group —CO—NH—CH(CO<sub>2</sub>H)— and Ar<sup>2</sup> or is a C<sub>1-2</sub> alkylene group;

Ar<sup>2</sup> is phenylene, tetrazolediyl, thiophenediyl, thiazolediyl, pyridinediyl or pyrimidinediyl which in the case of phenylene may optionally bear one or two substituents on the ring selected from halogeno, nitro, C<sub>1-4</sub> alkyl and C<sub>1-4</sub> alkoxy;

25 A<sup>2</sup> is a C<sub>1-3</sub> alkylene or C<sub>2-3</sub> alkenylene group; and

Y<sup>1</sup> is carboxyl, tetrazol-5-yl, N-(C<sub>1-4</sub> alkylsulfonyl)carbamoyl, N-(phenylsulfonyl)carbamoyl which may optionally bear one or two substituents on the phenyl ring selected from the group consisting of halogeno, nitro, C<sub>1-4</sub> alkyl and C<sub>1-4</sub> alkoxy, tetrazol-5-ylthio, tetrazol-5-ylsulfinyl or tetrazol-5-ylsulfonyl; or

Y<sup>1</sup> is a group of the formula:

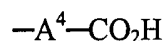


5 in which R is hydrogen, C<sub>1-4</sub> alkyl, C<sub>3-4</sub> alkenyl or C<sub>3-4</sub> alkynyl;

Y<sup>2</sup> is carboxy, tetrazol-5-yl, N-(C<sub>1-4</sub> alkylsulfonyl)carbamoyl, N-(phenylsulfonyl)carbamoyl which may optionally bear one or two substituents on the phenyl ring selected from the group consisting of halogeno, nitro, C<sub>1-4</sub> alkyl and C<sub>1-4</sub> alkoxy, tetrazol-5-ylthio, tetrazol-5-ylsulfinyl or tetrazol-5-ylsulfonyl; and

10 Y<sup>3</sup> is the residue of a naturally occurring amino acid NH<sub>2</sub>CH(CO<sub>2</sub>H)Y<sup>3</sup>; or

Y<sup>3</sup> is a group of the formula:



15 in which A<sup>4</sup> is a C<sub>2-6</sub> alkylene group;

R<sup>3</sup> is a group of the formula:

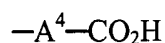


20 in which A<sup>5</sup> is a C<sub>1-6</sub> alkylene group and R is as defined above;

Y<sup>4</sup> is carboxy, tetrazol-5-yl, N-(C<sub>1-4</sub> alkylsulfonyl)carbamoyl, N-(phenylsulfonyl)carbamoyl which may optionally bear one or two substituents on the phenyl ring selected from the group consisting of halogeno, nitro, C<sub>1-4</sub> alkyl and C<sub>1-4</sub> alkoxy, tetrazol-5-ylthio, tetrazol-5-ylsulfinyl or tetrazol-5-ylsulfonyl; and

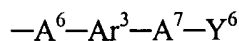
25 Y<sup>5</sup> is the residue of a naturally occurring amino acid NH<sub>2</sub>CH(CO<sub>2</sub>H)Y<sup>5</sup>; or

Y<sup>5</sup> is a group of the formula:



30 in which A<sup>4</sup> is as defined above; or

Y<sup>5</sup> is a group of the formula:



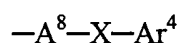
in which A<sup>6</sup> is a bond between the  $\alpha$ -carbon atom of the group —A<sup>5</sup>—CON(R)CH(Y<sup>4</sup>)— and Ar<sup>3</sup> or is a C<sub>1-2</sub> alkylene group;

Ar<sup>3</sup> is phenylene, tetrazolediyl, thiophenediyl, thiazolediyl, pyridinediyl or pyrimidinediyl which in the case of phenylene may optionally bear one or two  
5 substituents on the ring selected from halogeno, nitro, C<sub>1-4</sub> alkyl and C<sub>1-4</sub> alkoxy;

A<sup>7</sup> is a C<sub>1-3</sub> alkylene or C<sub>2-3</sub> alkenylene group; and

Y<sup>6</sup> is carboxy, tetrazol-5-yl, *N*-(C<sub>1-4</sub> alkylsulfonyl)carbamoyl, *N*-(phenylsulfonyl)carbamoyl which may optionally bear one or two substituents on the phenyl ring selected from the group consisting of halogeno, nitro, C<sub>1-4</sub> alkyl and C<sub>1-4</sub> alkoxy,  
10 tetrazol-5-ylthio, tetrazol-5-ylsulfinyl or tetrazol-5-ylsulfonyl; or

R<sup>3</sup> is a group of the formula:



15 in which A<sup>8</sup> is a C<sub>1-4</sub> alkylene group;

X is sulfinyl, sulfonyl or methylene; and

Ar<sup>4</sup> is 1,2,3-triazol-4-yl, 1,2,4-triazol-3-yl or, except when X is methylene, tetrazol-5-yl;

the compound (I) optionally being in the form of a pharmaceutically acceptable salt  
20 or ester.

2. A cyclopenta[g]quinazoline as claimed in claim 1 wherein:

R<sup>0</sup> and R<sup>1</sup> are each independently hydrogen or C<sub>1-4</sub> alkyl;

R<sup>2</sup> is ethyl, propyl, prop-2-enyl, prop-2-ynyl, 2-hydroxyethyl, 2-fluoroethyl,  
25 2-bromoethyl or 2-cyanoethyl;

Ar<sup>1</sup> is 1,4-phenylene which may optionally bear one or two substituents selected from the group consisting of chloro, fluoro, thiophene-2,5-diyl, thiazole-2,5-diyl or pyridine-2,5-diyl;

R<sup>3</sup> is a group of the formula:

30



in which A<sup>5</sup> is a C<sub>1-6</sub> alkylene group and R is as defined in claim 1;

- $Y^4$  is carboxy, tetrazol-5-yl, *N*-( $C_{1-4}$  alkylsulfonyl)carbamoyl, *N*-(phenylsulfonyl)carbamoyl which may optionally bear one or two substituents on the phenyl ring selected from the group consisting of halogeno, nitro,  $C_{1-4}$  alkyl and  $C_{1-4}$  alkoxy, tetrazol-5-ylthio, tetrazol-5-ylsulfinyl or tetrazol-5-ylsulfonyl; and
- 5  $Y^5$  is the residue of a naturally occurring amino acid  $NH_2CH(CO_2H)Y^5$ .
3. A cyclopenta[g]quinazoline as claimed in claim 2 wherein:  
 A is a group  $OR^0$  in which  $R^0$  is hydrogen or methyl;  
 $R^2$  is ethyl or prop-2-ynyl; and  
 10  $Ar^1$  is 1,4-phenylene or 1,4-phenylene having a 2-fluoro.
4. A cyclopenta[g]quinazoline as claimed in claim 3 in which  $R^3$  is the residue of an acid  $NH_2.CH(COOH)R^3$  which comprises L-Glu- $\gamma$ -D-Glu, i.e. *N*-L- $\gamma$ -glutamyl-D-glutamic acid.  
 15
5. A cyclopenta[g]quinazoline as claimed in any preceding claim wherein p is 1.
6. A cyclopenta[g]quinazoline as claimed in claim 1 comprising:  
 $N$ -{*N*-{4-[*N*-((6*RS*)-2-methoxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[g]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L- $\gamma$ -glutamyl}-D-glutamic acid;  
 20  $N$ -{*N*-{4-[*N*-((6*RS*)-2-hydroxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[g]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L- $\gamma$ -glutamyl}-D-glutamic acid; or  
 25  $N$ -{*N*-{4-[*N*-((6*RS*)-2-hydroxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[g]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L- $\gamma$ -glutamyl}-*N*-methyl-L-glutamic acid;  
 or a pharmaceutically acceptable salt or ester thereof.
- 30 7. A cyclopenta[g]quinazoline according to any of the preceding claims for use in therapy.

8. A pharmaceutical composition comprising a cyclopenta[g]quinazoline according to any of Claims 1 to 6 together with a pharmaceutically acceptable diluent or carrier.
- 5 9. The use of a cyclopenta[g]quinazoline according to any of claims 1 to 6 for the manufacture of a medicament for use in the treatment of cancer.
10. The use according to claim 9 wherein the cancer comprises a carcinoma of ovarian origin.
- 10 11. A method for aiding regression and palliation of cancer in a patient in need of such treatment which comprises administering to said patient an effective amount of a cyclopenta[g]quinazoline according to any of Claims 1 to 6.

## INTERNATIONAL SEARCH REPORT

PCT/GB 02/03979

| <b>A. CLASSIFICATION OF SUBJECT MATTER</b>  |  |   |
|---|--|---|
| IPC 7   | C07K5/02<br>A61P35/00  | C07D239/90<br>C07D403/12<br>A61K38/05<br>A61K31/517   |
| According to International Patent Classification (IPC) or to both national classification and IPC   |  |   |
| <b>B. FIELDS SEARCHED</b>   |  |   |
| Minimum documentation searched (classification system followed by classification symbols)   |  |   |
| IPC 7   | C07K A61K C07D   |   |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched   |  |   |
| Electronic data base consulted during the international search (name of data base and, where practical, search terms used)  |  |   |
| EPO-Internal, WPI Data, PAJ, CHEM ABS Data  |  |   |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>   |  |   |
| Category *  | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.   |
| X   | WO 95 30673 A (BRITISH TECH GROUP ; ZENECA LTD (GB); BAVETSIAS VASSILIOS (GB); BOY)<br>16 November 1995 (1995-11-16)<br>cited in the application<br>claim 7<br>---         | 1-11  |
| A   | WO 94 11354 A (ZENECA LTD ; BRITISH TECH GROUP (GB); BOYLE FRANCIS THOMAS (GB); CR)<br>26 May 1994 (1994-05-26)<br>cited in the application<br>claims<br>example 17<br>--- | 1-11  |
| A   | WO 00 50417 A (JACKMAN ANN ; BAVETSIAS VASSILIS (GB); CANCER RES CAMPAIGN TECH (GB)<br>31 August 2000 (2000-08-31)<br>the whole document<br>---                            | 1-11  |
|   | -/--   |   |
| <input checked="" type="checkbox"/>   | Further documents are listed in the continuation of box C.   | <input checked="" type="checkbox"/> Patent family members are listed in annex.  |
| * Special categories of cited documents :   |  |   |
| *A* document defining the general state of the art which is not considered to be of particular relevance<br>*E* earlier document but published on or after the international filing date<br>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)<br>*O* document referring to an oral disclosure, use, exhibition or other means<br>*P* document published prior to the international filing date but later than the priority date claimed |  | *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention<br>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone<br>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.<br>*&* document member of the same patent family |
| Date of the actual completion of the international search   |  | Date of mailing of the international search report  |
| 20 November 2002  |  | 28/11/2002  |
| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.<br>Fax: (+31-70) 340-3016  |  | Authorized officer<br><br>Schmid, J-C   |

## INTERNATIONAL SEARCH REPORT

PCT/GB 02/03979

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |   |                       |
|--|---|-----------------------|
| Category °   | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
| A  | <p>BAVETSIAS V ET AL: "Design and synthesis of cyclopenta'g!quinazoline-based antifolates as inhibitors of thymidylate synthase and potential antitumor agents" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 43, no. 10, 2000, pages 1910-1926-26, XP002187697<br/>ISSN: 0022-2623<br/>cited in the application<br/>the whole document</p> <p style="text-align: center;">---</p>  | 1-11                  |
| A  | <p>MELIN C. ET AL.: "Novel cyclopenta'g!quinazoline dipeptide antifolates: Thymidylate synthase inhibitors with activity independent of the reduced folate carrier and folylpolyglutamate synthetase" CHEMISTRY AND BIOLOGY OF PTERIDINES AND FOLATES 1997, PROCEEDINGS OF THE INTERNATIONAL SYMPOSIUM ON PTERIDINES AND FOLATES, 11TH, BERCHTESGADEN, GERMANY . EDITOR(S): PFLEIDERER, WOLFGANG;ROKOS, HARTMUT. PUBLISHER: BLACKWELL WISSENSCHAFTS-VERLAG, 15 - 20 June 1997, pages 139-144, XP009000407<br/>the whole document</p> <p style="text-align: center;">---</p> | 1-11                  |
| A  | <p>BAVETSIAS V. ET AL.: "Synthetic and antitumour activity of cyclopenta'g!quinazoline-based antifolates, a novel class of thymidylate synthase (TS) inhibitors " CHEMISTRY AND BIOLOGY OF PTERIDINES AND FOLATES 1997, PROCEEDINGS OF THE INTERNATIONAL SYMPOSIUM ON PTERIDINES AND FOLATES, 11TH, BERCHTESGADEN, GERMANY, . EDITOR(S): PFLEIDERER, WOLFGANG;ROKOS, HARTMUT. PUBLISHER: BLACKWELL WISSENSCHAFTS-VERLAG, 15 - 20 June 1997, pages 205-208, XP009000408<br/>the whole document</p> <p style="text-align: center;">-----</p>                                  | 1-11                  |

# INTERNATIONAL SEARCH REPORT

PCT/GB 02/03979

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  

Although claims 11 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound.
2.  Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

PCT/GB 02/03979

| Patent document<br>cited in search report |   | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|---|---|---------------------|----------------------------|---------------------|
| WO 9530673                                | A | 16-11-1995          | CA 2187449 A1              | 16-11-1995          |
|   |   |                     | EP 0758328 A1              | 19-02-1997          |
|   |   |                     | WO 9530673 A1              | 16-11-1995          |
|   |   |                     | GB 2290082 A ,B            | 13-12-1995          |
|   |   |                     | JP 9512812 T               | 22-12-1997          |
|   |   |                     | US 5747499 A               | 05-05-1998          |
|   |   |                     | -----                      |                     |
| WO 9411354                                | A | 26-05-1994          | AT 158578 T                | 15-10-1997          |
|   |   |                     | AU 5376094 A               | 08-06-1994          |
|   |   |                     | DE 69314183 D1             | 30-10-1997          |
|   |   |                     | DE 69314183 T2             | 15-01-1998          |
|   |   |                     | DK 667864 T3               | 04-05-1998          |
|   |   |                     | EP 0667864 A1              | 23-08-1995          |
|   |   |                     | ES 2107688 T3              | 01-12-1997          |
|   |   |                     | WO 9411354 A1              | 26-05-1994          |
|   |   |                     | GB 2272217 A ,B            | 11-05-1994          |
|   |   |                     | GR 3024875 T3              | 30-01-1998          |
|   |   |                     | JP 3271667 B2              | 02-04-2002          |
|   |   |                     | JP 8505838 T               | 25-06-1996          |
|   |   |                     | US 5789417 A               | 04-08-1998          |
|   |   |                     | ZA 9307756 A               | 11-05-1994          |
| -----                                     |   |                     |                            |                     |
| WO 0050417                                | A | 31-08-2000          | AU 2683800 A               | 14-09-2000          |
|   |   |                     | EP 1155012 A1              | 21-11-2001          |
|   |   |                     | WO 0050417 A1              | 31-08-2000          |
| -----                                     |   |                     |                            |                     |