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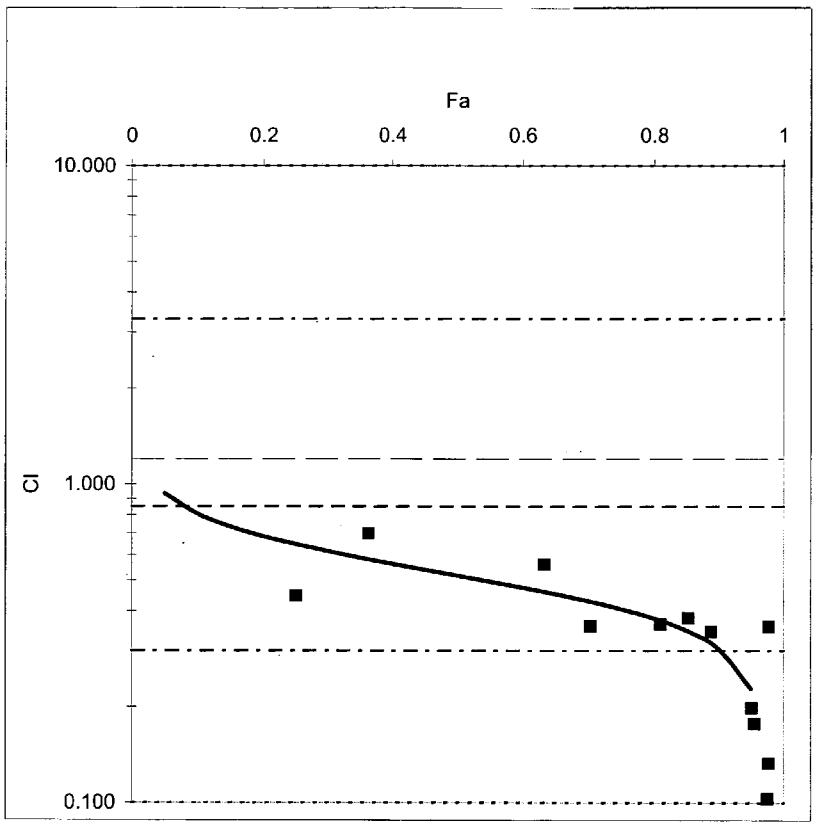

Figure 1

(57) Abstract: This invention relates to a combination product comprising a MEK inhibitor and a B-raf inhibitor, and to methods for the production of an anti-cancer effect in a patient, which is accordingly useful in the treatment of cancer in a patient. More specifically the present invention relates to: a combination product comprising a MEK inhibitor and a B raf inhibitor; a combination product comprising a kit of parts comprising a MEK inhibitor and a B-raf inhibitor; use of the combination product in the treatment of cancer; a method of treating cancer comprising administering the combination product to a patient. The combination product and methods of the invention are also useful in the treatment of other diseases associated with the activity of MEK, and/or B-raf.

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COMBINATION OF A MEK- INHIBITOR AND A B-RAF INHIBITOR FOR THE TREATMENT OF CANCER

This invention relates to a combination product comprising a MEK inhibitor and a B-raf inhibitor, and to methods for the production of an anti-cancer effect in a patient, which is accordingly useful in the treatment of cancer in a patient. More specifically the present invention relates to: a combination product comprising a MEK inhibitor and a B-raf inhibitor; a combination product comprising a kit of parts comprising a MEK inhibitor and a B-raf inhibitor; the use of the combination product in the treatment of cancer; a method of treating cancer comprising administering the combination product to a patient. The combination product and methods of the invention are also useful in the treatment of other diseases associated with the activity of MEK, and/or B-raf.

The Ras, Raf, MAP protein kinase/extracellular signal–regulated kinase kinase (MEK), extracellular signal–regulated kinase (ERK) pathway plays a central role in the regulation of a variety of cellular functions dependent upon cellular context, including cellular proliferation, differentiation, survival, immortalization, invasion and angiogenesis (reviewed in Peyssonnaux and Eychene, Biology of the Cell, 2001, 93,3-62). Indeed, the ras-dependent raf-MEK-MAPK cascade is one of the key signalling pathways responsible for conveying both mitogenic and invasive signals from the cell surface to the nucleus resulting in changes in gene expression and cell fate.

The Ras/Raf/MEK/ERK pathway has been reported to contribute to the tumorigenic phenotype by inducing immortalisation, growth factor-independent growth, insensitivity to growth-inhibitory signals, ability to invade and metastasis, stimulating angiogenesis and inhibition of apoptosis (reviewed in Kolch et al., Exp.Rev. Mol. Med., 2002, 25 April, http://www.expertreviews.org/02004386h.htm). In fact, ERK phosphorylation is enhanced in approximately 30% of all human tumours (Hoshino et al., Oncogene, 1999, 18, 813-822). This may be a result of overexpression and/or mutation of key members of the pathway, including *RAS* and *BRAF* genes.

B-Raf is reportedly the major isoform involved in cell proliferation and the primary target of oncogenic Ras. Activating somatic missense mutations have been identified exclusively for B-Raf, occurring with a frequency of 66% in malignant cutaneous melanomas (Davies et al., Nature, 2002, 417, 949-954) and also present in a wide range of human

cancers, including but not limited to papillary thyroid tumours (Cohen et al., J. Natl. Cancer Inst., 2003, 95, 625-627), cholangiocarcinomas (Tannapfel et al., Gut, 2003, 52, 706-712), colon and ovarian cancers (Davies et al., Nature, 2002, 417, 949-954). The most frequent mutation in B-Raf (80%) is a glutamic acid for valine substitution at position 600. These mutations increase the basal kinase activity of B-Raf and are thought to uncouple Raf/MEK/ERK signalling from upstream proliferation drivers including Ras and growth factor receptor activation resulting in constitutive activation of ERK.

Accordingly, it has been recognised that an inhibitor of a protein of the MAPK kinase pathway should be of value both as an anti-proliferative, pro-apoptotic and anti-invasive agent for use in the containment and/or treatment of proliferative or invasive disease.

It is expected that inhibition of a single protein of the MAPK kinase pathway alone would inhibit the downstream activity of the pathway. It is expected that inhibition of MEK alone would inhibit downstream activation of the MAPK kinase pathway. So, where MEK is inhibited, it is expected that inhibition of an upstream protein in the pathway, such as B-raf, as well would have no additional inhibitory effect on the MAPK kinase pathway, given that B-raf signals through MEK.

Surprisingly, we have found that concurrent inhibition of both MEK and B-raf yields a beneficial inhibitory effect on the growth or viability of COLO-205, SW620, A375 and A549 tumour cell lines, in comparison with the inhibition of MEK or B-raf alone. Moreover this beneficial effect is detected in tumour cell lines that exhibit both a range of sensitivities to B-raf or MEK inhibition alone and either BRAF or RAS activating gene mutations. It is proposed that inhibition of any two components of the MAPK kinase pathway, such as Raf, MEK or ERK will yield a beneficial effect in comparison to that which would be achieved by the inhibition of either Raf, MEK or ERK alone.

The present invention provides a combination product comprising a MEK inhibitor and a B-raf inhibitor. The combination product of the invention is useful in a method for the production of an anti-cancer effect in a patient, which is accordingly useful in the treatment of cancer in a patient.

According to a first aspect of the present invention there is provided a combination product comprising

a MEK inhibitor, or a pharmaceutically acceptable salt thereof, and

a B-raf inhibitor, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

The combination product of the present invention provides for the administration of a MEK inhibitor in conjunction with a B-raf inhibitor. The combination product may be in the form of a combined preparation of a MEK inhibitor and a B-raf inhibitor. The combination product may comprise a kit of parts comprising separate formulations of a MEK inhibitor and a B-raf inhibitor. The separate formulations of a MEK inhibitor and a B-raf inhibitor may be administered sequentially, separately and/or simultaneously. In one embodiment the separate formulations of a MEK inhibitor and a B-raf inhibitor of the combination product are administered simultaneously (optionally repeatedly). In one embodiment the separate formulations of a MEK inhibitor and a B-raf inhibitor of the combination product are administered sequentially (optionally repeatedly). In one embodiment the separate formulations of a MEK inhibitor and a B-raf inhibitor of the combination product are administered separately (optionally repeatedly). Where the administration of the separate formulations of a MEK inhibitor and a B-raf inhibitor of the combination product is sequential or separate, the delay in administering the second formulation should not be such as to lose the beneficial effect of the combination therapy. Thus, the present invention provides a combination product comprising a MEK inhibitor, or a pharmaceutically-acceptable salt thereof, and a B-raf inhibitor, or a pharmaceutically-acceptable salt thereof, for use sequentially, separately and/or simultaneously in the treatment of cancer.

In another aspect there is provided a combination product which comprises a kit of parts comprising the following components:

a MEK inhibitor, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier; and

a B-raf inhibitor, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier,

wherein the components are provided in a form which is suitable for sequential, separate and/or simultaneous administration.

In one embodiment the kit of parts comprises

a first container comprising a MEK inhibitor, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier; and

a second container comprising a B-raf inhibitor, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier, and

a container means for containing said first and second containers.

In one embodiment the kit of parts further comprises instructions on how to administer the components sequentially, separately and/or simultaneously. In one embodiment the kit of parts further comprises instructions indicating that the combination product can be used in the treatment of cancer.

In one embodiment the MEK inhibitor is a small molecular weight compound. In one embodiment the MEK inhibitor is selected from any one of an ATP-competitive MEK inhibitor, a non-ATP competitive MEK inhibitor, or an ATP-uncompetitive MEK inhibitor. In one embodiment the MEK inhibitor is selected from any one of AZD6244 (Example 10 of International Patent Publication Number WO03/077914) or a pharmaceutically acceptable salt thereof, 2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide or a pharmaceutically acceptable salt thereof, 4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide or a pharmaceutically acceptable salt thereof, PD-0325901 (Pfizer), PD-184352 (Pfizer), XL-518 (Exelixis), AR-119 (Ardea Biosciences, Valeant Pharmaceuticals), AS-701173 (Merck Serono), AS-701255 (Merck Serono), 360770-54-3 (Wyeth). In one embodiment the MEK inhibitor is selected from AZD6244 or a pharmaceutically acceptable salt thereof, 2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide or a pharmaceutically acceptable salt thereof or 4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide or a pharmaceutically acceptable salt thereof as described below. In one embodiment the MEK inhibitor is selected from AZD6244 or a pharmaceutically acceptable salt thereof or 2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide or a pharmaceutically acceptable salt thereof, as described below. In one embodiment the MEK inhibitor is AZD6244 hydrogen sulphate salt.

AZD6244 hydrogen sulphate salt may be synthesised according to the process described in International Patent Publication Number WO07/076245.

In another embodiment the MEK inhibitor may inhibit gene expression, for example by interfering with mRNA stability or translation. In one embodiment the MEK inhibitor is selected from small interfering RNA (siRNA), which is sometimes known as short interfering RNA or silencing RNA, or short hairpin RNA (shRNA), which is sometimes known as small hairpin RNA.

In one embodiment the B-raf inhibitor is a small molecular weight compound. In one embodiment the B-raf inhibitor is selected from any one of an ATP-competitive B-raf inhibitor, a non-ATP competitive B-raf inhibitor, or an ATP-uncompetitive B-raf inhibitor. In one embodiment the B-raf inhibitor is selected from any one of the small molecular weight compounds disclosed in International Patent Publication Number WO2006/024834 or WO2006/067446 or International Patent Application Number PCT/GB2006/004756, or is selected from any one of CHIR-265 (Novartis), XL281 (Exelixis) or PLX4032 (Plexxikon, Roche). In one embodiment the B-raf inhibitor is selected from any one of

3-(1-cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

3-(1,1-dimethylprop-2-yn-1-yl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

3-(1-cyano-1-methylethyl)-5-fluoro-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

3-(1-cyano-1-methylethyl)-5-[(dimethylamino)methyl]-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

4-dimethylaminomethyl-*N*-[4-methyl-3-(3-methyl-4-oxo-3,4-dihydro-quinazolin-6-ylamino)-phenyl]-3-trifluoromethyl-benzamide;

2-(1-cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}isonicotinamide;

3-(1-cyano-1-methylethyl)-2-fluoro-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

N-(3-{[3-(3-aminopropyl)-4-oxo-3,4-dihydroquinazolin-6-yl]amino}-4-methylphenyl)-3-(1-cyano-1-methylethyl)benzamide;

3-{[methoxy(methyl)amino]sulfonyl}-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide; and
3-*tert*-butyl-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide.

In one embodiment the B-raf inhibitor is selected from any one of
3-(cyano-dimethyl-methyl)-*N*-[3-(7-methoxy-quinazolin-4-ylamino)-4-methyl-phenyl]-benzamide;
3-(cyano-dimethyl-methyl)-5-fluoro-*N*-[3-(7-methoxy-quinazolin-4-ylamino)-4-methyl-phenyl]-benzamide;
3-(1-cyano-1-methylethyl)-2-fluoro-*N*-{3-[(7-methoxy quinazolin-4-yl)amino]-4-methylphenyl}benzamide;
3-(cyano-dimethyl-methyl)-*N*-[3-(5,7-dimethoxy-quinazolin-4-ylamino)-4-methyl-phenyl]-benzamide;
3-(1-cyano-1-methylethyl)-*N*-{3-[(7-isopropoxyquinazolin-4-yl)amino]-4-methyl phenyl}benzamide;
N-{3-[6,7-dimethoxyquinazolin-4-ylamino]-4-methylphenyl}-3-fluoro-5-isopropylbenzamide;
2-(cyano-dimethyl-methyl)-*N*-[3-(7-methoxy-quinazolin-4-ylamino)-4-methyl-phenyl]-isonicotinamide;
3-(cyano-dimethyl-methyl)-*N*-{3-[7-(3-dimethylamino-propoxy)-quinazolin-4-ylamino]-4-methyl-phenyl}-benzamide;
4-dimethylaminomethyl-*N*-[3-(7-methoxy-quinazolin-4-ylamino)-4-methyl-phenyl]-3-trifluoromethyl-benzamide; and
3-(cyano-dimethyl-methyl)-*N*-[3-(7-methyl-quinazolin-4-ylamino)-4-methyl-phenyl]-benzamide.

In one embodiment the B-raf inhibitor is selected from any one of
N-(6-amino-5-chloropyridin-3-yl)-5-{[3-(1-cyano-1-methylethyl)benzoyl]amino}-2-methylbenzamide;
N-(6-amino-5-chloropyridin-3-yl)-5-{[3-(1-cyano-1-methylethyl)-5-fluorobenzoyl]amino}-2-methylbenzamide;

5-{[3-(1-cyano-1-methylethyl)benzoyl]amino}-*N*-(5-methoxypyridin-3-yl)-2-methyl benzamide;

N-(6-amino-5-chloropyridin-3-yl)-2-methyl-5-{[3-(trifluoromethyl)benzoyl]amino} benzamide;

5-{[3-(1-cyano-1-methylethyl)benzoyl]amino}-*N*-(5,6-dimethylpyridin-3-yl)-2-methylbenzamide;

N-(3-{[(6-amino-5-chloropyridin-3-yl)amino]carbonyl}-4-methylphenyl)-2-(1-cyano-1-methylethyl)isonicotinamide;

N-(6-amino-5-chloropyridin-3-yl)-2-chloro-5-{[3-(trifluoromethyl)benzoyl]amino} benzamide;

N-(6-acetylamino-pyridin-3-yl)-5-[3-(cyano-dimethyl-methyl)-benzoylamino]-2-methyl-benzamide;

N-[6-(acetylamino)pyridin-3-yl]-2-chloro-5-{[3-(trifluoromethyl)benzoyl]amino}benzamide; and

N-(6-amino-5-methylpyridin-3-yl)-2-chloro-5-{[3-(1-cyano-1-methylethyl)benzoyl]amino} benzamide.

In one embodiment the B-raf inhibitor is selected from any one of

CHIR265;

XL281;

PLX4032;

3-(1-Cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

(R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide; and

3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide.

In a further embodiment the B-raf inhibitor is selected from any one of

CHIR265;

XL281; and

PLX4032.

In a further embodiment, the B-raf inhibitor is selected from XL281 or PLX4032.

In a further embodiment, the B-raf inhibitor is selected from any one of

3-(1-Cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

(R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide; and

3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide.

In one embodiment the B-raf inhibitor is a mutant B-raf selective inhibitor. Examples of mutant B-raf selective inhibitors are described in International Patent Publication Number WO2008020203. Particular mutant B-raf selective inhibitors include

(R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide; and

3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide.

In another embodiment the B-raf inhibitor may inhibit gene expression, for example by interfering with mRNA stability or translation. In one embodiment the B-raf inhibitor is selected from for example siRNA or shRNA.

In one embodiment there is provided a combination product comprising

AZD6244, or a pharmaceutically acceptable salt thereof, and

a B-raf inhibitor, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier, wherein the B-raf inhibitor is selected from any one of

XL281;

PLX4032;

3-(1-cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

(R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide; and

3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide.

In one embodiment there is provided a combination product comprising

2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and

a B-raf inhibitor, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier, wherein the B-raf inhibitor is selected from any one of

XL281;

PLX4032;

3-(1-cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

(R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide; and

3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl} ethyl)propanamide.

In one embodiment there is provided a combination product comprising 4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and a B-raf inhibitor, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier, wherein the B-raf inhibitor is selected from any one of

XL281;

PLX4032;

3-(1-cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

(R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide; and

3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl} ethyl)propanamide.

In one embodiment there is provided a combination product comprising AZD6244, or a pharmaceutically acceptable salt thereof, and XL281, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one embodiment there is provided a combination product comprising AZD6244, or a pharmaceutically acceptable salt thereof, and PLX4032, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one embodiment there is provided a combination product comprising AZD6244, or a pharmaceutically acceptable salt thereof, and

3-(1-cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one embodiment there is provided a combination product comprising

AZD6244, or a pharmaceutically acceptable salt thereof, and

(R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one embodiment there is provided a combination product comprising

AZD6244, or a pharmaceutically acceptable salt thereof, and

3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one embodiment there is provided a combination product comprising

2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and

XL281, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one embodiment there is provided a combination product comprising

2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and

PLX4032, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one embodiment there is provided a combination product comprising

2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and

3-(1-cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one embodiment there is provided a combination product comprising

2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and

(R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one embodiment there is provided a combination product comprising

2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and

3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one embodiment there is provided a combination product comprising

4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and

XL281, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one embodiment there is provided a combination product comprising

4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and

PLX4032, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one embodiment there is provided a combination product comprising

4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and

3-(1-cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one embodiment there is provided a combination product comprising

4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and

(R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one embodiment there is provided a combination product comprising

4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and

3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one embodiment there is provided a combination product comprising

a AZD6244, or a pharmaceutically acceptable salt thereof, and

a mutant B-raf inhibitor, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one embodiment there is provided a combination product comprising

a AZD6244, or a pharmaceutically acceptable salt thereof, and

a mutant B-raf inhibitor, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier, wherein the mutant B-raf inhibitor is selected from any one of

(R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide; and

3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide.

In one embodiment there is provided a combination product comprising

2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and

a mutant B-raf inhibitor, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier, wherein the mutant B-raf inhibitor is selected from any one of

(R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide; and

3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide.

In one embodiment there is provided a combination product comprising

4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide, or a pharmaceutically acceptable salt thereof, and

a mutant B-raf inhibitor, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier, wherein the mutant B-raf inhibitor is selected from any one of

(R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide; and

3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding pharmaceutically-acceptable salt of the inhibitor. A suitable pharmaceutically-acceptable salt of a MEK inhibitor or a B-raf inhibitor may be, for example, an acid-addition salt which is sufficiently basic, for example an acid-addition salt with an inorganic or organic acid. A suitable pharmaceutically-acceptable salt of a MEK inhibitor or a B-raf inhibitor may be, for example, a salt which is sufficiently acidic, for example an alkali or alkaline earth metal salt.

In another aspect of the present invention there provided a combination product comprising

a MEK inhibitor, or a pharmaceutically acceptable salt thereof,

linked to a B-raf inhibitor, or a pharmaceutically acceptable salt thereof,

in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

The combination product of the present invention is expected to produce a synergistic or beneficial effect through the production of an anti-cancer effect in a patient, which is accordingly useful in the treatment of cancer in a patient. A beneficial effect is achieved if the effect is therapeutically superior, as measured by, for example, the extent of the response, the response rate, the time to disease progression or the survival period, to that achievable on dosing one or other of the components of the combination treatment at its conventional dose. The beneficial effect may be synergistic, if the combined effect is therapeutically superior to the sum of the individual effect achievable with a MEK inhibitor or a B-raf inhibitor. Further, a beneficial effect is obtained if an effect is achieved in a group of patients that does not respond (or responds poorly) to an antagonist of the biological activity of a MEK inhibitor or a B-raf inhibitor alone. In addition, the effect is defined as affording a beneficial effect if one of the components is dosed at its conventional dose and the other component(s) is/are dosed at a reduced dose and the therapeutic effect, as measured by, for example, the extent of the response, the response rate, the time to disease progression or the survival period, is equivalent to that achievable on dosing conventional amounts of the components of the

combination treatment. In particular, a beneficial effect is deemed to be achieved if a conventional dose of a MEK inhibitor or a B-raf inhibitor may be reduced without detriment to one or more of the extent of the response, the response rate, the time to disease progression and survival data, in particular without detriment to the duration of the response, but with fewer and/or less troublesome side-effects than those that occur when conventional doses of each component are used.

In another aspect of the present invention there is provided a method of treating cancer, which comprises administration of a therapeutically effective amount of a combination product of the invention to a patient having or suspected of having cancer. In one embodiment the MEK inhibitor, or a pharmaceutically acceptable salt thereof, is administered sequentially, separately and/or simultaneously with the B-raf inhibitor, or a pharmaceutically acceptable salt thereof. In one embodiment the method additionally comprises selecting a patient in need of treatment of cancer, and administration to the patient of a therapeutically effective dose of a combination product of the invention.

In another aspect of the present invention there is provided a method of inhibiting MEK and B-raf, which comprises administration of a therapeutically effective amount of a combination product of the invention to a patient. In one embodiment the MEK inhibitor, or a pharmaceutically acceptable salt thereof, is administered sequentially, separately and/or simultaneously with the B-raf inhibitor, or a pharmaceutically acceptable salt thereof. In one embodiment the method additionally comprises selecting a patient in need of MEK and/or B-raf inhibition, and administration to the patient of a therapeutically effective dose of a combination product of the invention.

In another aspect of the present invention there is provided use of a combination product of the invention in the production of an anti-cancer effect in a patient, which is accordingly useful in the treatment of cancer. In one embodiment there is provided use of a combination product of the invention in the treatment of cancer.

In another aspect of the present invention there is provided use of a combination product of the invention in the inhibition of MEK and/or B-raf in a patient, which is accordingly useful in the treatment of cancer. In one embodiment there is provided use of a combination product of the invention for the inhibition of MEK and/or B-raf.

In one embodiment there is provided a method or use as described hereinabove wherein the patient is not resistant to MEK inhibition.

In another embodiment there is provided a method or use as described hereinabove wherein B-raf and MEK are not sequentially targeted in the method to overcome resistance to MEK inhibition.

The dosage of the MEK inhibitor and/or the B-raf inhibitor for a given patient will be determined by the attending physician, taking into consideration various factors known to modify the action of drugs including severity and type of disease, body weight, sex, diet, time and route of administration, other medications and other relevant clinical factors. Therapeutically effective dosages may be determined by either *in vitro* or *in vivo* methods.

The therapeutically effective amount of a MEK inhibitor or a B-raf inhibitor, as described herein, to be used will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it is preferred for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. A typical daily dosage might range from about 0.0001mg/kg to up to 100mg/kg or more, depending on the factors mentioned above. Typically, the clinician will administer the combination product until a dosage is reached that achieves the desired effect. Where separate formulations are administered, the sequence in which the MEK inhibitor, or pharmaceutically acceptable salt thereof, and the B-raf inhibitor, or pharmaceutically acceptable salt thereof, may be administered (i.e. whether and at what point sequential, separate and/or simultaneous administration takes place) may be determined by the physician or skilled person.

The combination product of the present invention is expected to be particularly useful for the treatment of patients with cancers, including, but not limited to, non-solid tumours such as leukaemia, for example acute myeloid leukaemia, multiple myeloma, haematologic malignancies or lymphoma, and also solid tumours and their metastases such as melanoma, non-small cell lung cancer, glioma, hepatocellular (liver) carcinoma, glioblastoma, carcinoma of the thyroid, bile duct, bone, gastric, brain/CNS, head and neck, hepatic, stomach, prostate, breast, renal, testicular, ovarian, skin, cervical, lung, muscle, neuronal, oesophageal, bladder, lung, uterine, vulval, endometrial, kidney, colorectal, pancreatic, pleural/peritoneal membranes, salivary gland, and epidermoid tumours and haematological malignancies.

The combination product of the invention is expected to be especially useful for the treatment of patients with lung cancer, melanoma, gastric cancer, colorectal cancer, ovarian cancer, thyroid cancer, pancreatic cancer, liver cancer, and their metastases, and also for the treatment of patients with acute myeloid leukaemia or multiple myeloma.

The combination product of the present invention is also expected to be particularly useful for the treatment of patients with a tumour which is associated with the Ras-Raf-MEK-ERK pathway or which is dependent alone, or in part, on the biological activity of the Ras-Raf-MEK-ERK pathway.

The combination product of the present invention is also expected to be particularly useful for the treatment of patients with a tumour which is associated with MEK or which is dependent alone, or in part, on the biological activity of MEK.

The combination product of the present invention is also expected to be particularly useful for the treatment of patients with a tumour which is associated with B-raf or which is dependent alone, or in part, on the biological activity of B-raf.

The combination product of the present invention may be used as a sole therapy or may involve additional surgery or radiotherapy or an additional chemotherapeutic agent or a therapeutic antibody in addition. Such chemotherapeutic agents may include one or more of the following categories of anti tumor agents:

(i) other antiproliferative/antineoplastic drugs and combinations thereof, as used in medical oncology, such as alkylating agents (for example cis-platin, oxaliplatin, carboplatin, cyclophosphamide, nitrogen mustard, melphalan, chlorambucil, busulphan, temozolamide and nitrosoureas); antimetabolites (for example gemcitabine and antifolates such as fluoropyrimidines like 5-fluorouracil and tegafur, raltitrexed, methotrexate, cytosine arabinoside, and hydroxyurea); antitumour antibiotics (for example anthracyclines like adriamycin, bleomycin, doxorubicin, daunomycin, epirubicin, idarubicin, mitomycin-C, dactinomycin and mithramycin); antimitotic agents (for example vinca alkaloids like vincristine, vinblastine, vindesine and vinorelbine and taxoids like taxol and taxotere and polokinase inhibitors); and topoisomerase inhibitors (for example epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan and camptothecin);

(ii) cytostatic agents such as antioestrogens (for example tamoxifen, fulvestrant, toremifene, raloxifene, droloxifene and iodoxyfene), antiandrogens (for example bicalutamide, flutamide,

nilutamide and cyproterone acetate), LHRH antagonists or LHRH agonists (for example goserelin, leuprorelin and buserelin), progestogens (for example megestrol acetate), aromatase inhibitors (for example as anastrozole, letrozole, vorazole and exemestane) and inhibitors of 5α-reductase such as finasteride;

(iii) anti-invasion agents (for example c-Src kinase family inhibitors like 4-(6-chloro-2,3-methylenedioxyanilino)-7-[2-(4-methylpiperazin-1-yl)ethoxy]-5-tetrahydropyran-4-yloxyquinazoline (AZD0530; International Patent Application WO 01/94341) and *N*-(2-chloro-6-methylphenyl)-2-{6-[4-(2-hydroxyethyl)piperazin-1-yl]-2-methylpyrimidin-4-ylamino}thiazole-5-carboxamide (dasatinib, BMS-354825; J. Med. Chem., 2004, 47, 6658-6661), and metalloproteinase inhibitors like marimastat, inhibitors of urokinase plasminogen activator receptor function or antibodies to Heparanase);

(iv) inhibitors of growth factor function: for example such inhibitors include growth factor antibodies and growth factor receptor antibodies (for example the anti-erbB2 antibody trastuzumab [Herceptin™], the anti-EGFR antibody panitumumab, the anti-erbB1 antibody cetuximab [Erbitux, C225] and any growth factor or growth factor receptor antibodies disclosed by Stern *et al.* Critical reviews in oncology/haematology, 2005, Vol. 54, pp11-29); such inhibitors also include tyrosine kinase inhibitors, for example inhibitors of the epidermal growth factor family (for example EGFR family tyrosine kinase inhibitors such as *N*-(3-chloro-4-fluorophenyl)-7-methoxy-6-(3-morpholinopropoxy)quinazolin-4-amine (gefitinib, ZD1839), *N*-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine (erlotinib, OSI-774) and 6-acrylamido-*N*-(3-chloro-4-fluorophenyl)-7-(3-morpholinopropoxy)-quinazolin-4-amine (CI 1033), erbB2 tyrosine kinase inhibitors such as lapatinib, inhibitors of the hepatocyte growth factor family, inhibitors of the platelet-derived growth factor family such as imatinib, inhibitors of serine/threonine kinases (for example Ras signalling inhibitors such as farnesyl transferase inhibitors, for example sorafenib (BAY 43-9006)), inhibitors of cell signalling through AKT kinases, inhibitors of the hepatocyte growth factor family, c-kit inhibitors, abl kinase inhibitors, IGF receptor (insulin-like growth factor) kinase inhibitors; aurora kinase inhibitors (for example AZD1152, PH739358, VX-680, MLN8054, R763, MP235, MP529, VX-528 AND AX39459) and cyclin dependent kinase inhibitors such as CDK2 and/or CDK4 inhibitors;

(v) antiangiogenic agents such as those which inhibit the effects of vascular endothelial growth factor, [for example the anti-vascular endothelial cell growth factor antibody bevacizumab (Avastin™) and VEGF receptor tyrosine kinase inhibitors such as 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-ylmethoxy)quinazoline (ZD6474; Example 2 within WO 01/32651), 4-(4-fluoro-2-methylindol-5-yloxy)-6-methoxy-7-(3-pyrrolidin-1-ylpropoxy)quinazoline (AZD2171; Example 240 within WO 00/47212), vatalanib (PTK787; WO 98/35985) and SU11248 (sunitinib; WO 01/60814), compounds such as those disclosed in International Patent Applications WO97/22596, WO 97/30035, WO 97/32856 and WO 98/13354 and compounds that work by other mechanisms (for example linomide, inhibitors of integrin αvβ3 function and angiostatin)];

(vi) vascular damaging agents such as Combretastatin A4 and compounds disclosed in International Patent Applications WO 99/02166, WO 00/40529, WO 00/41669, WO 01/92224, WO 02/04434 and WO 02/08213;

(vii) antisense therapies, for example those which are directed to the targets listed above, such as ISIS 2503, an anti-ras antisense;

(viii) gene therapy approaches, including for example approaches to replace aberrant genes such as aberrant p53 or aberrant BRCA1 or BRCA2, GDEPT (gene-directed enzyme pro-drug therapy) approaches such as those using cytosine deaminase, thymidine kinase or a bacterial nitroreductase enzyme and approaches to increase patient tolerance to chemotherapy or radiotherapy such as multi-drug resistance gene therapy; and

(ix) immunotherapy approaches, including for example ex-vivo and in-vivo approaches to increase the immunogenicity of patient tumour cells, such as transfection with cytokines such as interleukin 2, interleukin 4 or granulocyte-macrophage colony stimulating factor, approaches to decrease T-cell anergy, approaches using transfected immune cells such as cytokine-transfected dendritic cells, approaches using cytokine-transfected tumour cell lines and approaches using anti-idiotypic antibodies.

Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate dosing of the individual components of the treatment.

Anti-cancer effects which are accordingly useful in the treatment of cancer in a patient include, but are not limited to, anti-tumour effects, the response rate, the time to disease progression and the survival rate. Anti-tumour effects of a method of treatment of the present

invention include but are not limited to, inhibition of tumour growth, tumour growth delay, regression of tumour, shrinkage of tumour, increased time to regrowth of tumour on cessation of treatment, slowing of disease progression. It is expected that when a combination product of the present invention is administered to a patient in need of treatment for cancer, said combination product will produce an effect, as measured by, for example, one or more of: the extent of the anti-tumour effect, the response rate, the time to disease progression and the survival rate. Anti-cancer effects include prophylactic treatment as well as treatment of existing disease.

The following terms, unless otherwise indicated, shall be understood to have the following meanings:

An inhibitor may be a polypeptide, nucleic acid, carbohydrate, lipid, small molecular weight compound, an oligonucleotide, an oligopeptide, siRNA, antisense, a recombinant protein, an antibody, a peptibody, or conjugates or fusion proteins thereof. For a review of siRNA see Milhavet O, Gary DS, Mattson MP. (Pharmacol Rev. 2003 Dec;55(4):629-48. For a review of antisense see Opalinska JB, Gewirtz AM. Sci STKE. 2003 Oct 28; 2003 (206) : pe47.

A small molecular weight compound refers to a compound with a molecular weight of less than 2000 Daltons, 1000 Daltons, 700 Daltons or 500 Daltons.

A B-raf inhibitor is any inhibitor of the biological activity of wild-type or any mutant form of B-raf, including inhibitors that inhibit the biological activity of B-raf and other wild-type or mutant Raf serine/threonine protein kinase family members including Raf-1 /c-Raf, and/or A-Raf. A B-raf inhibitor may additionally inhibit VEGFR-2 and/or c-kit.

A patient is any warm-blooded animal, such as a human.

The term treatment includes therapeutic and/or prophylactic treatment.

The MEK inhibitor 4-(4-Bromo -2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide can be prepared according to the following method

Step A: Preparation of diethyl 2-(2-methylhydrazono)malonate: To a solution of diethyl ketomalonate (95 g, 546 mmol) in EtOH (600 mL) (2 L 3-neck flask equipped with thermocouple, °C (internal temperature, heated by a heating mantle) and stirred for 6 hours.

The reaction mixture was cooled to room temperature and stirred overnight. The reaction mixture was concentrated under reduced pressure to give the crude material along with solid precipitates that was purified by a silica gel plug (3:2 hexanes:EtOAc) to afford 81 g (74%) of the desired product. N_2 line, condenser and mechanical stirrer) was added $MeNHNH_2$ (32 mL, 600 mmol) in one portion at room temperature. The reaction mixture was warmed to 60

Step B: Preparation of diethyl 2-(2-methyl-2-propionylhydrazono)malonate: To a solution of 2-(2-methylhydrazono)malonate (100 g, 494 mmol) in THF (1 L) at 0 °C was added LiHMDS (643 mL, 643 mmol) by an addition funnel over 45 minutes. The reaction mixture was stirred for 45 minutes at 0 °C. Propionyl chloride (51.6 mL, 593 mmol) was added in one portion). The resulting mixture was warmed to room temperature and stirred for 20 hours. The reaction mixture was quenched with saturated aqueous NH_4Cl (85 mL) and water (85 mL). The reaction mixture was concentrated under reduced pressure and additional water (300 mL) was added. The resulting mixture was extracted with EtOAc (3 x 250 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (2 x 250 mL) followed by brine (250 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give 112 g (88%) of the crude product that was used directly in the next step without further purification.

Step C: Preparation of 4-hydroxy-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxylic acid: To a solution of LiHMDS (331 mL, 331 mmol, 1 M solution in THF) in THF (430 mL) at -78 °C was added a solution of 2-(2-methyl-2-propionylhydrazono)malonate (21.40 g, 82.86 mmol) in THF (10 mL). The resulting mixture was slowly warmed to -40 °C over 1 hour and stirred for 1.5 hours at -40 °C. To the reaction mixture was added water (500 mL) at -40 °C. The reaction mixture was warmed to room temperature and stirred for 3 hours. The reaction mixture was concentrated under reduced pressure, quenched with 6 N aqueous HCl at 0 °C, and acidified to pH 1 to 2. The resulting mixture was stirred for 16 hours at room temperature. The precipitates were filtered off and triturated with CH_2Cl_2 to afford 7.21 g (47%) of the desired product. The filtrate was extracted with EtOAc (3x). The combined organic layers were washed with water, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude material that was triturated with CH_2Cl_2 to afford additional 3.56 g (23%) of the desired product. The aqueous layer was extracted again with EtOAc (3x). The combined organic layers were washed with

water, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude material that was triturated with CH_2Cl_2 to afford additional 1.32 g (9%) of the desired product. A total of 12.09 g (79%) of the desired product was obtained.

Step D: Preparation of 4-chloro-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxylic acid: A mixture of 4-hydroxy-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxylic acid (35.4 g, 192 mmol), catalytic amount of DMF (3 drop), and $POCl_3$ (178 mL, 1.92 mol) was heated for 2 days at 90 °C, and then the $POCl_3$ was removed under reduced pressure. The crude material was quenched with ice, and the reaction mixture was stirred for 2 hours at room temperature. The precipitates formed out of the solution was filtered off and washed with ether. The precipitates collected were triturated with ether to afford 11.7 g (30%) of the desired product. The filtrate was extracted with EtOAc (2x). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product that was triturated with ether and dried under reduced pressure to afford additional 9.56 g (24%) of the desired product. A total of 21.29 g (55%) of the desired product was obtained.

Step E: Preparation of 4-(4-bromo-2-fluorophenylamino)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxylic acid: To a solution of 4-bromo-2-fluoroaniline (22.6 g, 116 mmol) in THF (165 mL) at -78 °C was slowly added a solution of LiHMDS (174 mL, 174 mmol, 1 M solution in THF). The resulting mixture was stirred for 1 hour at -78 °C. To this mixture was added 4-chloro-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxylic acid (11.0 g, 54.4 mmol) as a solid at -78 °C. The reaction mixture was slowly warmed to room temperature and stirred for 21 hour. The reaction was quenched and acidified with 10% aqueous HCl (250 mL) at 0 °C. To this mixture was added water (100 mL), EtOAc (350 mL), and brine (50 mL). The reaction mixture was warmed to room temperature and stirred for 30 minutes. The organic layer was separated and the acidic aqueous layer was extracted with EtOAc (2 x 300 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude material that was triturated with ether (5x), filtered, washed with ether, and dried under reduced pressure to afford 14.51 g (75%) of the desired product.

Step F: Preparation of 4-(4-bromo-2-fluorophenylamino)-1,5-dimethyl-6-oxo-N-(2-(vinyloxy)ethoxy)-1,6-dihydropyridazine-3-carboxamide: To a suspension of 4-(4-bromo-2-

fluorophenylamino)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxylic acid (14.51 g, 40.74 mmol) and HOBt (11.01 g, 81.48 mmol) in DMF (165 mL) was added EDCI (15.62 g, 81.48 mmol) at room temperature. The resulting mixture was stirred for 1.5 hours. O-(2-(Vinyloxy)ethyl)hydroxylamine (8.36 mL, 81.48 mmol) and TEA (11.36 mL, 81.48 mmol) was added to the activated ester at room temperature. After stirring for 1.5 hours, the reaction mixture was diluted with EtOAc and washed with saturated aqueous NH_4Cl, brine, saturated aqueous $NaHCO_3$ (2x), and brine. The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product that was used directly without further purification.

Step G: Preparation of 4-(4-bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide: A mixture of 4-(4-bromo-2-fluorophenylamino)-1,5-dimethyl-6-oxo-N-(2-(vinyloxy)ethoxy)-1,6-dihydropyridazine-3-carboxamide (17.98 g, 40.75 mmol) and 6 N aqueous HCl (13.58 mL, 81.50 mmol) in EtOH/THF (50 mL/50 mL) was stirred for 3 hours at room temperature. The reaction mixture was concentrated under reduced pressure and diluted with water (50 mL). The resulting mixture was extracted with EtOAc (2x). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude material that was purified by silica gel flash column chromatography (100% CH_2Cl_2 to 2.5% MeOH in CH_2Cl_2) to afford 9.41 g (56% for two steps) of the desired product. MS APCI (-) m/z 413, 415 (M-1, Br pattern) detected; ^1H NMR (400 MHz, CD_3OD) δ 7.38 (dd, 1H), 7.27 (d, 1H), 6.79 (t, 1H), 3.99 (t, 2H), 3.80 (s, 3H), 3.74 (t, 2H), 1.77 (s, 3H).

The MEK inhibitor 2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide can be prepared according to the following method

Step A. Preparation of 2-chloro-6-oxo-1,6-dihydro-pyridine-3-carboxylic acid: 2-Chloro-6-oxo-1,6-dihydro-pyridine-3-carboxylic acid was prepared from dichloronicotinic acid (3.00 g, 15.6 mmol, Aldrich) according to the procedure described in U.S. Patent No. 3,682,932 to yield 1.31 g (48%) of the desired product.

Step B. Preparation of 2-chloro-1-methyl-6-oxo-1,6-dihydro-pyridine-3-carboxylic acid methyl ester: To a solution of 2-chloro-6-oxo-1,6-dihydro-pyridine-3-carboxylic acid

(0.644 g, 3.71 mmol) in DMF (20 mL) was added lithium hydride (95%, 0.078 g, 9.28 mmol) and the reaction mixture was stirred for 40 minutes under N_2. Methyl iodide (0.508 mL, 1.16 g, 8.16 mmol) was then added and the reaction mixture was stirred for an additional 45 minutes. The reaction mixture was quenched with 2 M HCl until the pH was 6-7. The reaction mixture was diluted with EtOAc and saturated NaCl and the layers separated. The aqueous layer was back extracted with EtOAc (1x). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to yield a crude yellow solid. HPLC analysis showed two products in a 4:1 ratio that were separated by flash column chromatography (methylene chloride/EtOAc, 15:1 to 10:1) to give 0.466 g (62%) pure desired product as a white crystalline solid.

Step C. Preparation of methyl 5-bromo-2-chloro-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxylate: To a solution of methyl 2-chloro-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxylate (0.100 g, 0.496 mmol) in DMF (5 mL) was added N-bromosuccinimide (0.177 g, 0.992 mmol) and the reaction mixture was stirred for 4 hours at room temperature under N_2. The reaction mixture was quenched with saturated sodium bisulfite and then diluted with EtOAc and H_2O and the layers separated. The aqueous layer was back extracted with EtOAc (2x). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to yield a yellow solid in quantitative yield.

Step D. Preparation of methyl 2-chloro-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxylate: To a suspension of methyl 5-bromo-2-chloro-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxylate (0.400 g, 1.43 mmol) and **1,1'-**bis(diphenylphosphino)ferrocenedichloropalladium(II) (0.0587 g, 0.0713 mmol) in dioxane (8 mL) at 0 °C under N_2 was added dimethylzinc (0.713 mL, 1.43 mmol, 2 M solution in toluene). The reaction mixture was immediately heated to 100 °C for 30 minutes. The reaction mixture was cooled to 0 °C and quenched with MeOH (0.800 mL). The reaction mixture was diluted with EtOAc and washed with 1 M HCl. The aqueous layer was back extracted with EtOAc (1x). The combined organic layers were washed with saturated NaCl, dried (Na_2SO_4) and concentrated under reduced pressure to a dark yellow gum. Purification by flash column chromatography (methylene chloride/EtOAc, 15:1) gave 0.164 g (53%) pure desired product as a yellow crystalline solid.

Step E: Preparation of methyl - (2-fluoro-4-iodophenylamino)-1,5-dimethyl-6-oxo-

1,6-dihydropyridine-3-carboxylate: To a solution of 2-fluoro-4-iodobenzenamine (0.058 g, 0.31 mmol) in THF (2 mL) at –78 °C under N_2 was added lithium bis(trimethylsilyl)amide (0.56 mL, 0.56 mmol, 1 M solution in hexanes) dropwise. The reaction mixture was stirred for one hour at –78 °C. Methyl 2-chloro-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxylate (0.060 g, 0.28 mmol) was then added dropwise as a solution in THF (1 mL) and the reaction mixture was stirred for 25 minutes at –78 °C. The reaction mixture was quenched by the addition of H_2O and the pH was adjusted with 0.1M HCl and then diluted with EtOAc and saturated NaCl and the layers separated. The aqueous layer was back extracted with EtOAc (1x). The combined EtOAc layers were dried (Na_2SO_4) and concentrated under reduced pressure. Purification by flash column chromatography (methylene chloride/EtOAc, 20:1) gave 0.086 g (84%) pure desired product as a white crystalline solid. MS ESI (+) m/z 417 (M+1) detected; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.56 (s, 1H), 7.79 (s, 1H), 7.49 (d, 1H), 7.36 (d, 1H), 6.43 (t, 1H), 3.85 (s, 3H), 3.30 (s, 3H), 2.15 (s, 3H).

Step F: Preparation of 2-(2-fluoro-4-iodophenylamino)-1,5-dimethyl-6-oxo-N-(2-(vinyloxy)ethoxy)-1,6-dihydropyridine-3-carboxamide: To a solution of methyl 2-(2-fluoro-4-iodophenylamino)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxylate (0.500 g, 1.20 mmol) in THF (60 mL) was added O-(2-vinyloxy-ethyl)-hydroxylamine (0.149 g, 1.44 mmol). The solution was cooled to 0 °C and lithium bis(trimethylsilyl)amide (4.81 ml, 4.81 mmol) (1 M solution in hexanes) was added dropwise. The reaction mixture was warmed to room temperature. After stirring for 10 minutes the reaction mixture was quenched by the addition of 1 M HCl and partitioned between EtOAc and saturated NaCl. The layers were separated and the organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to yield a crude yellow solid that was used without purification in the next step.

Step G: Preparation of 2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide: To a solution of crude 2-(2-fluoro-4-iodophenylamino)-1,5-dimethyl-6-oxo-N-(2-(vinyloxy)ethoxy)-1,6-dihydropyridine-3-carboxamide (0.585 g, 1.20 mmol) in ethanol (10 mL) was added aqueous 2 M HCl (3 mL). The reaction mixture was stirred for 45 minutes at room temperature. The pH of the reaction mixture was adjusted to pH 7 with 1 M NaOH. The reaction mixture was diluted with EtOAc and H_2O. The organic layer was separated and washed with saturated NaCl. The combined

aqueous layers were back extracted with EtOAc (1x). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. Purification by silica gel flash column chromatography (methylene chloride/MeOH, 15:1) gave 2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide (0.421 g; 76% over two steps) as a pale yellow solid. MS ESI (+) m/z 462 (M+1) pattern detected; ^1H NMR (400 MHz, $CDCl_3$) δ 9.77 (s, 1H), 8.50 (s, 1H), 7.47 (d, 1H), 7.36 (d, 1H), 6.43 (t, 1H), 4.04 (br s, 2H), 3.85 (br s, 1H), 3.74 (br s, 2H), 3.29 (s, 3H), 2.14 (s, 3H). MS ESI (+) m/z 462 (M+1) pattern detected.

The invention will now be illustrated by the following non-limiting examples, which are provided for illustrative purposes only and are not to be construed as limiting upon the teachings herein, in which:

Figure 1. Combination Index curve for combination of AZD6244 and B-raf inhibitor Compound A in COLO-205 cell line.

Figure 2. Combination Index curve for combination of AZD6244 and B-raf inhibitor Compound A in SW620 cell line.

Figure 3. Combination Index curve for combination of AZD6244 and B-raf inhibitor Compound A in A375 cell line.

Figure 4. Shows dose response curves for combination of AZD6244 and B-raf inhibitor Compound A in the A549 cell line (concentration vs. fraction unaffected (Fu)).

Figure 5. Shows dose response curves for combination of AZD6244 and B-raf inhibitor Compound A in the A549 cell line (concentration vs. % cell viability).

Figure 6. Shows dose response curves for combination of 4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide and B-raf inhibitor Compound A in the A549 cell line (concentration vs. % cell viability).

Figure 7. Shows dose response curves for 2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide and B-raf inhibitor Compound A plotting concentration vs. % cell viability in the A549 cell line.

In Figures 5, 6 and 7; the circles represent AZD6244 monotherapy; the triangles represent AZD6244 plus 20nM B-raf inhibitor Compound A; the inverted triangles represent

AZD6244 plus 167nM B-raf inhibitor Compound A; the diamonds represent AZD6244 plus 333nM B-raf inhibitor Compound A.

Figure 8. Combination Index curve for combination of AZD6244 and B-raf inhibitor Compound A in SW620 cell line.

Figure 9. Combination Index curve for combination of 4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide and B-raf inhibitor Compound A in SW620 cell line.

Figure 10. Combination Index curve for combination of 2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide and B-raf inhibitor Compound A in SW620 cell line.

Figure 11. Combination Index curve for combination of AZD6244 and B-raf inhibitor Compound A in A375 cell line.

Figure 12. Combination Index curve for combination of 4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide and B-raf inhibitor Compound A in A375 cell line.

Figure 13. Combination Index curve for combination of 2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide and B-raf inhibitor Compound A in A375 cell line.

Figure 14. Combination Index curve for combination of AZD6244 and B-raf inhibitor Compound A in COLO-205 cell line.

Figure 15. Combination Index curve for combination of 4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide and B-raf inhibitor Compound A in COLO-205 cell line.

Figure 16. Combination Index curve for combination of 2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide and B-raf inhibitor Compound A in COLO-205 cell line.

Figure 17. Combination Index curve for combination of AZD6244 and (R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide in A375 cell line.

Figure 18. Shows dose response curves for AZD6244 and (R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide plotting concentration vs. % cell growth in the COLO-205 cell line. The closed circles represent 200nM AZD6244 monotherapy; the

closed diamonds represent 20nM AZD6244 monotherapy; the open triangles represent (R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide monotherapy; the closed squares represent 20nM AZD6244 plus (R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide; the closed triangles represent 200nM AZD6244 plus (R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide.

Figure 19. Shows dose response curves for AZD6244 and 3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide plotting concentration vs. % cell growth in the COLO-205 cell line. The closed circles represent 200nM AZD6244 monotherapy; the closed diamonds represent 20nM AZD6244 monotherapy; the open triangles represent 3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide monotherapy; the closed squares represent 20nM AZD6244 plus 3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide; the closed triangles represent 200nM AZD6244 plus 3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide.

Example 1: In vitro combination study of AZD6244 with 3-(1-Cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide, a B-raf inhibitor

The effect on cell viability of a mek inhibitor, AZD6244, was evaluated in combination with a B-raf inhibitor, 3-(1-Cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide ("B-raf inhibitor Compound A"), in several cell lines. A correlation between B-raf mutational status and sensitivity to B-raf inhibition has been seen previously, so cell lines with different B-raf and ras status were assayed to determine whether this status would have an effect on whether the combination of inhibitors was antagonistic, additive, or synergistic. The COLO-205 and A375 cell lines, which are mutant for B-raf and wild type for K-ras, are sensitive to both inhibitors. The SW620 cell line is less sensitive and the A549 cell line is resistant to both inhibitors. The SW620 and A549 cell lines are wild type for B-raf and mutant for K-ras.

Cells were seeded in 96-well plates on day 0 and treated with either a single drug or simultaneously with both drugs for 72 hours beginning on Day 1. For cell lines in which an IC50 could be determined for the single compounds (COLO-205, SW620, andA375), the

MEK inhibitor and B-raf inhibitor were combined at a constant ratio of $[IC50]_{raf\ inhibitor}$:$[IC50]_{mek\ inhibitor}$. For the less sensitive cell line, in which IC50s could not be determined (A549), the inhibitors were combined in a 1:1 ratio. See Table 1 for an example of the drug concentrations used in each cell line.

Table 1 **IC50 values Used to Determine AZD6244: B-raf Inhibitor Compound A Ratio and Top Concentration Used in Combination (uM)**

Drug	COLO-205 IC50	COLO-205 Top Conc.	SW620 IC50	SW620 Top Conc	A375 IC50	A375 Top Conc	A549 IC50	A549 Top Conc
AZD6244	0.075	9.61	0.175	1.66	0.02	0.6	----	16.7
Compd. A	0.078	10.0	1.05	10.0	0.055	1.65	----	16.7

Cell viability was measured on day 4 using an MTS assay. A dose response curve was generated for each drug alone and in combination. For cell lines COLO-205, SW620, and A375, a combination index (CI; Chou and Talalay, 1984), which compares the response of single agents to the combination, was calculated from the dose response curves, and a CI curve generated which indicates whether the drug combinations were antagonistic (CI value >1), additive (CI values ~1), or synergistic (CI values <1).

Figures 1-3 show the representative Combination Index curves for treatment of the COLO-205, SW620, and A375 cell lines with the MEK inhibitor and B-raf inhibitor. In all cases, treatment of cells with the combined drugs was beneficial. At the ratios tested, combined treatment resulted in synergy at all concentrations >IC15.

For the A549 cell line, a CI could not be determined because the compounds do not have single agent activity at the concentrations tested. For this cell line, the dose-response curves for the single agents were compared to that for the combination. For any given concentration of drug (uM), Fu (fraction unaffected) is lower for the combination than for either single agent. The combined drug treatment had an IC50 (Inhibitory Concentration producing 50% inhibition) of ~0.6uM (0.3 uM AZD6244 + 0.3 uM B-raf Inhibitor Compound A) whereas the cells were unaffected by either single agent at this concentration, indicating

that the compounds acted synergistically. Example data for the A549 cell line is shown in Figure 4.

The combination of a MEK inhibitor and a B-raf inhibitor was found to be synergistic in all cell lines tested, regardless of B-raf and K-ras mutational status. The combination of a MEK inhibitor and a B-raf inhibitor showed synergy even in a cell line A549, which is relatively insensitive to both inhibitors when used as single agents.

Example 2: In vitro combination study of AZD6244 or 2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide or 4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide with B-raf inhibitor Compound A

The effect on cell viability of MEK inhibitors, AZD6244, 2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide and 4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide was evaluated in combination with a B-raf inhibitor, 3-(1-Cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide ("B-raf inhibitor Compound A"), in several cell lines. A correlation between B-raf mutational status and sensitivity to B-raf inhibition has been seen previously, so cell lines with different B-raf and ras status were assayed to determine whether this status would have an effect on whether the combination of inhibitors was antagonistic, additive, or synergistic. The COLO-205 and A375 cell lines, which are mutant for B-raf and wild type for K-ras, are sensitive to both inhibitors. The SW620 cell line is less sensitive and the A549 cell line is resistant to both inhibitors. The SW620 and A549 cell lines are wild type for B-raf and mutant for K-ras.

Cells were seeded in 96-well plates on day 0 and treated with either a single drug or simultaneously with both drugs for 96 hours beginning on Day 0. For cell lines in which an IC50 could be determined for the single compounds (COLO-205, SW620, andA375), the MEK inhibitor and B-raf inhibitor were combined using equal inhibitory effect ratios of the two agents (IC_{60}, IC_{50}, IC_{40}, IC_{30}, IC_{20}, IC_{10}). For the less sensitive cell line, in which IC50s could not be determined (A549), a fixed dose of the BRAF inhibitor was applied across the

full dose range of the MEK inhibitor. The dose of the BRAF inhibitor was choosen by its pharmacodynamic effect against pMAPK ($^{Thr202/Tyr204}$).

Cell viability was measured on day 5 using an MTS assay. A dose response curve was generated for each drug alone and in combination. For cell lines COLO-205, SW620, and A375, a combination index, which compares the response of single agents to the combination, was calculated from the dose response curves, and a CI curve generated which indicates whether the drug combinations were antagonistic.

Figures 8-16 show the representative CI curves for treatment of the COLO-205, SW620, and A375 cell lines with a MEK inhibitor and B-raf inhibitor Compound A. In all cases, treatment of cells with the MEK inhibitor and B-raf inhibitor did achieve synergy.

For the A549 cell line, a CI could not be determined because the compounds do not have single agent activity at the concentrations tested. For this cell line, the dose-response curves for the single agents were compared to that for the combination. For any given concentration of drug (uM), Fu (fraction unaffected) is lower for the combination than for either single agent.

For any given concentration of drug (uM), Fu (fraction unaffected) is lower for the combination than for either single agent. The combination of a MEK inhibitor and a B-raf inhibitor was found to be synergistic in all cell lines tested, regardless of B-raf and K-ras mutational status. This combination of a MEK inhibitor and a B-raf inhibitor showed synergy even in a cell line A549, which is relatively insensitive to both inhibitors when used as single agents. Example data for the A549 cell line is shown in Figures 5, 6 and 7.

Table 2 IC50 (μM) values of MEK inhibitor + B-Raf inhibitor Compound A in A549 Cells

	AZD6244	**4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide**	**2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide**
Monotherapy	15.08 (CIR 4.9, n = 3)	1.33 (CIR 1.5, n = 3)	0.13 (CIR 1.43, n = 3)
+ Cpd. A 20nm	3.12 (CIR 1.7, n = 3)	0.85 (CIR 4.3, n = 3)	0.07 (CIR 1.2, n=3)
+ Cpd. A 167nm	0.84 (CIR 2.4, n = 3)	0.30 (CIR 1.9, n = 3)	0.02 (CIR 2.2, n =2)
+ Cpd. A 333nm	0.42 (CIR 2.1, n = 3)	0.22 (CIR 2.2, n = 3)	0.01 (CIR 1.5, n = 3)

CIR = Confidence Interval Ratio. n = number of replicate experiments.

Example 3: In vitro combination study of AZD6244 with a mutant B-raf inhibitor in a homozygous mutant B-raf (V600E) cell line

The effect on cell viability of a mek inhibitor, AZD6244, was evaluated in combination with a mutant B-raf inhibitor, (R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide, in the A375 cell line, which is homozygous for mutant B-raf. These cells are sensitive to both compounds.

Cells were seeded in 96-well plates on day 0 and treated with either a single drug or simultaneously with both drugs for 72 hours beginning on day 1. The MEK inhibitor and the B-raf inhibitor were combined at a constant ratio of $[IC50]_{raf\ inhibitor}$:$[IC50]_{mek\ inhibitor}$.

Cell viability was measured on day 4 using an MTS assay. A dose response curve was generated for each drug alone and in combination. A combination index (CI; Chou and Talalay, 1984), which compares the response of cells to single agents vs. the combination, was calculated from the dose response curves, and a CI curve generated which indicates whether the drug combinations were antagonistic (CI value >1), additive (CI value ~1), or synergistic (CI value <1).

Figure 17 shows the Combination Index curves for treatment of A375 cells with the MEK inhibitor and the B-raf inhibitor. Treatment with the combination achieves synergy.

Example 4: In vitro combination study of AZD6244 with mutant B-raf inhibitors in a cell line heterozygous for mutant B-raf (V600E)

The effect on cell viability of a MEK inhibitor, AZD6244, was evaluated in combination with mutant B-raf inhibitors, (R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide and 3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide, in the COLO-205 cell line that is heterozygous for mutant B-raf (V600E). The COLO-205 cell line is sensitive to the MEK inhibitor AZD6244, and to the mutant B-raf inhibitors (R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide and 3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide.

Cells were seeded in 96-well plates on day 0 and treated with either a single drug or simultaneously with both drugs for 72 hours beginning on day 1. Cells treated with both drugs received a fixed dose of AZD6244 in combination with serially diluted mutant B-raf inhibitor. AZD6244 was used at two different concentrations, 20nM and 200nM. The dose of 200nM AZD6244 was selected as previous experiments had shown this dose can completely inhibit phosphorylation of ERK, a MEK substrate, in a number of cell lines by western blot analysis. Cell viability was measured on day 4 using an MTS assay. A dose response curve was generated for each drug alone and in combination. Dose response curves for the single agents were compared to that for the combination.

Figures 18 and 19 show the results of this experiment using COLO-205 cells. Treatment of cells with the combined drugs was more beneficial than monotherapy.

Claims

1. A combination product comprising

a MEK inhibitor selected from:

AZD6244;

2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide; or

4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide; or a pharmaceutically acceptable salt thereof, and

a B-raf inhibitor, or a pharmaceutically acceptable salt thereof,

in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

2. A combination product which comprises a kit of parts comprising components:

a MEK inhibitor selected from:

AZD6244;

2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide; or

4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide; or a pharmaceutically acceptable salt thereof in association with a pharmaceutically acceptable adjuvant, diluent or carrier; and

a B-raf inhibitor, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable adjuvant, diluent or carrier,

wherein the components are provided in a form which is suitable for sequential, separate and/or simultaneous administration, and further comprising instructions to administer the components sequentially, separately and/or simultaneously.

3. A combination product according to claim 1 or 2, wherein the MEK inhibitor is AZD6244.

4. A combination product according to claim 1 or 2, wherein the MEK inhibitor is 2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide.

5. A combination product according to claim 1 or 2, wherein the MEK inhibitor is 4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide.

6. A combination product according to any one of the preceding claims wherein the B-raf inhibitor is selected from any one of

3-(1,1-dimethylprop-2-yn-1-yl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

3-(1-cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

3-(1-cyano-1-methylethyl)-5-fluoro-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

3-(1-cyano-1-methylethyl)-5-[(dimethylamino)methyl]-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

4-dimethylaminomethyl-*N*-[4-methyl-3-(3-methyl-4-oxo-3,4-dihydro-quinazolin-6-ylamino)-phenyl]-3-trifluoromethyl-benzamide;

2-(1-cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}isonicotinamide;

3-(1-cyano-1-methylethyl)-2-fluoro-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

N-(3-{[3-(3-aminopropyl)-4-oxo-3,4-dihydroquinazolin-6-yl]amino}-4-methylphenyl)-3-(1-cyano-1-methylethyl)benzamide;

3-{[methoxy(methyl)amino]sulfonyl}-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

3-*tert*-butyl-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

3-(cyano-dimethyl-methyl)-*N*-[3-(7-methoxy-quinazolin-4-ylamino)-4-methyl-phenyl]-benzamide;

3-(cyano-dimethyl-methyl)-5-fluoro-*N*-[3-(7-methoxy-quinazolin-4-ylamino)-4-methyl-phenyl]-benzamide;

3-(1-cyano-1-methylethyl)-2-fluoro-*N*-{3-[(7-methoxy quinazolin-4-yl)amino]-4-methylphenyl}benzamide;

3-(cyano-dimethyl-methyl)-*N*-[3-(5,7-dimethoxy-quinazolin-4-ylamino)-4-methyl-phenyl]-benzamide;

3-(1-cyano-1-methylethyl)-*N*-{3-[(7-isopropoxyquinazolin-4-yl)amino]-4-methyl phenyl}benzamide;

N-{3-[6,7-dimethoxyquinazolin-4-ylamino]-4-methylphenyl}-3-fluoro-5-isopropylbenzamide;

2-(cyano-dimethyl-methyl)-*N*-[3-(7-methoxy-quinazolin-4-ylamino)-4-methyl-phenyl]-isonicotinamide;

3-(cyano-dimethyl-methyl)-*N*-{3-[7-(3-dimethylamino-propoxy)-quinazolin-4-ylamino]-4-methyl-phenyl}-benzamide;

4-dimethylaminomethyl-*N*-[3-(7-methoxy-quinazolin-4-ylamino)-4-methyl-phenyl]-3-trifluoromethyl-benzamide;

3-(cyano-dimethyl-methyl)-*N*-[3-(7-methyl-quinazolin-4-ylamino)-4-methyl-phenyl]-benzamide;

N-(6-amino-5-chloropyridin-3-yl)-5-{[3-(1-cyano-1-methylethyl)benzoyl]amino}-2-methylbenzamide;

N-(6-amino-5-chloropyridin-3-yl)-5-{[3-(1-cyano-1-methylethyl)-5-fluorobenzoyl]amino}-2-methylbenzamide;

5-{[3-(1-cyano-1-methylethyl)benzoyl]amino}-*N*-(5-methoxypyridin-3-yl)-2-methyl benzamide;

N-(6-amino-5-chloropyridin-3-yl)-2-methyl-5-{[3-(trifluoromethyl)benzoyl]amino} benzamide;

5-{[3-(1-cyano-1-methylethyl)benzoyl]amino}-*N*-(5,6-dimethylpyridin-3-yl)-2-methylbenzamide;

N-(3-{[(6-amino-5-chloropyridin-3-yl)amino]carbonyl}-4-methylphenyl)-2-(1-cyano-1-methylethyl)isonicotinamide;

N-(6-amino-5-chloropyridin-3-yl)-2-chloro-5-{[3-(trifluoromethyl)benzoyl]amino} benzamide;

N-(6-acetylamino-pyridin-3-yl)-5-[3-(cyano-dimethyl-methyl)-benzoylamino]-2-methyl-benzamide;

N-[6-(acetylamino)pyridin-3-yl]-2-chloro-5-{[3-(trifluoromethyl)benzoyl]amino}benzamide; and

N-(6-amino-5-methylpyridin-3-yl)-2-chloro-5-{[3-(1-cyano-1-methylethyl)benzoyl]amino} benzamide.

7. A combination product according to any one of the preceding claims, wherein the MEK inhibitor is selected from any one of

AZD6244;

2-(2-fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide; and

4-(4-Bromo-2-fluorophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridazine-3-carboxamide,

and the B-raf inhibitor is selected from any one of

XL281;

PLX4032;

3-(1-cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

(R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide; and

3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl} ethyl)propanamide.

8. A combination product according to claim 7, wherein the MEK inhibitor is AZD6244 and the B-raf inhibitor is selected from any one of

XL281;

PLX4032;

3-(1-cyano-1-methylethyl)-*N*-{4-methyl-3-[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)amino]phenyl}benzamide;

(R)-N-(1-(4-(6-(Pyridin-4-yl)quinazolin-2-ylamino)phenyl)ethyl)acetamide; and

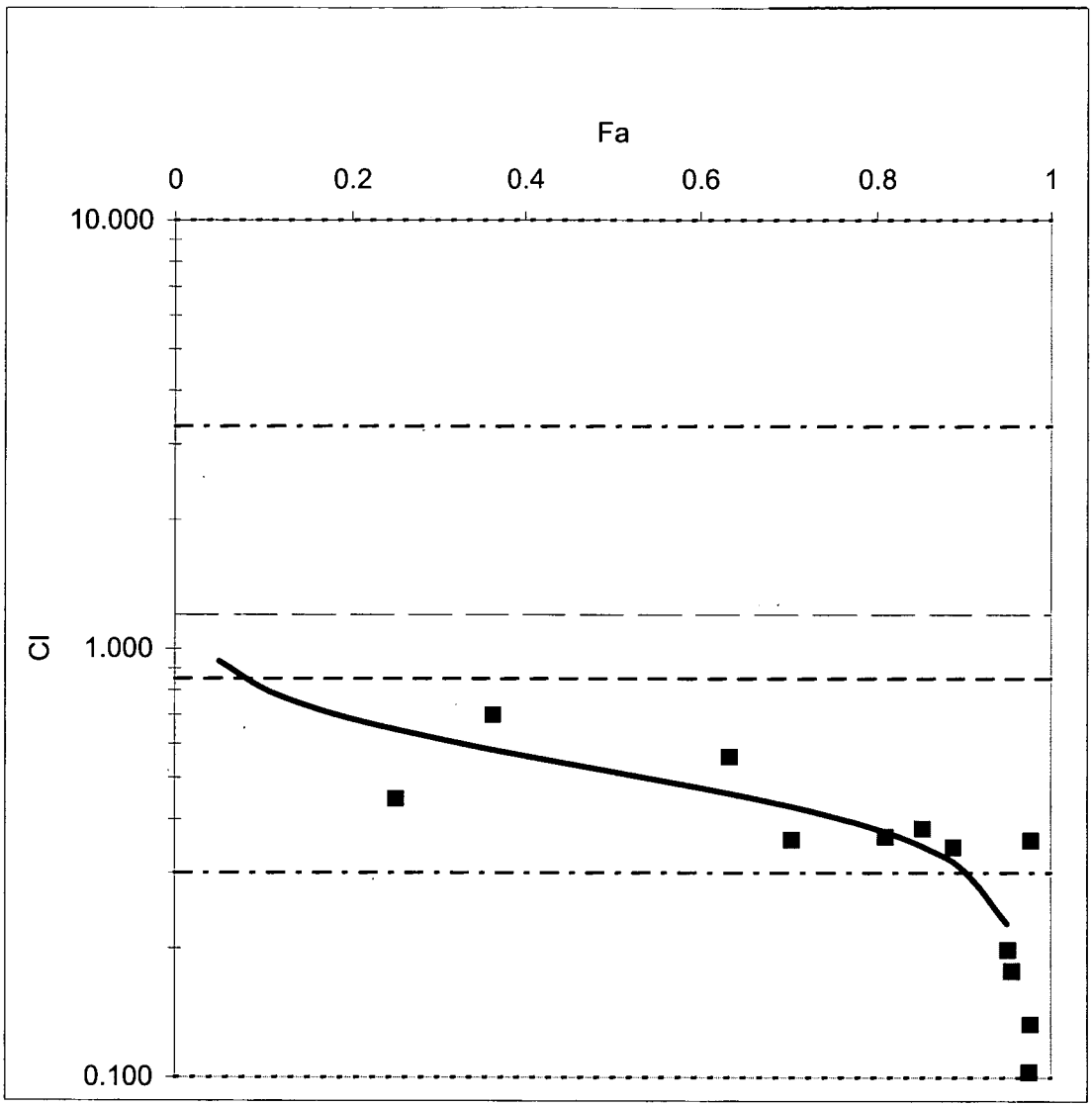
3-Methoxy-N-((1R)-1-{4-[(6-pyridin-4-ylquinazolin-2-yl)amino]phenyl}ethyl)propanamide..

9. Use of a combination product according to any one of the preceding claims in the treatment of cancer.

10. Use according to claim 9 wherein the cancer is selected from lung cancer, melanoma, colorectal cancer, ovarian cancer, gastric cancer, thyroid cancer, pancreatic cancer, liver cancer, acute myeloid leukaemia or multiple myeloma.

11. A method of treating cancer, which comprises administration of a combination product according to any one of claims 1-8 to a patient, having or suspected of having cancer.

12. A method of treating cancer according to claim 11 wherein the cancer is selected from lung cancer, melanoma, colorectal cancer, ovarian cancer, gastric cancer, thyroid cancer, pancreatic cancer, liver cancer, acute myeloid leukaemia or multiple myeloma.



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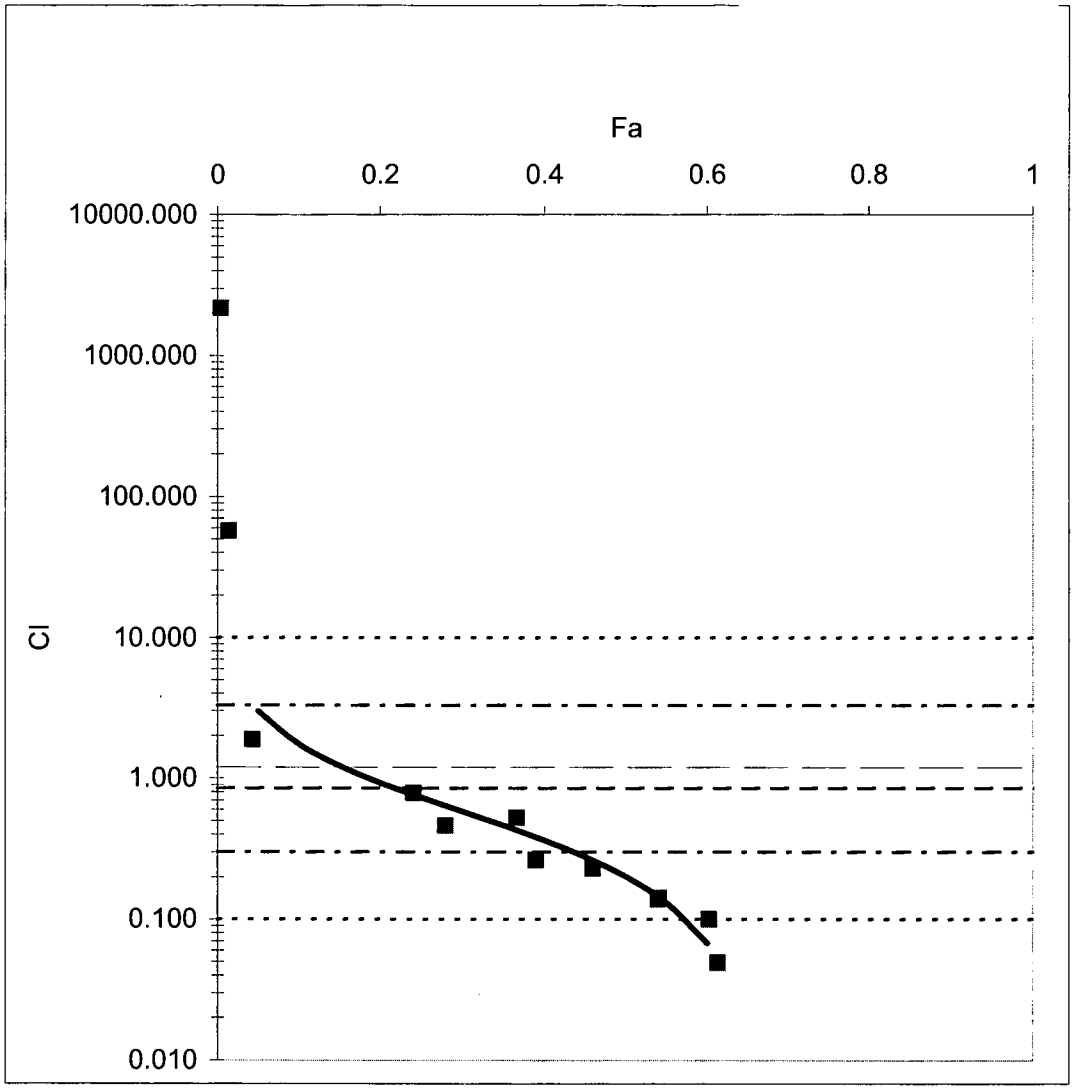
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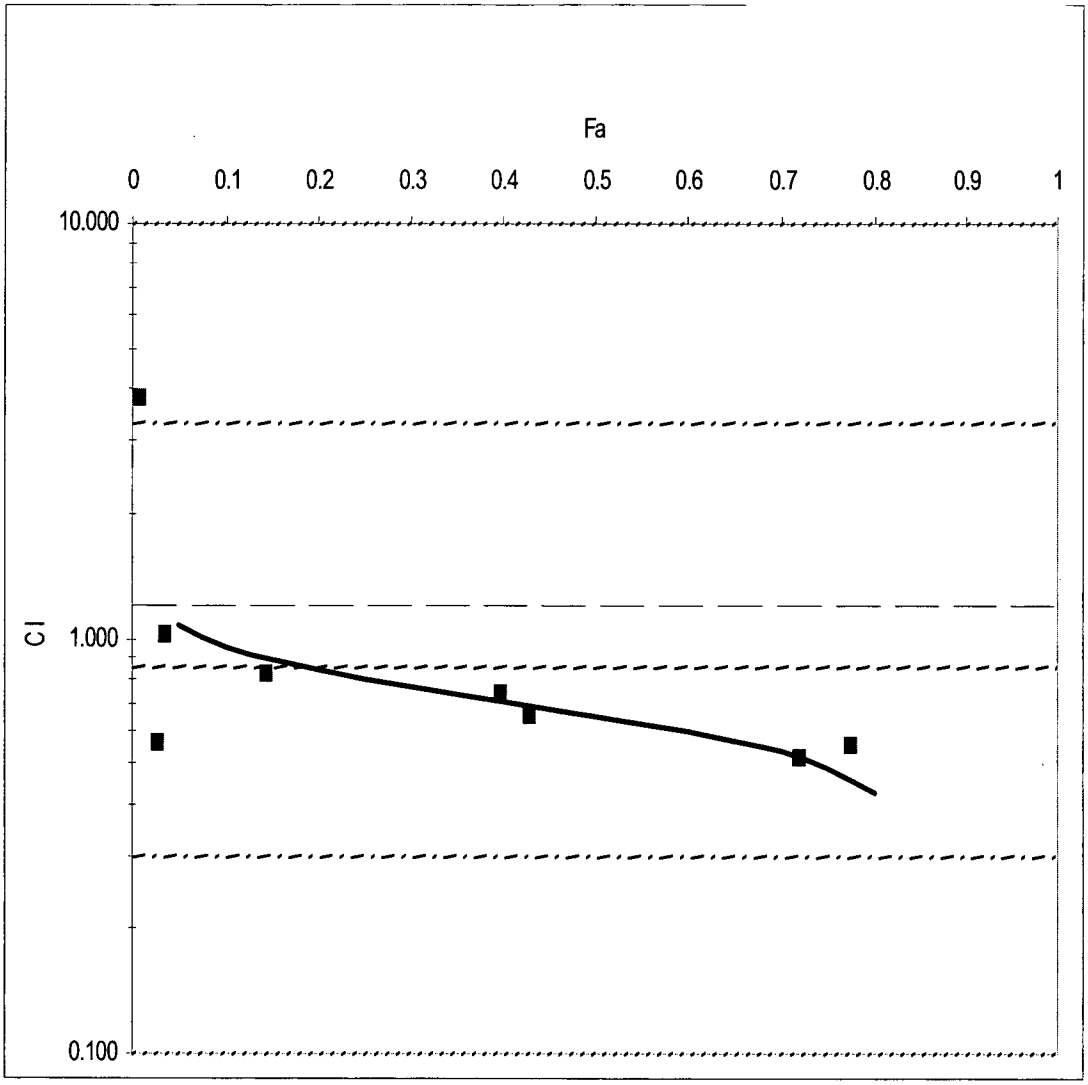
Figure 2



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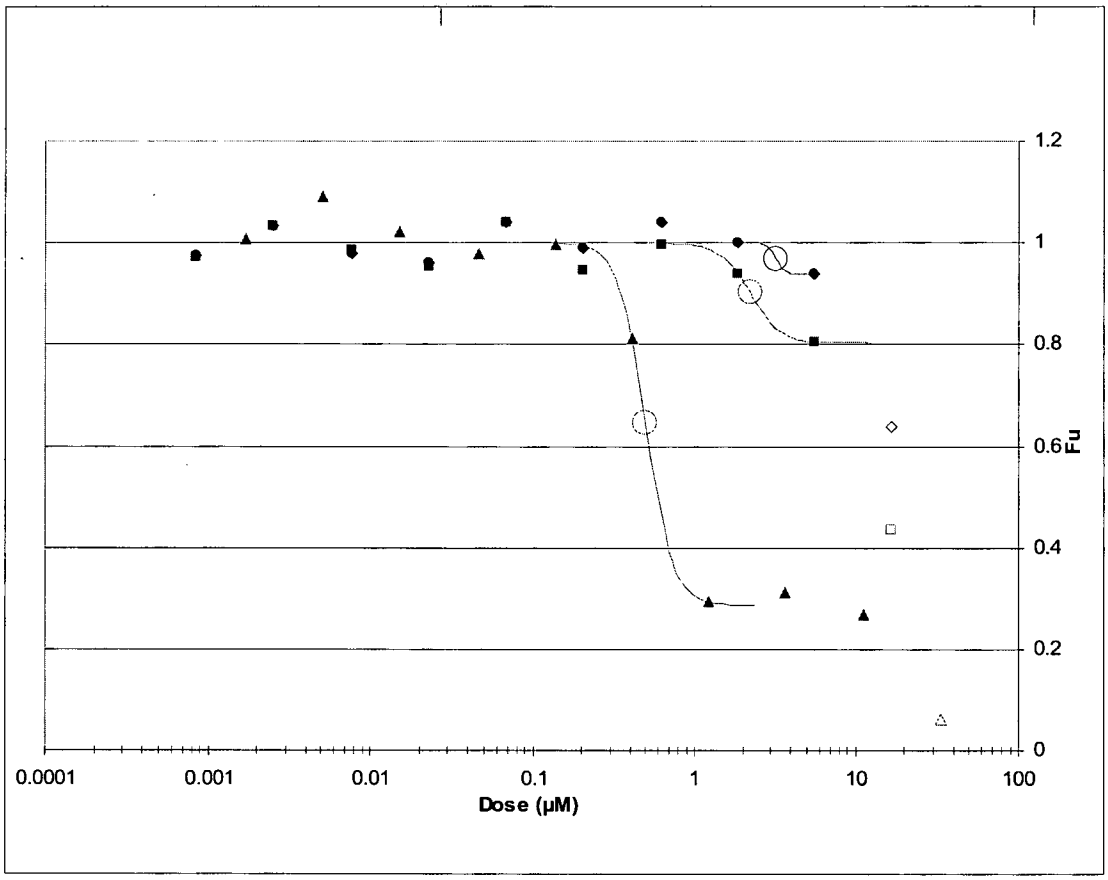

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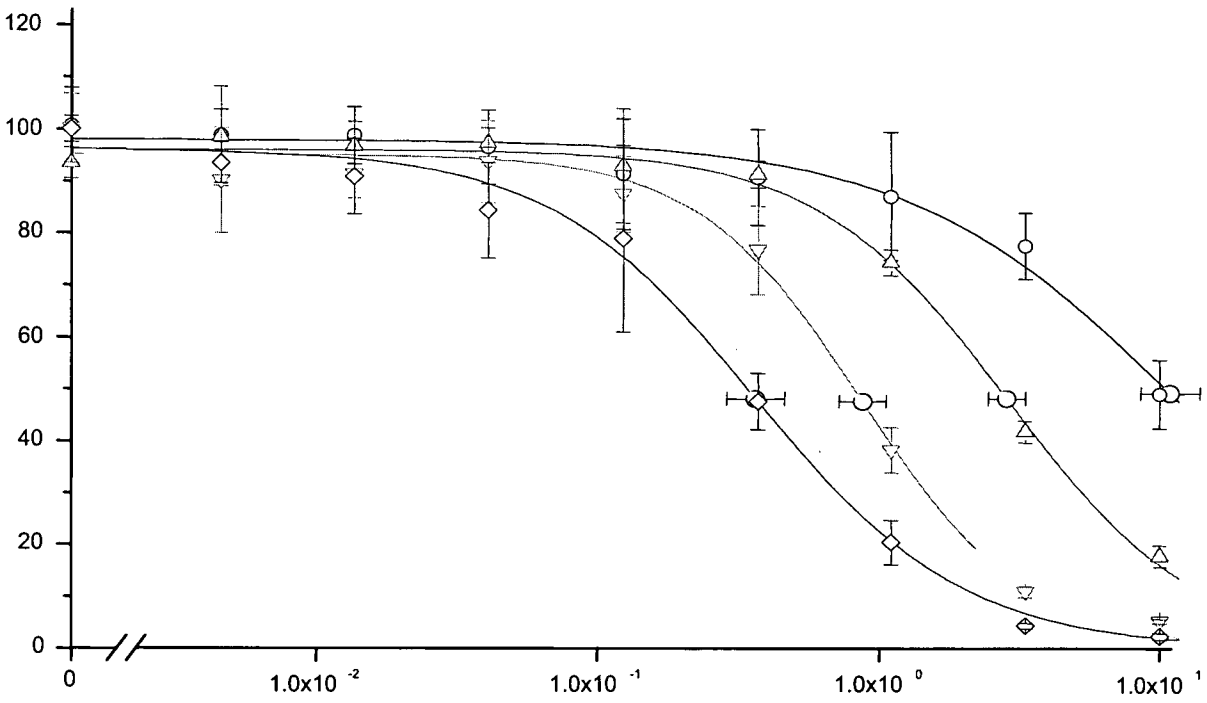

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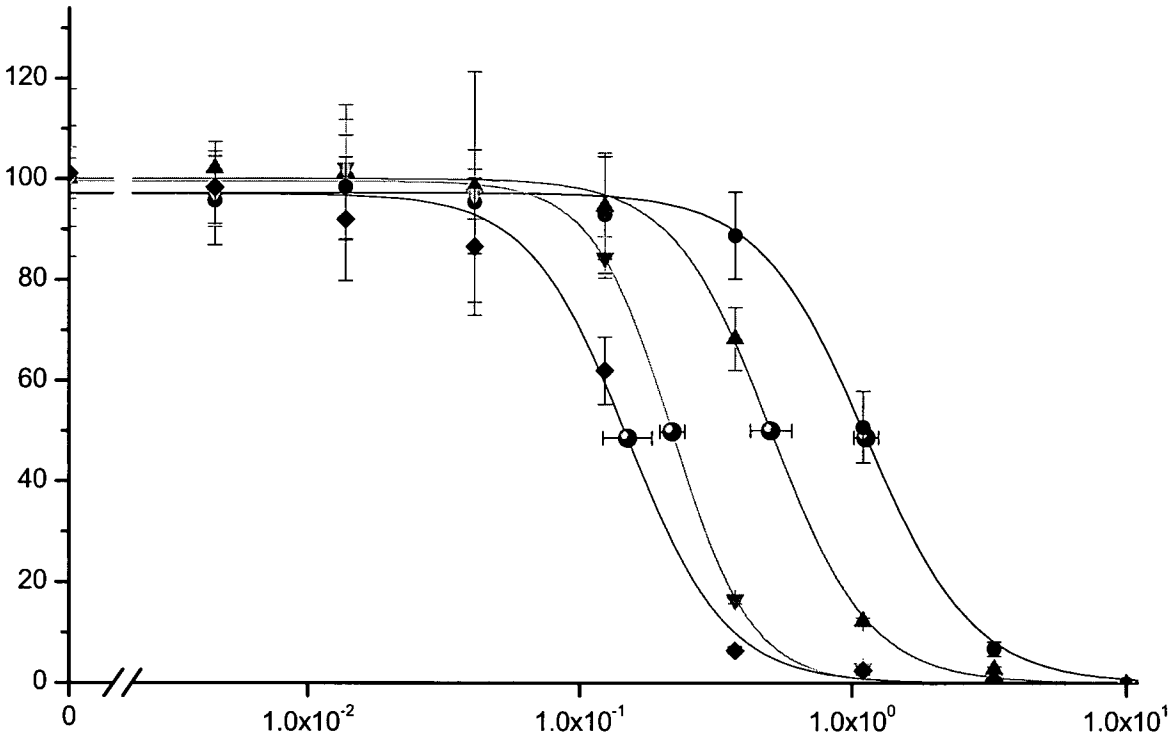

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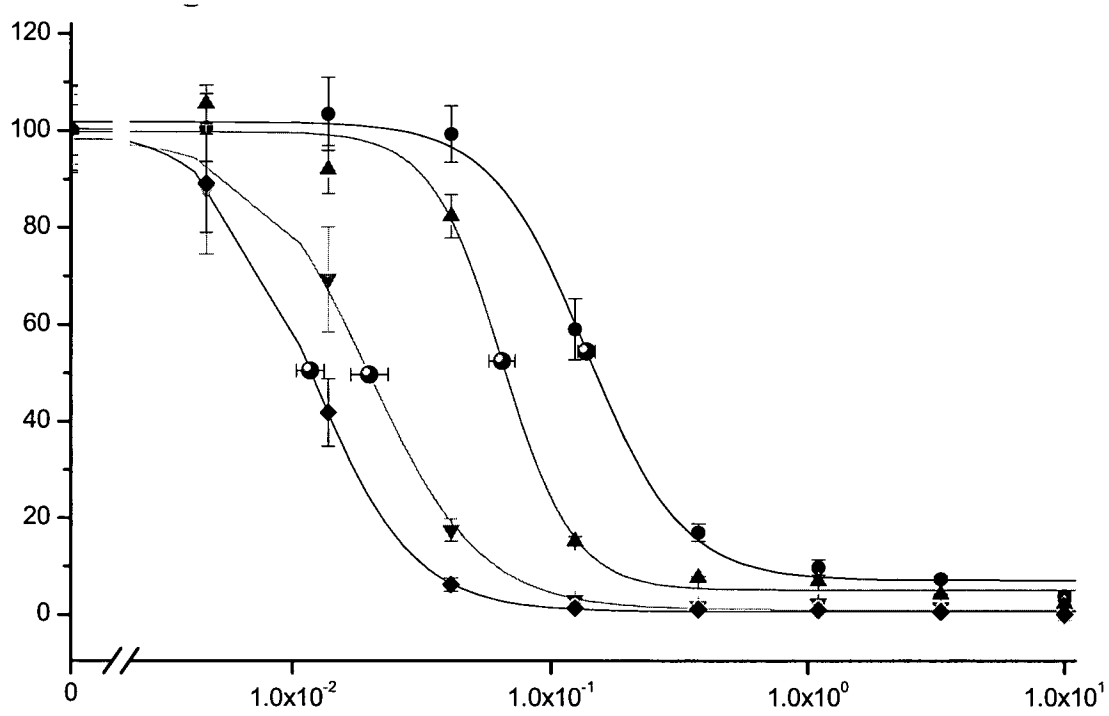

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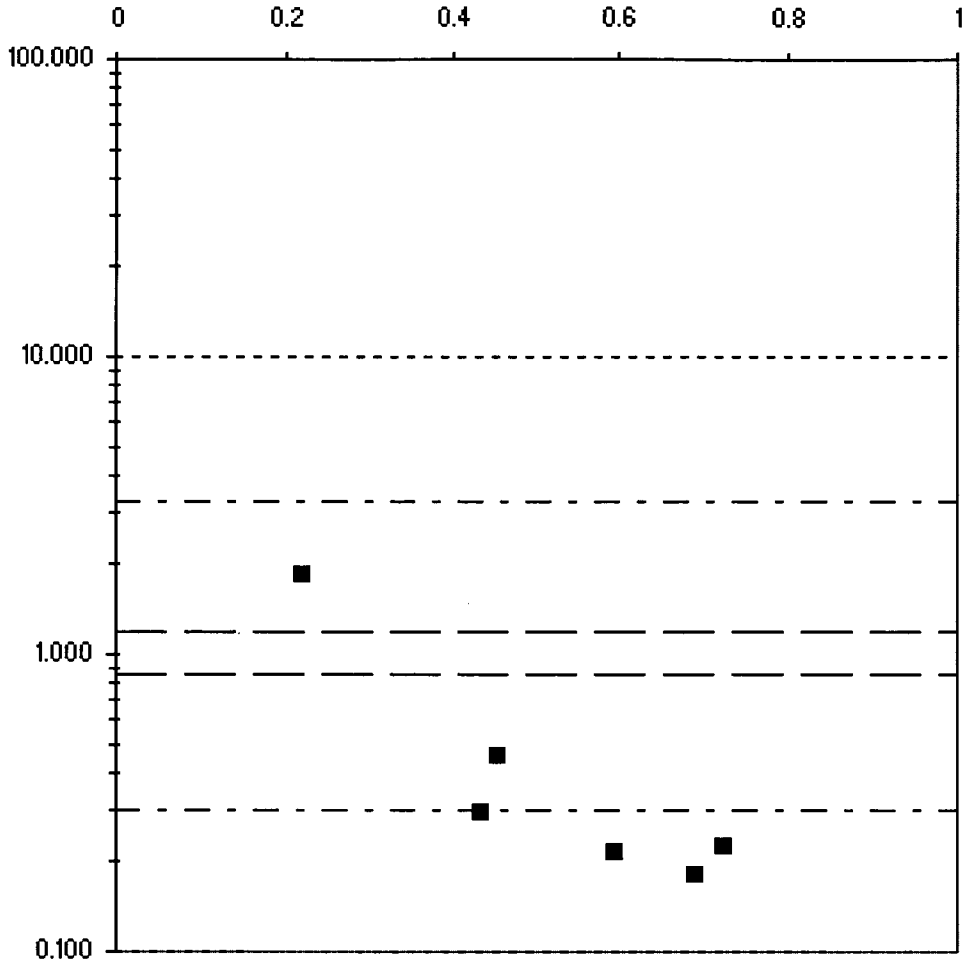
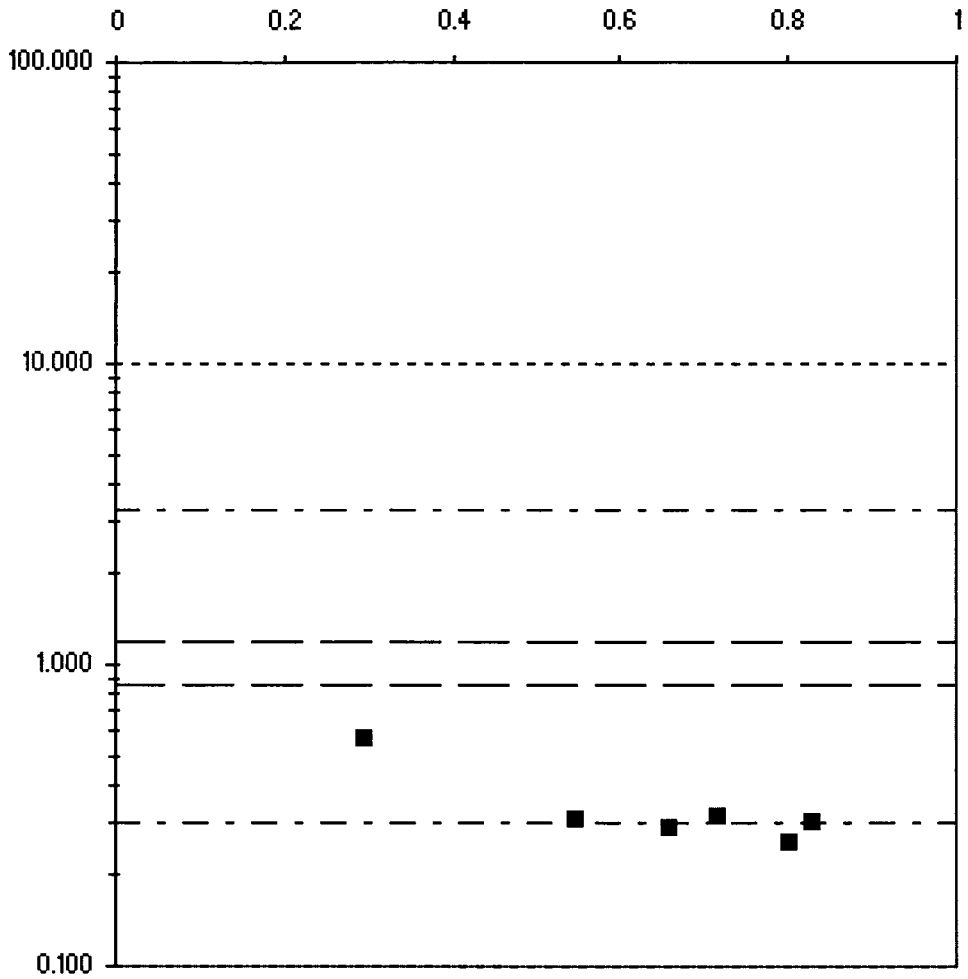


Figure 8



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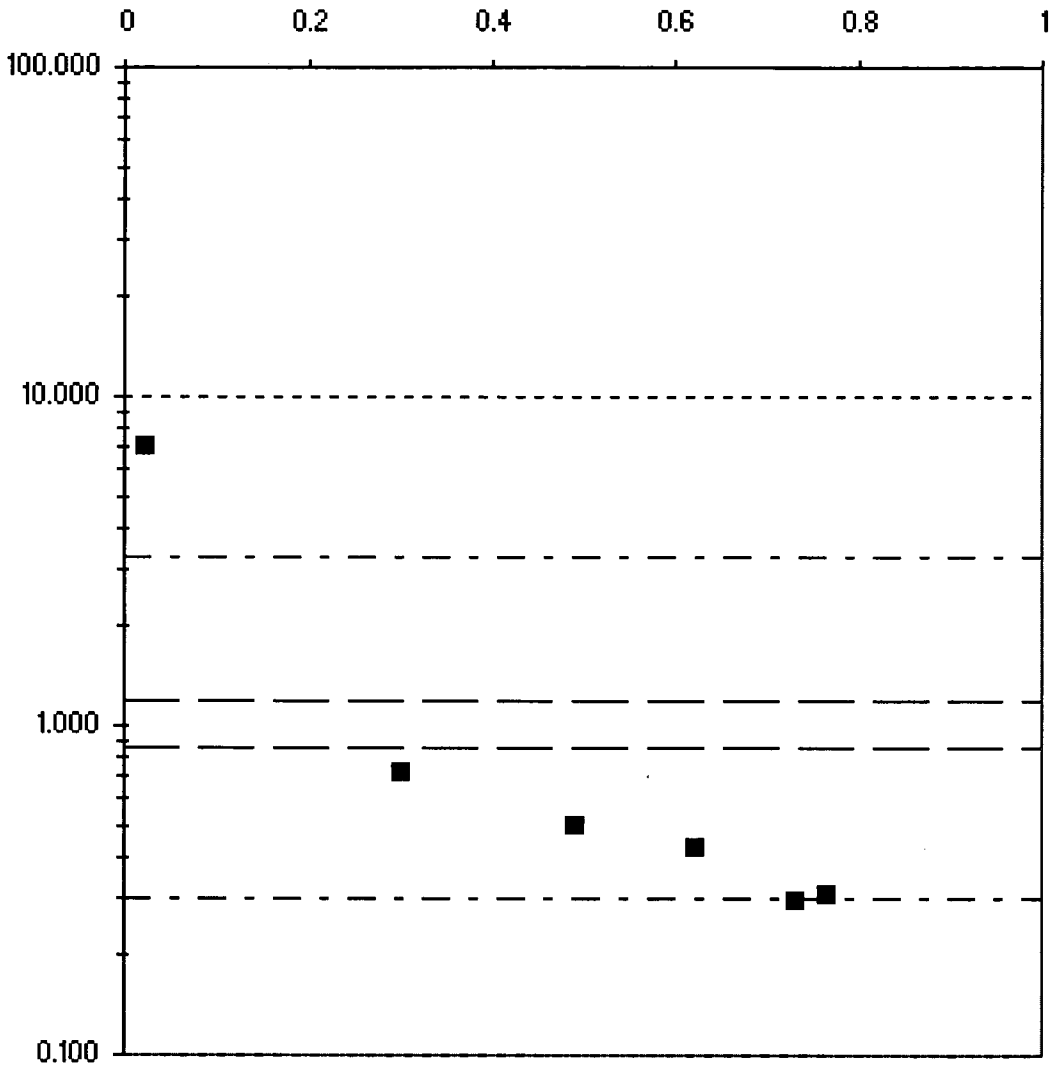

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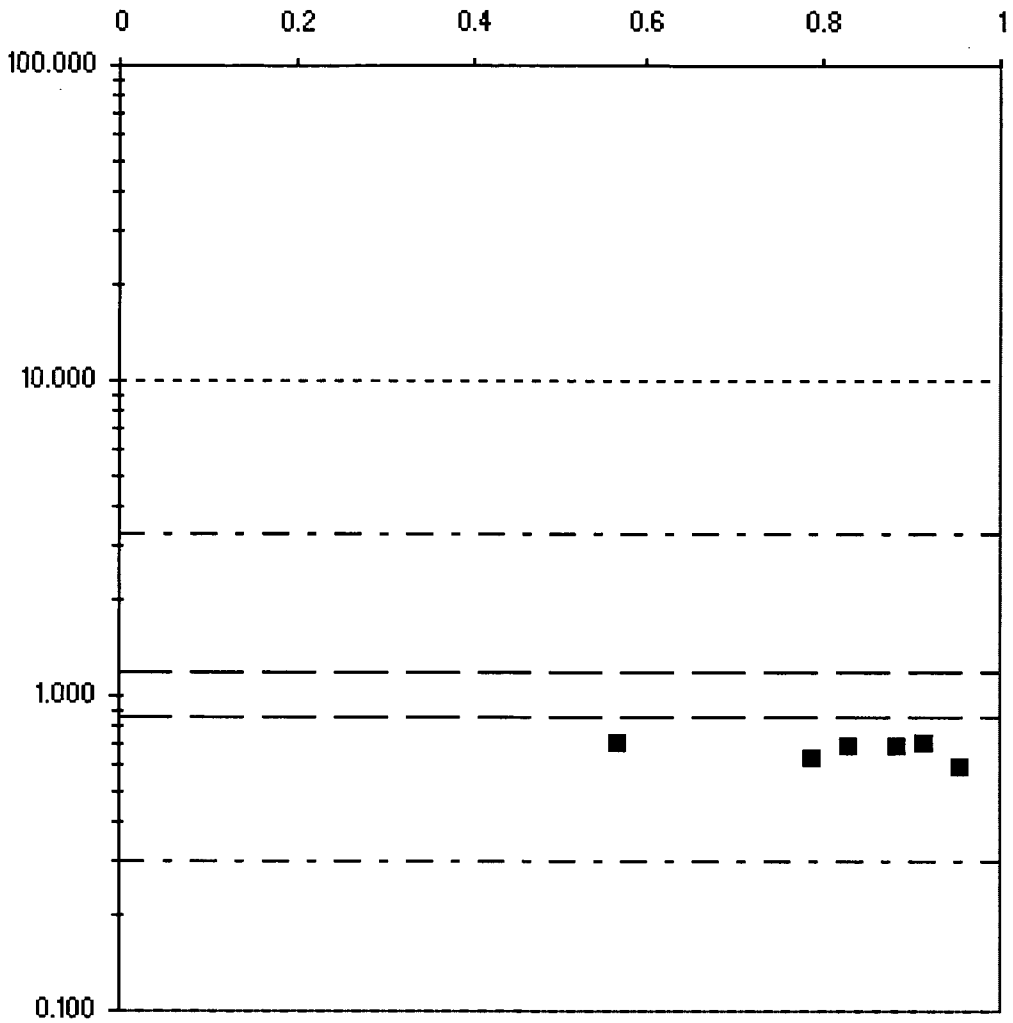


Figure 11

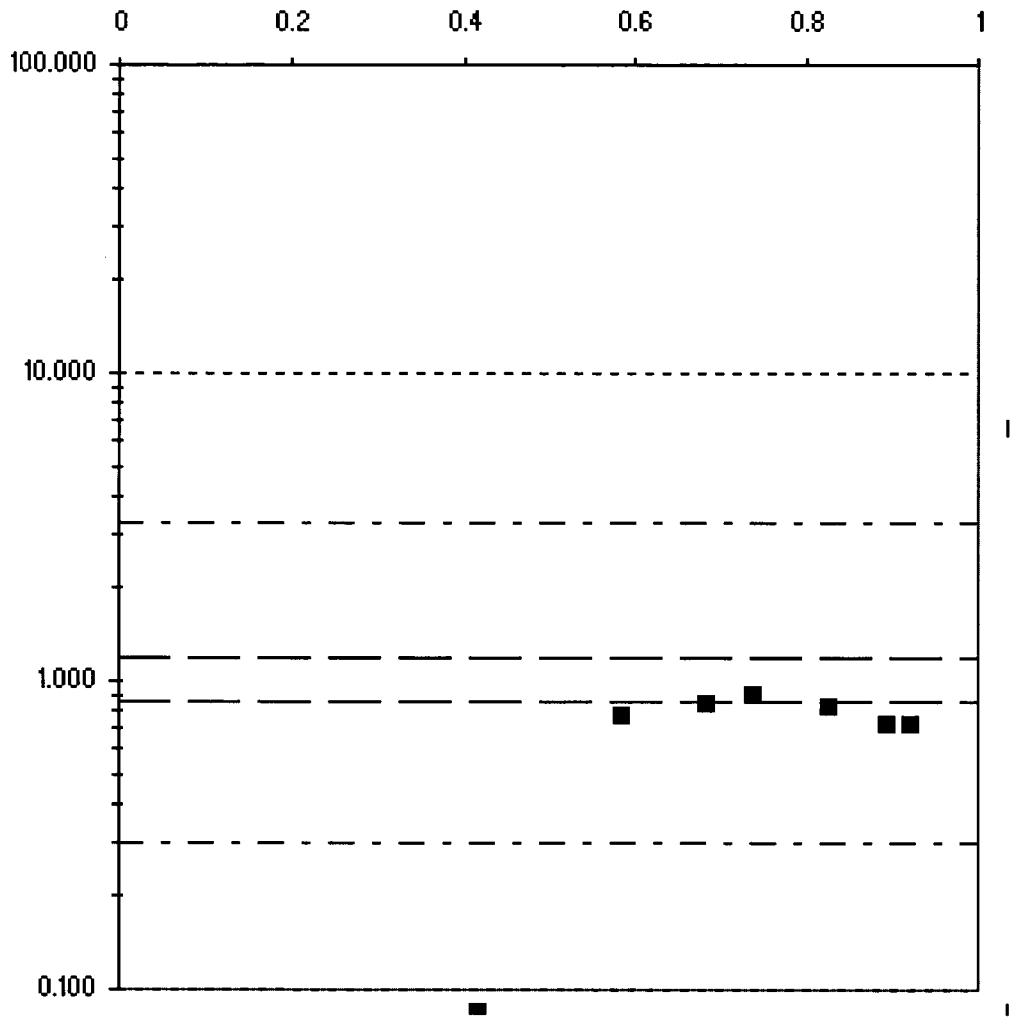


Figure 12

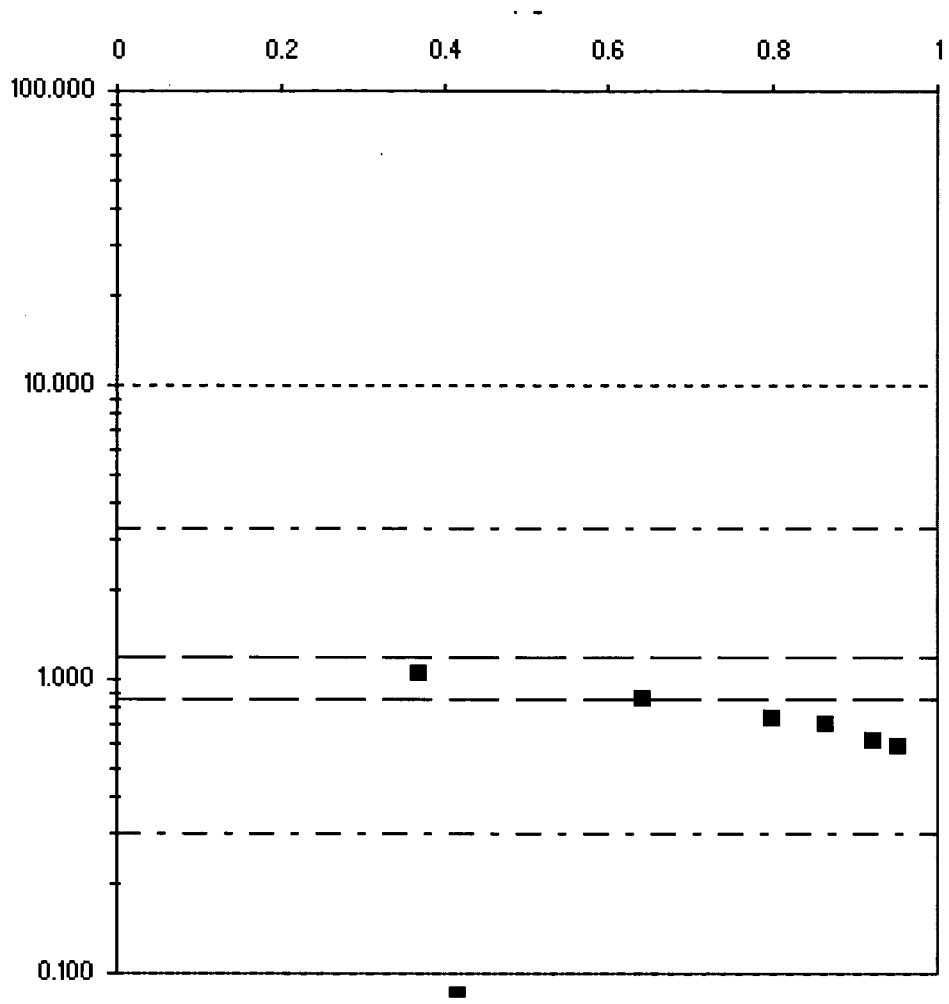


Figure 13

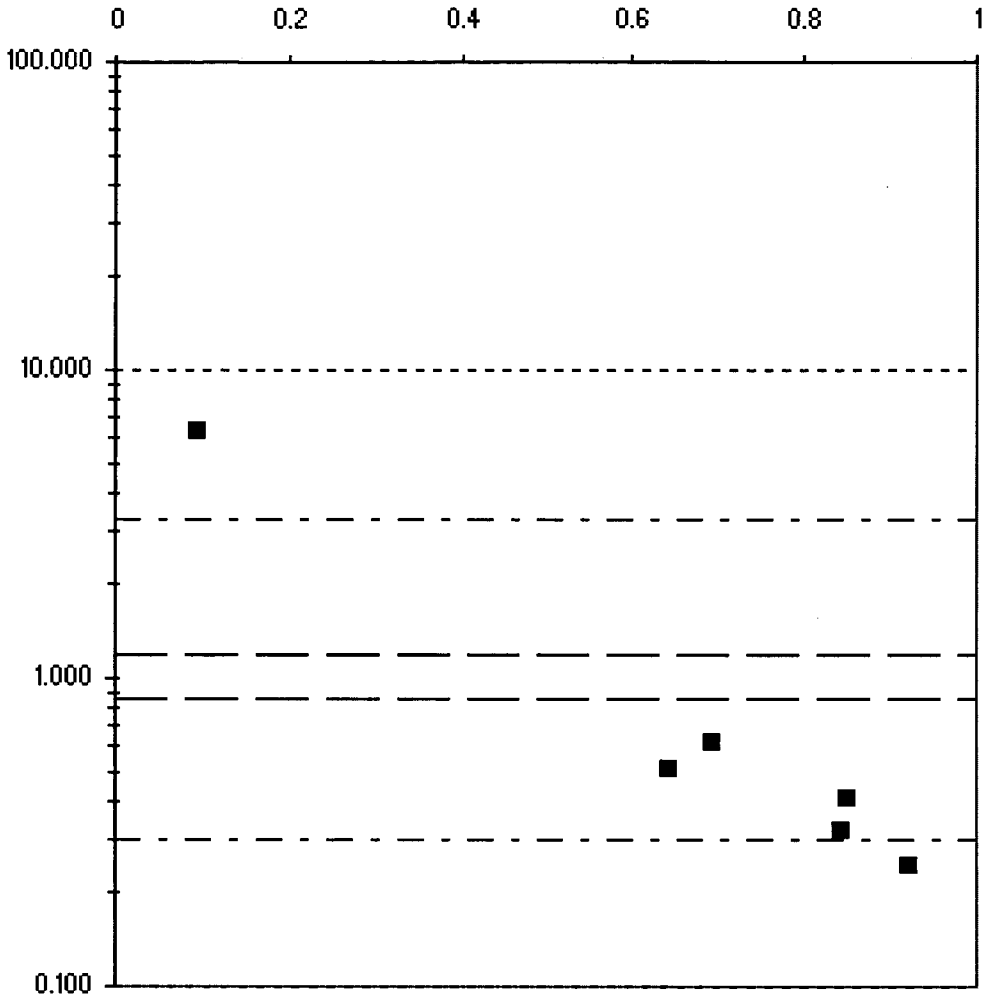


Figure 14

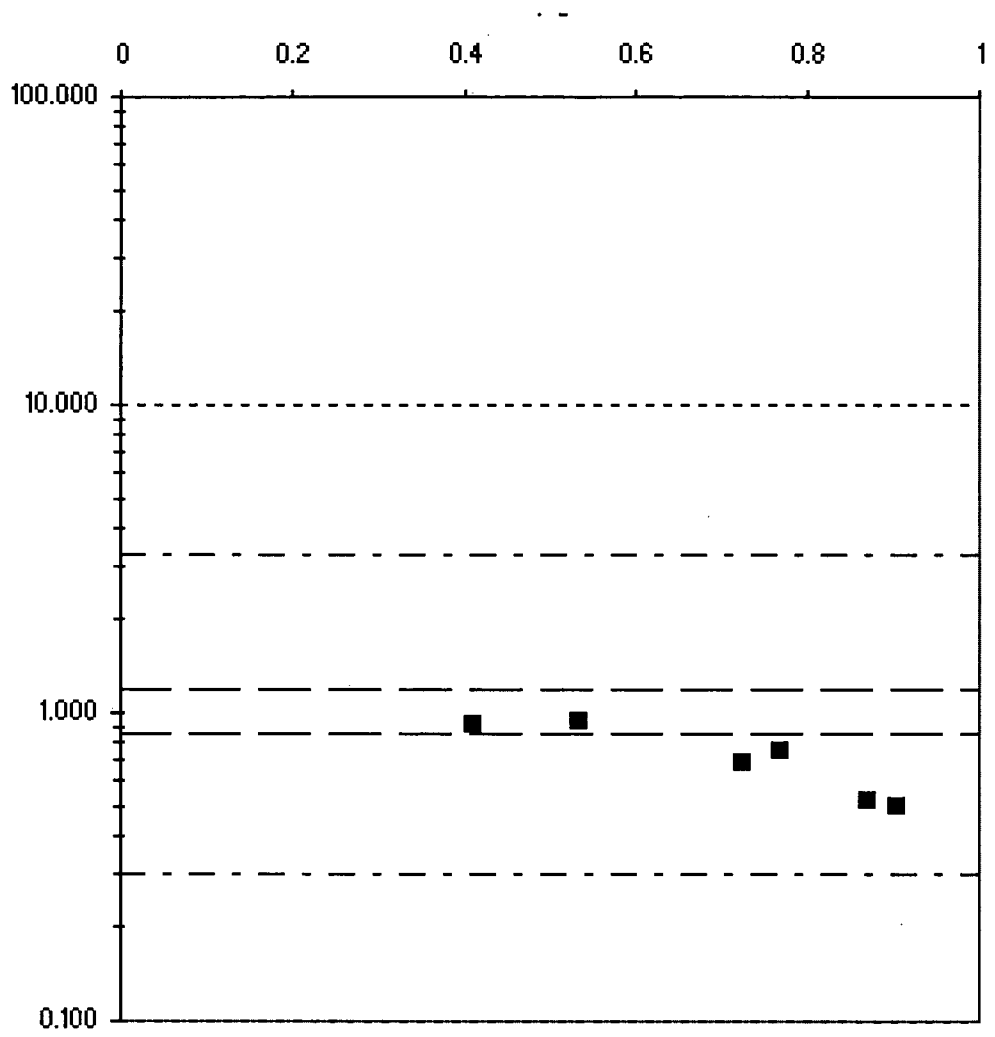


Figure 15

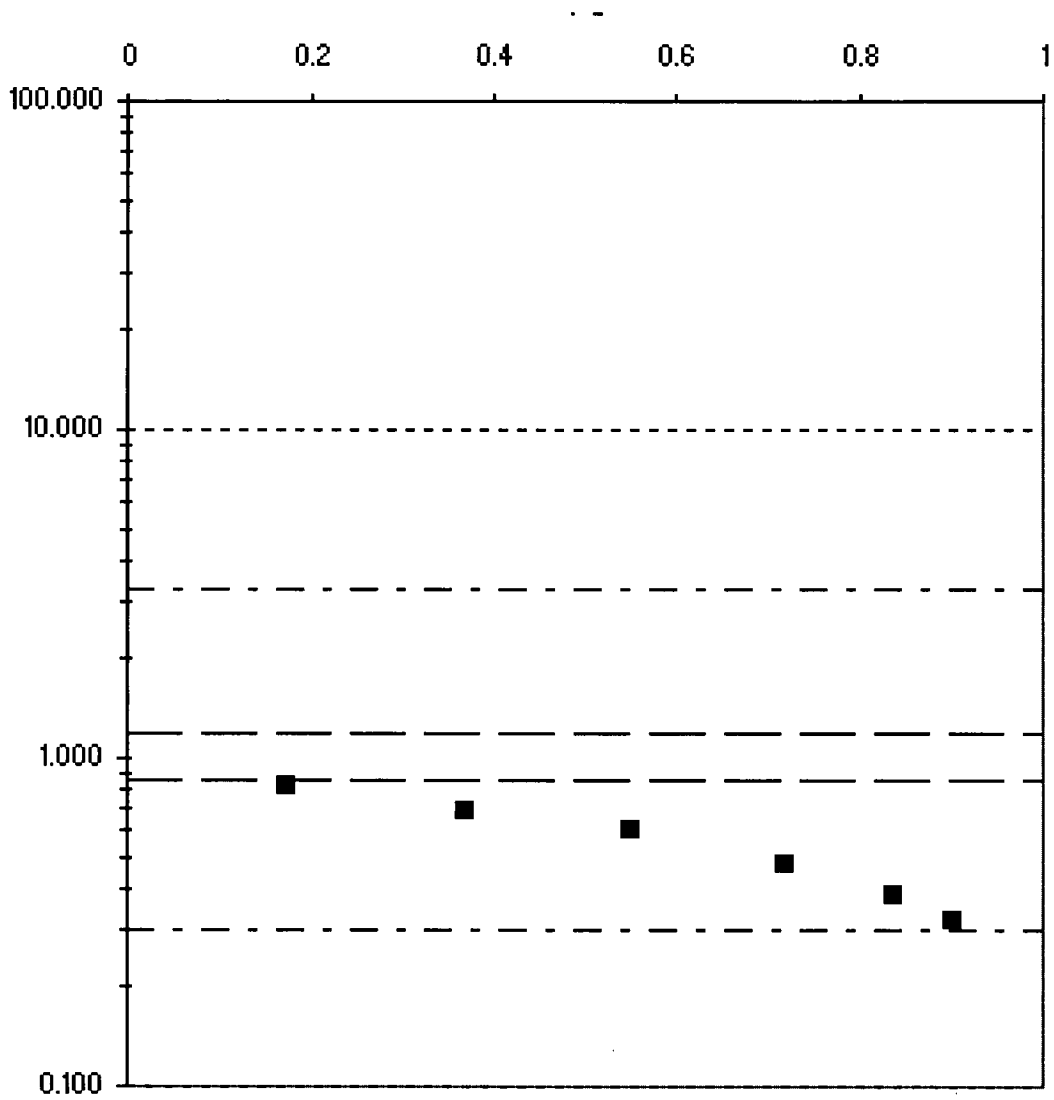
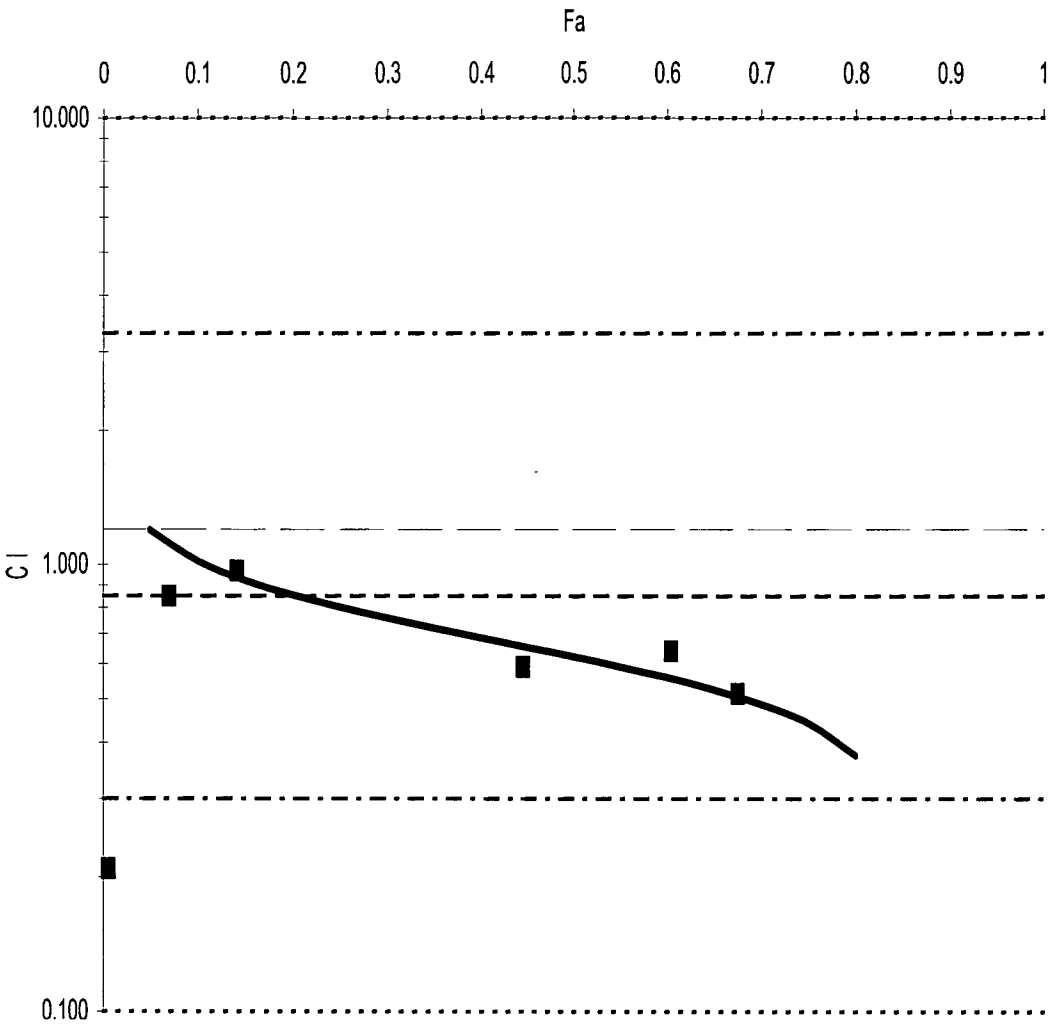


Figure 16



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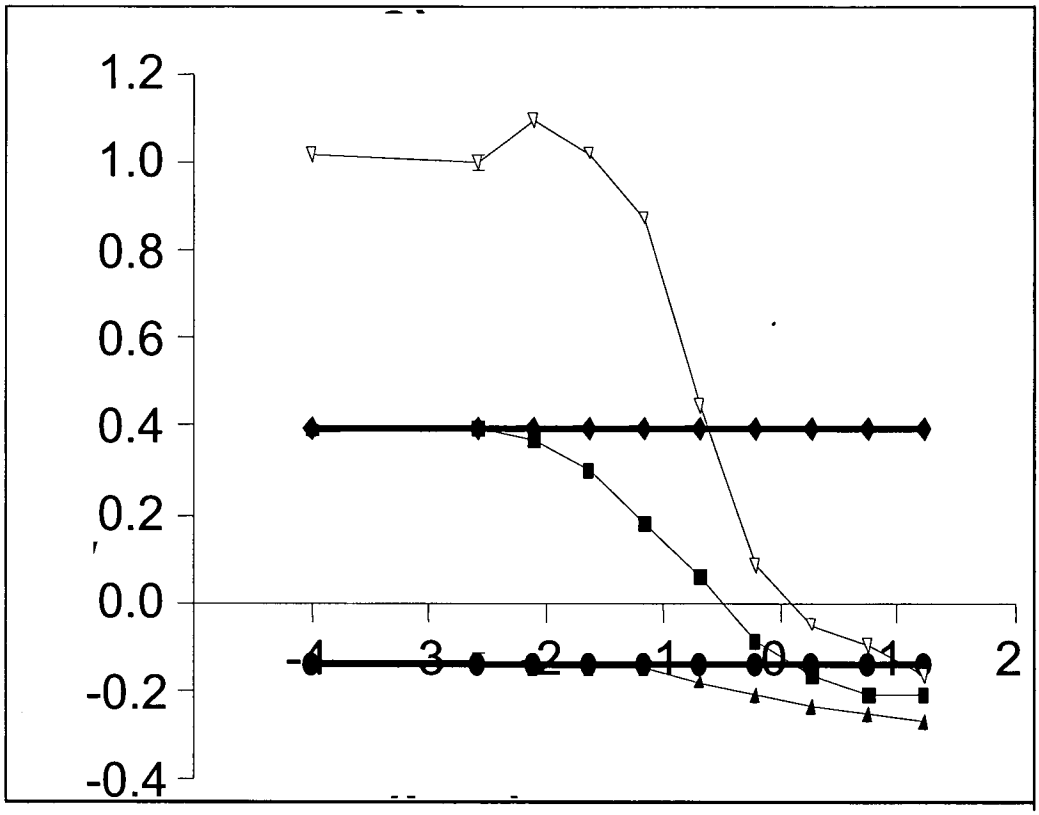

Figure 18

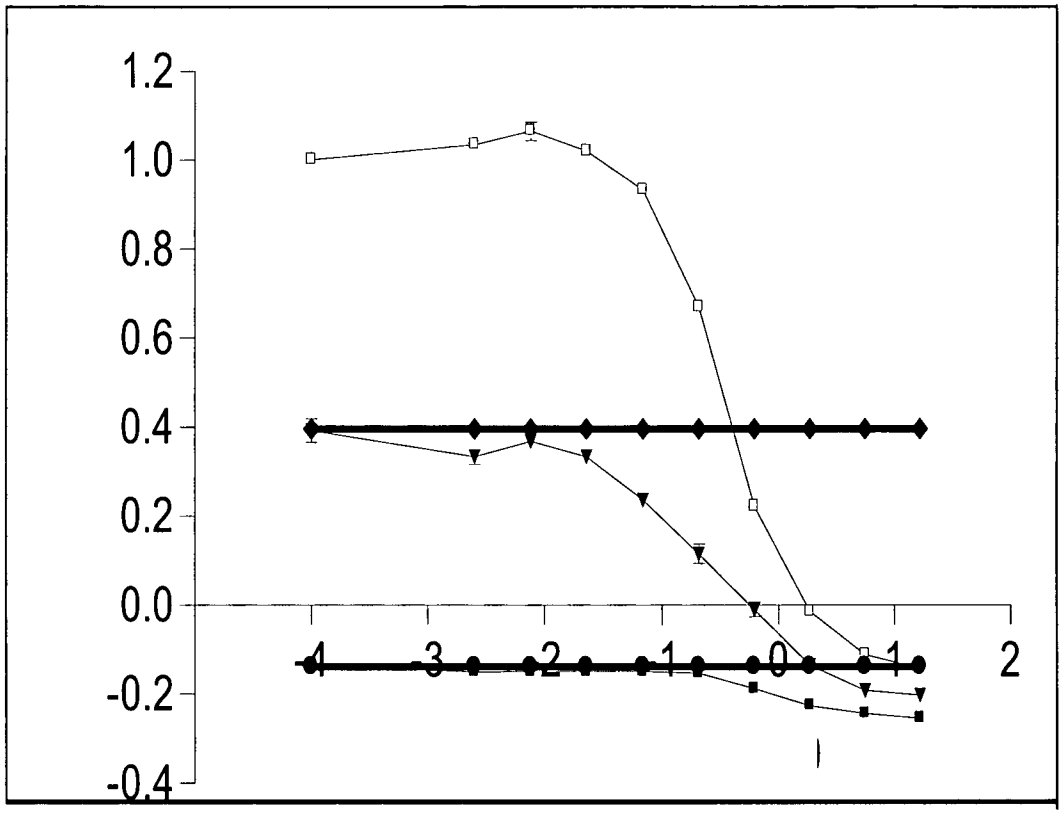

Figure 19

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2008/001184

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/4184 A61K31/4412 A61K31/50 A61K31/517 A61P35/00
A61K31/44 A61K31/4427

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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, EMBASE, BIOSIS, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2005/222163 A1 (ECK STEPHEN L [US] ET AL) 6 October 2005 (2005-10-06) abstract claims 1,3,7,8	1-3,7-12
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Y	WO 2006/067446 A (ASTRAZENECA AB [SE]; ASTRAZENECA UK LTD [GB]; ALMEIDA LYNSIE [US]; AQU) 29 June 2006 (2006-06-29) abstract page 30, line 29 - line 32 page 31, line 23 claim 1	1-12

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"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

"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

"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.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 July 2008	27/08/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Garabatos-Perera, J

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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2008/001184

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A	SHELTON J G ET AL: "Differential effects of kinase cascade inhibitors on neoplastic and cytokine-mediated cell proliferation." LEUKEMIA (BASINGSTOKE), vol. 17, no. 9, September 2003 (2003-09), pages 1765-1782, XP002489013 ISSN: 0887-6924 abstract page 1772, right-hand column, line 18 - line 31	1-12
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A,P	WO 2007/071951 A (ASTRAZENECA AB [SE]; ASTRAZENECA UK LTD [GB]; ROBERTS RONALD JOHN [GB]) 28 June 2007 (2007-06-28) abstract claim 1	1-12
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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2008/001184

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