Synthesis and Biological Activity of Akt/PI3K Inhibitors

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Abstract: Phosphatidylinositol 3-kinase (PI3K) and serine/threonine protein kinase B (PKB or Akt) pathways regulate important cellular processes and are related to a number of human pathologies, such as cancer. The development of kinase inhibitors, with particular attention to small molecule analogues of natural phosphoinositides for pathway interruption and therapeutic applications will be reviewed.

Key Words: Kinases, Akt, PI3K, cancer, kinase inhibitors, allosteric inhibitors, phosphatidylinositol, phosphatidylinositol analogues.

INTRODUCTION

Protein kinases comprise a large family of enzymes that catalyse the transfer of the terminal phosphate group from ATP (adenosine triphosphate) to protein substrates, specifically to the hydroxyl group of serine or threonine (Ser/Thr kinases) or tyrosine (Tyr kinases). The serine/threonine protein kinase B (PKB), also known as Akt, has recently gained great attention as a promising molecular target in cancer therapy for its central role in many important cellular processes controlling the balance of survival and apoptosis of mammalian cells. Till now, three members of the Akt family (Akt1, Akt2, and Akt3) have been identified and they are, in general, broadly expressed in all living organisms. A crucial step in Akt activation is its translocation to the plasma membrane. This process involves the phosphatidylinositol 3-kinase (PI3K) pathway; PI3K catalyses the phosphorylation of inositol phospholipids or phosphatidyl inositols (PtdIns) at position three (D3) of the inositol ring, leading to the formation of the corresponding 3-phosphorylated PtdIns, such as 3,4,5-triphosphate (PtdIns(3,4,5)P_3, Fig. **1**).

Phosphoinositides generated by PI3K interact with Akt pleckstrin homology (PH) domain inducing a conformational change in the molecule. The PH domain, found in many proteins that are involved in intracellular signalling, consists of an up-and-down beta-barrel made of 7 antiparallel beta-strands and a C terminal amphiphilic alpha-helix which caps one end of the barrel. Following the conformational change, Akt can be phosphorylated at Thr308 in the activation loop and at Ser473 [1]. Phosphoinositide-dependent kinase-1 (PDK-1) operates Thr308 phosphorylation, while different hypothesis have been made concerning the enzymes required for the Ser473 phosphorylation. In particular, the suggested candidate enzymes include: Akt itself through auto-phosphorylation [2], PKCα [3], PKCβII [4], DNA-dependent kinase [5] and the rictor-mTOR complex [6]. The PH domain mediates Akt membrane translocation. Activated Akt can phosphorylate several signalling proteins, which lead the cell to proliferation or death by apoptosis. Several researches indicate that activation of Akt is both necessary and sufficient for cell survival [7, 8]. In particular, it has been reported that Akt promotes survival through the inactivation of the caspase-9 and the activation of the transcription factor NF-kB [9, 10]. In addition, it inhibits apoptosis through the phosphorylation of different components of the apoptotic machinery, such as BAD and the forkhead transcription factor, FKHRL 1 [11, 12].

For all of these reasons, Akt is considered the major mediator of survival signals protecting the cells from apoptosis, after activation in response to various stimuli, in a PI3K dependent manner [13-23].

Inappropriate activation of the PI3K/Akt pathway has been linked to the development of several human pathological states, including type II diabetes, atherosclerosis, tuberous sclerosis and diseases involving aberrant immune responses [12, 24]. In addition, several components of the PI3K/Akt pathway is deregulated and over expressed in a wide range of human carcinomas and Akt is considered a critical player in tumorigenesis, tumour progression and growth, invasion, metastasis, angiogenesis, and the tumour cells resistance to therapeutic treatments. The activation of PI3K/Akt-survival cascade in endothelial cells contributes, indeed, to augment radio and chemotherapy resistance [20, 25-27]. The crucial role of the PI3K/Akt pathway in cancer is further supported by the fact that the tumour suppressor PTEN, whose gene is deleted or mutated in a wide range of human cancers, possesses a 3'-phosphoinositide-phosphatase activity capable to inactivate the PI3K/Akt pathway. Furthermore, the gene encoding the p110a catalytic subunit of PI3K, PIK3Cα, is over expressed in several ovarian cancer cell lines [28], and an elevated PI3K activity has been found in lung cancer cell lines [29]. Finally, gain- or loss of function mutants of several components of the PI3K/Akt pathway leads to neoplastic transformation in various experimental models [18, 30, 31].

Akt and PI3K have thus emerged as therapeutic targets for many human diseases, and therapeutic strategies that target the Akt/PI3K pathway are currently in development [18, 32-35].

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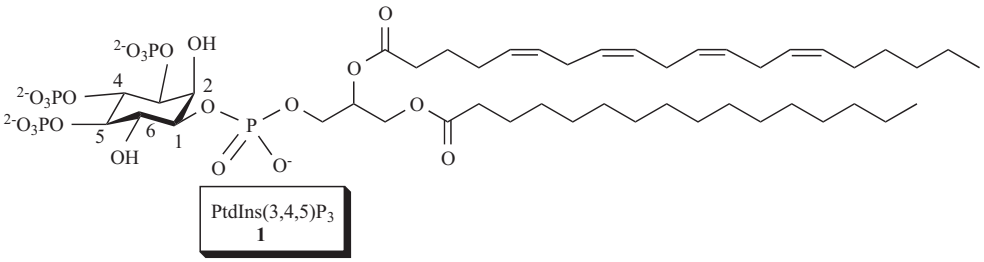


Fig. (1). Chemical structure of PtdIns(3,4,5)P_3.

PI3K/AkT INHIBITORS

Since the activation of PI3K/Akt pathway is a crucial step in many of the events leading to cancer, compounds that can modulate this pathway are therefore of clinical and therapeutic interest for single and/or combined anticancer treatments. The interest in the chemistry, biochemistry, and pharmacology of kinase inhibitors is continually growing. Most of the drug-development efforts have been focused on ATP-binding-site inhibitors (allosteric inhibitors), and the vast majority of the allosteric inhibitors proposed have found a wide range of applications. It is well-known the antitumour activity *in vitro* and *in vivo* of many commercially allosteric PI3K inhibitors, such as the fungal metabolite wortmannin (**2**) and the flavonoid derivative LY294002 (**3**) [18, 36-42], or the Akt inhibitors such as some unnatural canthine alkaloids (2,3-diphenylquinoxaline, **6**), and (**7**), Fig. (**2**) [43, 44]; IC_{50} values as Akt and PI3K inhibitors are reported in Table **1**. Despite the high inhibitory activity showed by these compounds, their general toxicity, the lack of selectivity towards specific kinases, together with the instability of wortmannin in aqueous environment and the insolubility of LY294002 are limiting their pharmaceutical applications; however, these compounds still represent invaluable tools in elucidating kinase roles in signal transduction pathways. Currently, water-soluble wortmannin conjugates (**5**), derived from the condensation of *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer with 11-*O*-desacetylwortmannin (DAWM **4,** Fig. **2**), have been developed, but the activity of this conjugate has been approximately ten-fold lower than the activity of its free counterpart (Table **1**) [45, 46]. Furthermore, suitable kinase inhibitors ought to be selective and specific in order to avoid side effects in the clinic; targeting the highly conserved ATP-binding site is probably not the best approach to this respect. Even if kinase inhibitors that target the ATP-binding site in a down-regulated conformation might have better selectivity, inhibitors that bind out of this site should have better specificity profile. This strategy may offer the basis for the selective control of cancer cell growth, without disrupting the function of normal cells.

In light of these considerations, rational design of non-allosteric inhibitors with respect to ATP-binding-site is now considered as a potential alternative for PI3K/Akt pathway interruption. Since the natural substrates of PI3K and Akt are differently phosphorylated phosphatidyl inositols, non allosteric inhibitors have been designed as analogues of these natural substrates. Toward this aim different approaches have

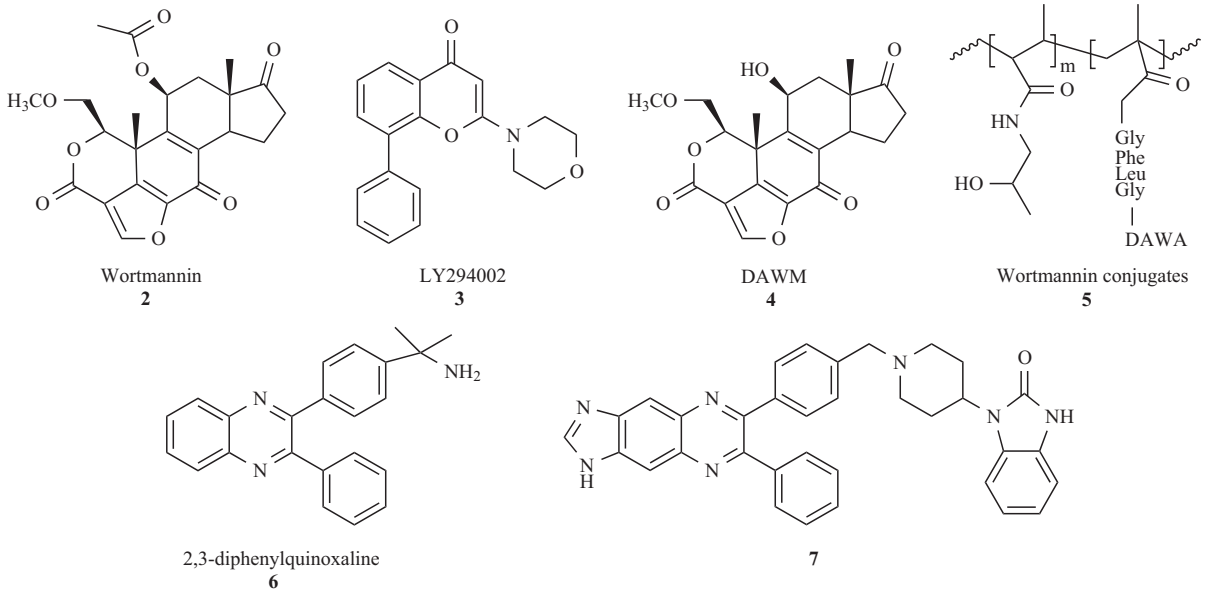


Fig. (2). PI3K and Akt allosteric inhibitors.

Table 1. IC_{50} Values for Inhibition of Akt and PI3K (More Then One Inhibition Value is Reported when Discrepancy was Found in Different Literature Reports)

Compound	IC_{50} (μM)		Refs.
	Akt	PI3K	
wortmannin (2)		2-4nM	[31-35]
LY294002 (3)		1.4	[31-34]
2,3-diphenylquinoxaline (6)	3.4 (Akt1) 23.1 (Akt2) > 50 (Akt3)		[39]
7	0.06 (Akt1) 0.21 (Akt2) 2.2 (Akt3)		[39]
DPI (8)	8 (Akt1)	> 250; 38	[52, 56]
DPIEL (9)	17.5 (Akt1); 1.5	5.3; 16.4; 14.8	[40, 51, 54, 56]
phosphate analogues (10)	7.8	31.0	[9, 49-52, 54, 58,]
phosphate analogues (11)	9.1	18.5	[9, 49-52, 54, 58]
DCIEL (12)	12.5	15.5	[54, 56]
carbonate analogue (13)	5.0	83.0	[54]
carbonate analogue (14)	32.0	21.3	[54]
15	4.5	5.7	[57]
16	2.5	8.8	[57]

been proposed: modification of the substituents naturally encountered on the inositol ring, substitution of the metabolically labile phosphate ester linkage between the inositol ring and the diacylglicerol moiety by mimetic groups, such as carbonates, modification of the diacylglicerol moiety by introduction of ether linkages instead of esters, and finally, substitution of the inositol ring by analogue structures. More than one modification can be found at the same time in the synthesised inhibitor.

PHOSPHATIDYLINOSITOL ETHER LIPID ANALOGUES

Although differently phosphorylated inositols were first proposed as potential inhibitors for the PI3K/Akt pathway [47-51], the metabolic instability of these compounds due to their hydrolysis by phospholipases, introduced the need to synthesise metabolically stable phosphatidylinositol analogues. In this context, the use of small molecule analogues of natural phosphoinositides, such as alkylphospholipids or phosphoinositide ether lipid analogues, has emerged over the past few years.

D-3-deoxy-myo-inositol Analogues (DPIs)

The first attempt to use phosphoinositide ether lipid as phosphatidylinositol analogue inhibitors for PI3K/Akt pathway was reported by Kozikowski and co-workers [52-55], with the aim to target the first kinase involved in the transduction pathway, that is PI3K, responsible for the phosphorylation at the D-3 position of the *myo*-inositol ring. The inhibitory activity should prevent the formation of inositide-3-phosphate second messengers, thus interrupting the signalling cascade. In the last decade, several different D-3-deoxy-substituted-*myo*-inositol analogues (**8, 9, 12,** Fig. **3**), that cannot be phosphorylated at the 3-position of the *myo*-inositol ring by PI3K, have been synthesised and their biological activity evaluated. Furthermore, additional changes in the diacylglycerol moiety (DAG) were performed [56].

Treatment of multiple cancer cell lines with DPIs demonstrated their ability to impair PI3K and Akt kinase activities, and inhibit cancer cell growth. The most effective analogue among this set, D-3-deoxy-phosphatidyl-*myo*-inositol (DPI, **8**), showed fairly good *in vitro* inhibition of Akt at low concentration (IC_{50} 8 μM), but resulted a weak inhibitor of Akt in the different cellular systems tested (IC_{50} 27.4 μM in NIH3T3) and of cancer cells growth (IC_{50} 35 μM in HT-29 human colon carcinoma cells, Tables **1** and **2**). Besides, no inhibition of PI3K activity was observed *in vitro* (Table **1**) [57].

The relatively low potency observed for these compounds may be due to their hydrolysis by phospholipases; in order to decrease the susceptibility of the DPIs, the DAG portion of this molecule was replaced by a metabolically stable ether lipid moiety. Among this new generation analogues, D-3-deoxy-phosphatidyl-*myo*-inositol ether lipid (DPIEL, **9**), was found to be a reasonably active inhibitor of both PI3K and Akt, blocking the growth of human colon cancer cell line with a 2.1 μM IC_{50}. Although discordant values of IC_{50} have been reported, it is possible to estimate a PI3K inhibitory activity with IC_{50} values in the 5-20 μM range, and Akt in-

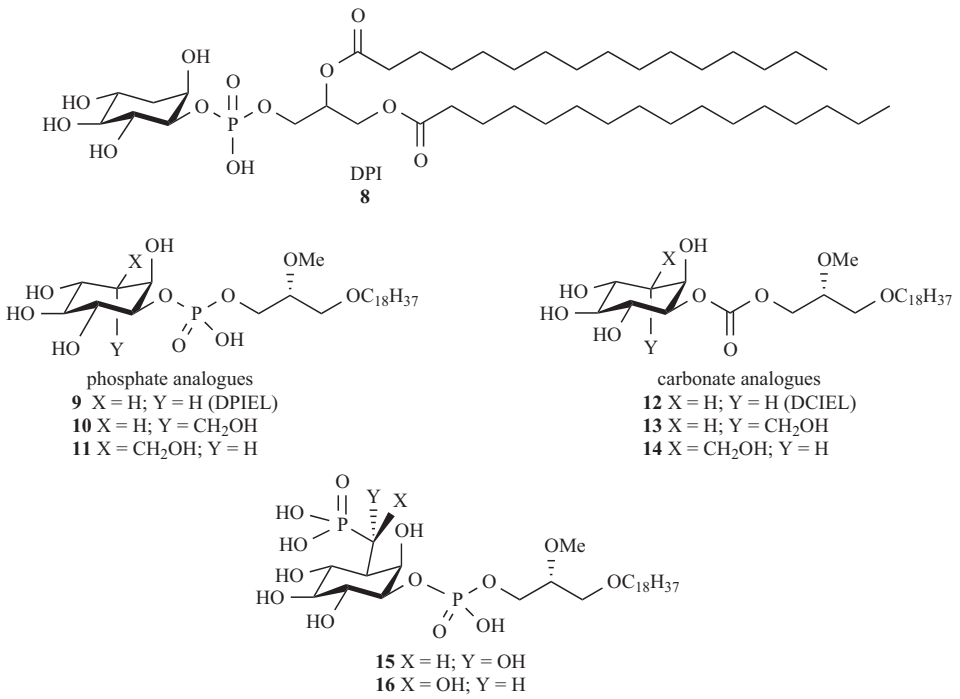


Fig. (3). Phosphatidylinositol ether lipid analogues.

hibitory activity with IC_{50} values in the 1-15 μM range [45, 56, 58, 59]. Furthermore when injected intraperitoneally, DPIEL (**9**) also inhibited tumour cell growth in xenograft model systems [60]. Unfortunately, the clinical use of DPIEL (**9**) has been strongly limited by its acid lability if orally administered, and by the massive hemolysis induction, when intravenously administered [61].

In order to further decrease the possible metabolic modifications of synthetic inhibitors, the carbonate analogues (**12-14**) were synthesised [59]. Although these compounds presented a good inhibitory activity of the Akt/PI3K pathway in HT-29 cell lines, comparable to those observed for DPIEL (**9**), the replacement of the phosphate group by a carbonate resulted in a decreased activity towards growth inhibition of other cancer cell lines, and in the inhibition of both kinases [59].

All evidences reported till now indicate that the phosphatidylinositol ether lipid analogues are able to act not only

Table 2. Cell Lines Growth Inhibition *In Vitro* by Different Inositol Analogues (72 h Exposure). (More Then One Inhibition Value is Reported when Discrepancy was Found in Different Literature Reports)

Compound	IC_{50} (μM)			Akt Activity (μM) (NIH3T3)	Refs.
	HT-29	MCF-7	NIH3T3		
7	35	ND	17.6	27.4	[26, 53, 57]
DPI (8)	45; 2.1	7.2	4.3	1.5	[26, 52, 55, 57]
DPIEL (9)	4.5	5.0			[51, 55]
(10)	7.5	2.0			[51, 55]
(11)	2.5	12.0	14.1	12.5	[26, 57, 55]
DCIEL 12)	10.0	1.2			[55]
carbonate analogue (13)	12.0	12.5			[55]
carbonate analogue (14)	7.8	9.0	19.8		[58]
15	7.8	8.0	19.8		[58]

ND = not determined.

as PI3K inhibitors but also as Akt inhibitors. It has been demonstrated on NIH3T3 cells that the cellular mechanism for Akt inhibition is based upon the interaction of DPIs with its PH domain, preventing the translocation of the kinase from the cytoplasm to the plasma membrane, and consequently its Ser473 and Thr308 phosphorylation.

Molecular modelling and docking studies rationalised these findings; comparison of the binding interactions of DPI (**8**), DPIEL (**9**), and DCIEL (**12**), Fig. (**4A-C**) [59], with the natural substrate, Fig. (**4D**) [31] showed that DPIEL (**9**) binds much more strongly to the PH domain of Akt than DPI (**8**) and DCIEL (**12**). The 3-deoxy-*myo*-inositol ring of DPIEL (**9**) is stabilised by hydrogen bond interactions with the terminal nitrogen atom of Arg25, while the 1-phosphate group exhibits a strong interaction with the positively charged pocket *via* a network of hydrogen bonds with Arg25, Arg23 and Lys14 residues. The methoxy group, in addition, exhibits a strong interaction between Lys14 and Thr21. These results are consistent with Akt inhibition data. The comparison of the binding mode of DPIEL (**9**) with DPI (**8**) and DCIEL (**12**) revealed that the 3-deoxy-*myo*-inositol ring of all the three compounds is similarly positioned in the enzyme, Fig. (**4A-C**), as also observed for PI(3,4)P_2, Fig. (**4D**). All hydroxyl groups of DPI (**8**) ring retained their interactions with Tyr38 and Arg48. In contrast, the interaction between the 1-phosphate group of DPI (**8**) and Lys14 is lost, due to a slightly different orientation of the DPI's ring, compared to that of the natural substrate. The position and orientation of the 3-deoxy-*myo*-inositol ring is stabilised by participation of the 4,5-OH groups in hydrogen bonding to Arg25 and Arg48. In conclusion, DIPIEL (**9**) was calculated to bind much more strongly to the Akt PH domain (-109.3 Kcal/mol), than DPI (**8**, -59.4 Kcal/mol) and DCIEL (**12**, -56.6 Kcal/mol) [59].

D-3-modified-myo-inositol Analogues

Despite the interesting activity showed by DPIEL (**9**), its inhibitory activity *in vivo* is limited by competition of the natural substrate *myo*-inositol, which is always present at physiological concentration.

Molecular modelling and docking studies demonstrate the importance of the hydrogen bond network between the 3-phosphate group of the inositol ring with the Akt PH domain, Fig. (**4D**) [31]. Hence, the electronic nature of the group at the 3-position of phosphatidylinositol analogues plays a crucial role for recognition and binding to the enzyme. In this context, several 3-modified-phosphatidylinositol ether lipid analogues (**10, 11, 13-16**) have been synthesised. Although the 3-(hydroxymethyl)-phosphatidylinositol ether lipid analogues (**10, 11, 13** and **14)** exhibited good attenuation of Akt activity and growth inhibition of various cancer cell lines with IC_{50} in the 1-12 μM range, they resulted less effective than DPIEL (**9**) [60]. It is worth to note that the PI analogue (**10**), bearing an axial hydroxymethyl group at position 3, was slightly more potent as PI3K inhibitor, than the one with the equatorial hydroxymethyl group (**11**), while in terms of Akt inhibition, the equatorial phosphate (**11)** is more active than its axial counterpart (**10**). The same trend was observed comparing the carbonate analogues (**13**) and (**14**). Among all of these analogues, carbonate (**13**) resulted to be more specific for Akt inhibition than DPIEL (**9**). The greater Akt inhibitory activity of the axial hydroxymethyl-bearing analogue (**13**), compared to its equatorial counterpart (**14**) can be rationalised in terms of different H-bonding patterns when bounded to the PH domain of Akt. The models obtained by molecular docking studies of (**13**) and (**14**) in complex with the Akt PH domain [60] showed that axially-oriented 3-hydroxymethyl group is able to interact with the Arg25 in the binding pocket, imposing the right orientation of the 1-carbonate group for its interaction with Arg23. In general, both carbonates are relatively poor inhibitors of PI3K, which suggests the importance of the phosphate moiety for the recognition by this enzyme. Although the cell growth inhibitory effects of these compounds are due to a combination of their PI3K and Akt inhibitory activities, on the basis of the reported data, growth inhibition appears to correlate best with the inhibition of the downstream target Akt.

The encouraging results showed by the 3-(hydroxymethyl)-phosphatidylinositol ether lipid analogues have driven the researchers to investigate the activity of other compounds structurally related to PI-3-phosphate such as (**15**) and (**16**), bearing a metabolically stable phosphonate group at position 3 [62]. Analogues (**15**) and (**16**) exhibited an increased PI3K and Akt inhibitory activity *in vitro*. However, these analogues have similar or lower cell growth inhibition effects on different cancer cell lines (Table **1**). Moreover, these compounds resulted toxic to many cell lines.

INOSITOLPHOSPATE-BASED INHIBITORS

The toxicity, the low chemical stability and solubility of the phosphatidylinositol ether lipid analogues so far described have limited their development as drug candidates.

Hence, the use of the natural compounds, such as differently phosphorylated inositols (Fig. **5**), was recently re-evaluated by Falasca and co-workers [63, 64]. In addition to their well-known roles as second messengers, they are also molecules of great therapeutic interest. In fact, Ins(1,2,3,4,5,6)P_6 (**19**) has been indicated as an effective cancer chemopreventive and chemotherapeutic agent, although at millimolar concentrations [65-68]. The authors propose that specific inositol polyphosphates, the water-soluble head groups of phosphoinositides, could antagonise the activation of PI3K/Akt pathways by competing with the binding of PtdIns(3,4,5)P_3 to PH domains of the protein kinases involved. These molecules probably act as inhibitors preventing the translocation of protein kinases to the plasma membrane or to any specific membrane compartment, as described above for the phosphatidylinositol ether lipid analogues [69]. Different inositol polyphosphates were evaluated as Akt inhibitors, and inositol 1,3,4,5,6-pentakisphosphate [Ins(1,3,4,5,6)P_5] (**18**) was found to inhibit Akt phosphorylation and kinase activity *in vivo* at very low concentrations (50mg/Kg), whereas all the other inositol polyphosphates had low or no effect [64]. All evidences reported by the authors indicate that the inhibitory effects of this inositol polyphosphate is probably due to a binding competition with PtdIns(3,4,5)P_3 in the Akt PH domains, highlighting that a rapid and efficient internalisation by cells is absolutely necessary. In fact, although pure synthetic inositol 1,4,5,6-tetrakisphosphate

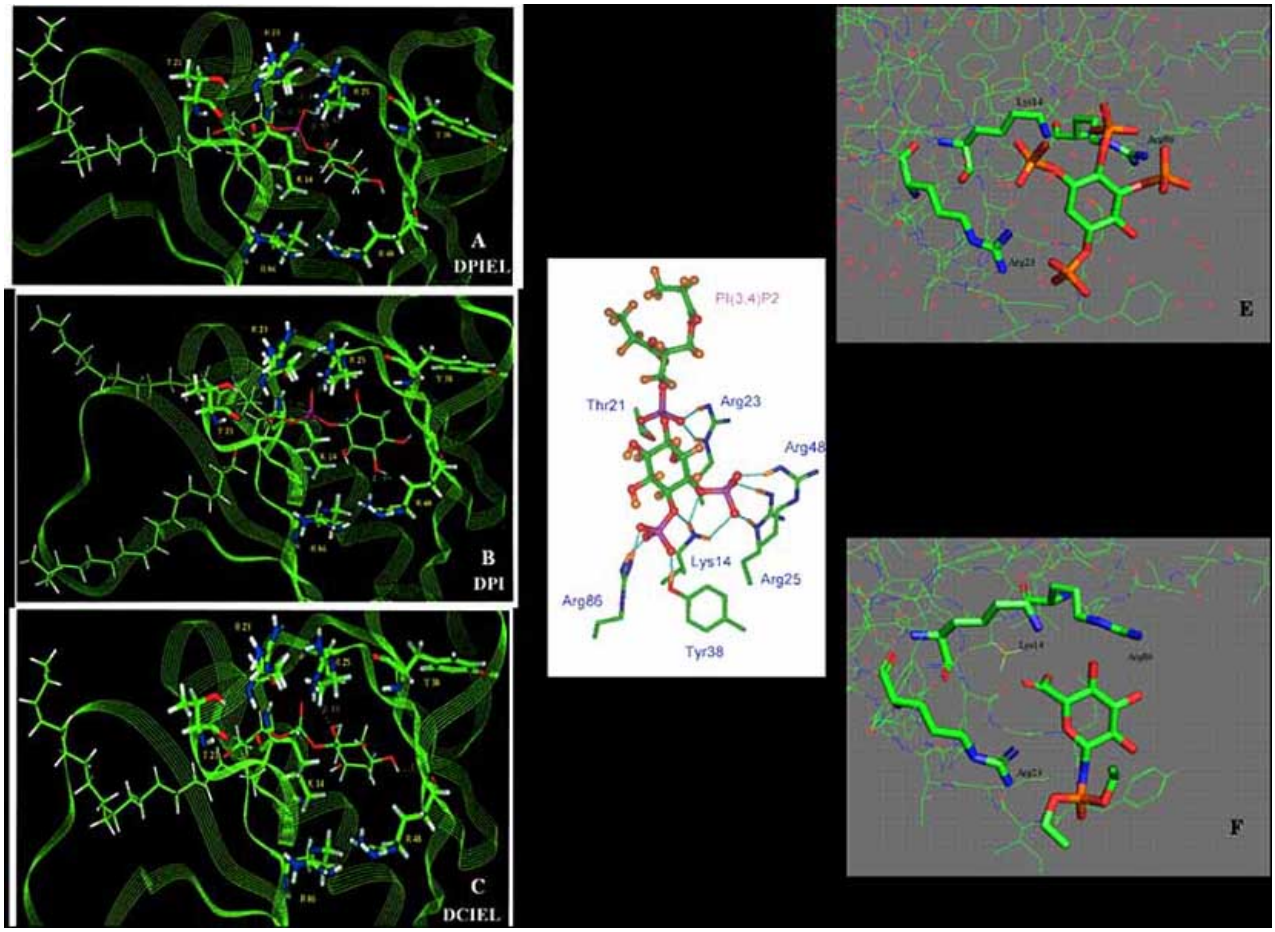


Fig. (4). *Interactions between Akt PH-domain with different compounds.* **A.** Akt PH domain and DPIEL (**9**); **B**. Akt PH domain and DPI (**8**); **C.** Akt PH domain and DCIEL (**12**). The ribbon of the PH domains is coloured in green. The amino acids thought to be involved in PtdIns(3,4,5)P_3 and/or PtdIns(3,4)P_2 binding in the PH domain are represented in ball and stick model and numbered in yellow. White dotted lines represent the hydrogen bonds occurring between the title compound and the amino acids of the protein. [Fig. **4A-C** are reprinted with permission from Meuillet, E.J.; Mahadevan, D.; Vankayalapati, H.; Berggren, M.;Williams, R.; Coon, A.; Kozikowski, A.P.; Powis G. *Mol. Cancer Ther.* **2003**, *2*, 389. Copyright (2003) American Association for Cancer Research]; **D**. Akt PH domain and PI(3,4)P_2. The lipid chain of PI(3,4)P2 was truncated to -$OPO_3CH_2CH(OCOC_2H_5)CH_2OCOC_2H_5$. The 3,4-phosphate groups are anchored via hydrogen bonds with Lys14, Arg25, Tyr38, Arg48, and Arg88. The 1-phosphate group forms hydrogen-bonding interactions with Thr21 and Arg23. [Reprinted in part with permission from Rong, S.-B.; Hu, Y.; Enyedy, I.; Powis, G.; Meuillet, E. J.; Wu, X.; Wang, R.; Wang, S.; Kozikowski, A. P. *J. Med. Chem.* **2001**, *44*, 898. Copyright (2001) American Chemical Society]; **E.** Akt PH domain and PtdIns(3,4,5)P_3. The structure was obtained by X-ray diffraction. **F.** Akt PH domain and (**23**). The structure was obtained by docking simulations; for the sake of clarity, the structure of phosphatidylinositol(3,4,5)-triphosphate and (**23**), as well as the side chains of Lys14, Arg23 and Arg86 are shown using the stick representation.

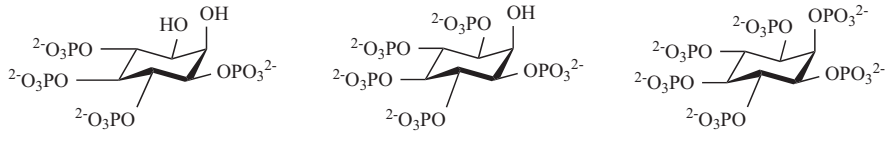
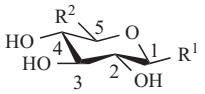


Fig. (5). Inositolphospate inhibitors.

[Ins(1,4,5,6)P_4, (**17**)][1] possesses anticarcinogenic action *in vitro*, *in vivo* human cancer cell lines growth inhibition was not observed [64, 70]. Specifically, Ins(1,3,4,5,6)P_5 promotes apoptosis in human lung, ovarian, and breast cancers, characterised by an elevated PI3K/Akt activity [63]. The antitumour properties of Ins(1,3,4,5,6)P_5 were also tested on SKOV-3 human ovarian carcinoma implanted s.c. in nude mice, where reduction of the tumour growth and no sign of toxicity have been observed. It is noteworthy that the effect of Ins(1,3,4,5,6)P_5 was comparable to that of cis platin, commonly used for ovarian cancer chemotherapy. Furthermore, the compound showed total inhibition of Akt phosphorylation at both residue Ser473 and Thr308 in Ins(1,3,4,5,6)P5-treated mice, after 12 days of treatment. Previous works indicated that inositol polyphosphates might be converted into different dephosphorylated metabolites in the cells [71], so the observed antitumour effects might be due to different dephosphorylated forms. In fact, the antitumour activity *in vitro* and *in vivo* of inositol hexakisphosphate Ins(1,2,3,4,5,6)P_6 (**19**) has been related to its intracellular delivery followed by dephosphorylation. These lower phosphorylated forms are more potent at inducing apoptosis, especially the pentakisphosphate [70, 71]. These results, supported by the high intracellular stability of Ins(1,3,4,5,6)P_5 even for prolonged times, demonstrate that it is directly responsible for Akt inhibition. Finally, the potential antineoplastic activity of the pentakisphosphate inositol should be supported by the evidence of its antiangiogenic properties both *in vivo* and *in vitro*, reported by the authors [64].



20: R^1 = SOPh; R^2= CH_2OH
21: R^1 = $NHCOCH_3$; R^2= CH_2OH
22: R^1 = $NHPO(CH_2CH_3)_2$; R^2= CH_2OH
23: R^1 = $NHPO(CH_2CH_3)_2$; R^2= COOH

Fig. (6). Phosphatidylinositol analogues derived from glucose.

3-phosphatidylinositol moiety. As discussed above, these compounds should increase the chance of a specific inhibition of the desired membrane-targeted protein, without interfering with other PI3K-mediated pathways. In this context, compounds (**20-23**) were synthesised from D-glucose (Fig. **6**). Carbohydrate-based mimics of inositols have already been reported [72]. The structure of phosphatidylinositol-3-phosphate (PIP3) produced by PI3K, and natural substrate of Akt, can be easily reconducted to suitably modified D-glucose, where specific functional groups have been introduced, taking into account the considerations already reported on structure/activity relationships of previously synthesised inhibitors [60, 62, 73, 74]. Compounds (**20-23**) lack the hydroxyl group in position 2 of the inositol ring, which is substituted by the pyranosidic oxygen of the sugar, while the phosphate group in position 3 of the inositol (position 5 of the sugar) is substituted by a different but still acidic group, and the phosphate group in position 1 of the inositol (position 1 of the sugar) is substituted by an electron-donating group with β-configuration at the anomeric center of D-glucose. In addition, the lipophilic acyl chains of the diacylglycerol moiety have been mimicked by small hydrophobic groups, assuming that the long fatty acid chain is not needed for biological activity, but only for membrane anchoring.

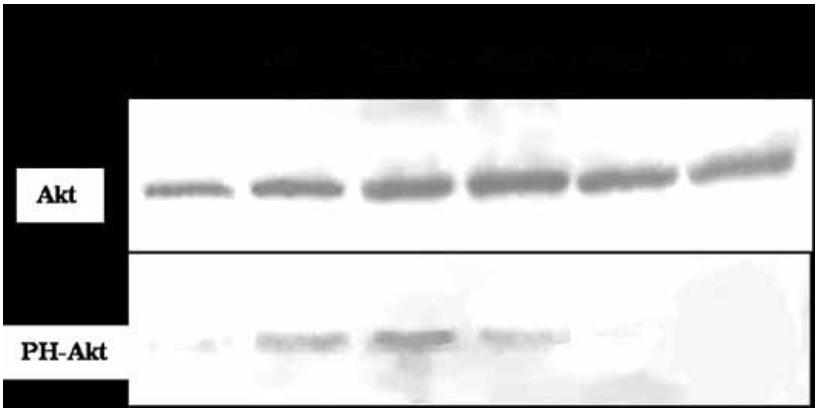


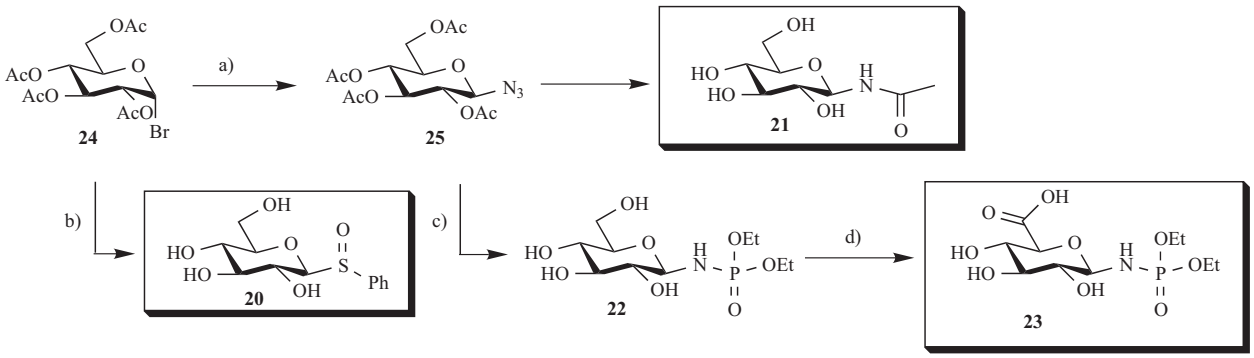
Fig. (7). *Western blot analysis of the inhibitory activity of compound (23)*. D1 cells were activated for 15 min. with LPS after or not 1.5 hours pretreatment with the inhibitor (**23**) at different concentrations, and with wortmannin (W). The protein levels of phosphorylated Akt (PH-Akt) and Akt were determined using monoclonal antibodies anti-phospho-Ser473-Akt or anti-Akt respectively ; NT, non-treated cells.

GLUCOSE-BASED INOSITOL ANALOGUES

Despite the large number of inhibitors synthesised, new molecules with increased inhibitory potency and better pharmacokinetics are highly desirable. Our research group is currently investigating new glucose-based inositol analogues, as potential kinase inhibitors. We focused our attention on the inhibitors of the PI3K/Akt pathway structurally related to the

Compounds **20-23**, Scheme **1**, have been synthesised by standard carbohydrate chemistry from tetra-*O*-acetyl-glucopyranosyl bromide **24** [75]. Sulfoxide **20** was obtained by thioglycosylation [76] with thiophenol, followed by TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxyl) oxidation [77, 78]

[1] commercial Ins(1,4,5,6)P_4 possessed inhibitory activity on cell growth, proliferation and a proapoptotic action to the same extent of those observed for Ins(1,3,4,5,6)P_5 [71] in contrast to the results herein reported The reason for this discrepancy is still completely unclear but it is probably due to a presence of impurities in commercial inositol.



Scheme 1. *Synthesis of glucose-based inositol analogues*: a) ref. [75]; b) ref. [76-78]; c) ref. [79]; d) ref. [80].

while compounds **21-23** were obtained from the corresponding glycosyl azide **25** [75], by standard procedures [79, 80].

The inhibitory activity of compounds (**20-23**) has been tested using the well-characterised murine dendritic cell line, D1. D1 cells are growth factor dependent long-term immature dendritic cells that can undergo maturation *in vitro* upon encounter of microbial cell products, such as lipopolysaccharides (LPS) and zymosan. Treatment of D1 cells with LPS and other microbial substances leads to the activation of PI3K/Akt pathway, involved in the upregulation of activation markers, such as B7.2, and in the expression of cytokines such as interleukin IL-2; activation of the Ser/Thr kinase Akt is required to have efficient IL-2 production. D1 cells were pre-treated with compounds **20-23** for 1.5 hours and then incubated with LPS or zymosan. D1 cell maturation was analysed 24 hours later by measuring the upregulation of costimulatory molecules and IL-2 production. None of the potential inhibitors tested (**20-23**) turned out to be toxic for the cells at the concentrations used for the assays. Among the four compounds assayed, only compound (**23**) showed a clear inhibitory activity in D1 cell maturation induced by LPS and zymosan, in terms of B7.2 upregulation and IL-2 production (data not shown). This effect was due to a down-regulation of Akt phosphorylation, evidenced by western blot analysis in D1 cells pre-treated with compound (**23**) and then exposed to LPS, Fig. (**7**).

In order to rationalise the biological activity of compound (**23**), preliminary docking simulation studies on Akt were performed (Fig. **4F**). The structure of the pleckstrin homology domain Akt complexed to phosphatidylinositol(3,4,5)-triphosphate was recently solved by X-ray diffraction, unravelling structure-activity relationships relevant to the design of enzyme inhibitors [81].

The X-ray structure of the pleckstrin homology domain of Akt is characterised by a PH domain fold in which seven β-strands form two antiparallel β-sheets closed at one end by the C-terminal-helix. Three loops, that are extremely variable in presently known PH domains, are placed at the other end of the β-barrel and form a bowl lined with basic residues into which phosphatidylinositol(3,4,5)-triphosphate binds (Fig. **4E**). Analysis of the binding site reveals that the 1-phosphate group interacts with Arg23 and the backbone nitrogen of Ile19. The 3-phosphate group interacts with the side chains of Lys14, Arg23, Arg25 and Asn53. Similarly, the 4-phosphate group interacts with Lys14, Asn53 and Arg86. Interestingly, the 5-phosphate group is oriented toward the solvent and it does not interact with any protein atom in the binding pocket.

Preliminary docking simulations were performed with the AutoDock 3.0 software package [82]. The grid spacing was set to 0.0375 nm in each dimension. The optimisation was started with a population of 100 randomly positioned individuals, with a maximum of 2.5×10^6 energy evaluations, and a maximum of 2.7×10^5 generations. During each docking experiment, 50 runs were carried out. Other parameters were set to default values. These preliminary experiments showed that the interaction between (**23**) and the pleckstrin homology domain of Akt has many analogies with the interaction of phosphatidylinositol(3,4,5)-triphosphate with the protein (Fig. **4F**). In particular, the role of the 1-phosphate group is played by the diethyl phosphoroamidate group at position 1 in (**23**), which can interact, *via* hydrogen bonding, with Arg23 side chain. In addition, the carboxylate group of (**23**) corresponds to the 3-phosphate and interacts with Lys14. The OH groups of (**23**) spatially corresponding to 4 and 5 positions of the D-*myo*-inositol ring are involved in H-bonding interaction with Arg86. Further studies on the biological activity of (**23**) are in due course.

CONCLUSIONS

In this review, different approaches have been reported for the design of non-allosteric kinase inhibitors; these class of compounds has great relevance as potential therapeutics for different pathologies; however, research is still far from the identification of good specific inhibitors of these key enzymes, involved in cell proliferation or death control.

ABBREVIATIONS

Akt	=	Protein kinase B (PKB)
ATP	=	Adenosine triphosphate
Bn	=	Benzyl
DAG	=	Diacylglycerol
DAWM	=	11-*O*-desacetylwortmannin
DCIEL	=	D-3-deoxy-carbonate-*myo*-inositol ether lipid

DPI	=	D-3-deoxy-substituted *myo*-inositol analogues
DPIEL	=	D-3-deoxy-phosphatidyl-*myo*-inositol ether lipid
FKHRL 1	=	Forkhead transcription factor
HPMA	=	*N*-(2-hydroxypropyl)methacrylamide
IL-2	=	Interleukin 2
$InsP_6$	=	Inositol hexaphosphate
LPS	=	Lipopolysaccharide
MOM	=	Methoxy methyl
MPM	=	*p*-methoxy benzyl
NF-kB	=	Kappa B transcription factor
PDK-1	=	Phosphoinositide-dependent kinase-1
PH	=	Pleckstrin homology
PI	=	Phosphatidylinositol
PI3K	=	Phosphatidylinositol 3-kinase
PIP3	=	Phosphatidylinositol-3-phosphate
PKB	=	Protein kinase B (Akt)
PKC	=	Protein kinase C
PtdIns	=	Phosphatidylinositols
PTEN	=	Tumour suppressor
SCLC-H69	=	Human small cell lung cancer
SKBR-3	=	Breast cancer cell line
SKOV3	=	Ovarian carcinoma cell line

REFERENCES

[1] Scheid, M.P.; Woodgett, J.R. *FEBS Lett*. **2003**, *546*, 108.
[2] Toker, A.; Newton, A.C. *J. Biol. Chem*. **2000**, *275*, 8271.
[3] Partovian, C.; Simons M. *Cell Signal*. **2004**, *16*, 951.
[4] Kawakami, Y.; Nishimoto, H.; Kitaura, J.; Maeda-Yamamoto, M.; Kato, R.M.; Littman, D.R.; Rawlings, D.J.; Kawakami, T. *J. Biol. Chem*. **2004**, *279*, 47720.
[5] Feng, J.; Park, J.; Cron, P.; Hess, D.; Hemmings, B.A. *J. Biol. Chem*. **2004**, *279*, 41189.
[6] Sarbassov, D.D.; Guertin, D.A.; Ali, S.M.; Sabatini, D.M. *Science* **2005**, *307*, 1098.
[7] Burgering, B.N.; Coffer, P.J. *Nature* **1995**; *376*, 599.
[8] Kennedy, S.G.; Wagner A.J.; Conzen, S.D.; Jordan, J.; Bellacosa, A.; Tsichlis, P.N.; Hay, N. *Genes Dev.* **1997**, *11*, 701.
[9] Baraud, C.; Henzel, W.J.; Baeuerle, P.A. Proc. *Natl. Acad. Sci. USA* **1999**, *96*, 429.
[10] Kane, L.P.; Shapiro, V.S.; Stokoe, D.; Weiss, A. *Curr. Biol*. **1999**, *9*, 601.
[11] Cardone, M.H; Roy, N.; Stennicke, H.R.; Salvesen, G.S.; Franke, T.F.; Stanbridge, E.; Frisch, S.; Reed, J.C. *Science* **1998**, *282*, 1318.
[12] Brunet, A.; Bonni, A.; Zigmond, M.J.; Lin, M.Z.; Juo, P.; Anderson, M.J.; Arden, K.C.; Blenis, J.; Greenberg, M.E. *Cell* **1999**, *19*, 857.
[13] Downward, J. *Cur. Opin. Cell Biol*. **1998**, *10*, 262.
[14] Qiao, L.; Hu, Y.; Nan, F.; Powis, G.; Kozikowski, P.A. *Org. Lett*. **2000**, *2*, 115.
[15] Saki, M.; Oshimura, M.; Ito, H. *Apoptosis* **2004**, *9*, 667.
[16] Brazil, D.P.; Hemmings B.A. *Trends Biochem. Sci*. **2001**, *26*, 657.
[17] Neri, M.L.; Borgatti, P.; Capitani, S.; Martelli A.M. *Biochim. Biophys. Acta* **2002**, *1584*, 73.
[18] Vivanco, I.; Sawyers, C.L. *Nature Rev. Cancer* **2002**, *2*, 489.
[19] Lawlor, M.A.; Alessi, D.R. *J. Cell Sci.* **2001**, *114*, 2903.
[20] Osaki, M.; Oshimura, M.; Ito, H. *Apoptosis* **2004**, *6*, 66.
[21] Parsone, R. *Semin. Cell Dev. Biol.* **2004**,*15*, 171.
[22] Downward, J. *Semin. Cell Dev. Biol.* **2004**,*15*, 177.
[23] Koyasu, S. *Nature Immunol.* **2003**, *4*, 313.
[24] Davis, S.J.; Ikemizu, S.; Evans, E.J.; Fugger, L. Bakker, T.R.; Van der Merwe, P. A. *Nature Immunol.* **2003**, *4*, 217.
[25] West, K.A.S.; Castillo, S.; Dennis, P.A. *Drug Resist. Updat.* **2002**, *5*, 234.
[26] Tanno, S.;Yanagawa, N.; Habiro, A.; Koizumi, K.; Nakano, Y.; Osanai, M.; Mizukami, Y.; Okumura, T.; Testa, J.R.; Kohgo, Y. *Cancer Res.* **2004**, *64*, 3486.
[27] Clark, A.S.; West, K.; Streicher, S.; Dennis, P.A. *Mol. Cancer Ther.* **2002**, *1*, 707.
[28] Shayesteh, L.; Lu, Y.; Kuo, W.L.; Balocchi, R.; Godfrey, T.; Collins, C.; Pinkel, D.; Powell, B.; Mills, G.B.; Gray, J.W. *Nat. Genet*. **1999**, *21*, 99.
[29] Moore, S.M.; Rintoul, R.C.; Walker, T.R.; Chilvers, E.R.; Haslett, C.; Sethi, T. *Cancer Res.* **1998**, *58*, 5239.
[30] Donahue, A. C.; Fruman D.A *Semin. Cell Dev. Biol.* **2004**, *15*, 183.
[31] Rong, S.-B.; Hu, Y.; Enyedy, I.; Powis, G.; Meuillet, E. J.; Wu, X.; Wang, R.; Wang, S.; Kozikowski, A.P. *J. Med. Chem.* **2001**, *44*, 898.
[32] Gill, A. *Drug Discov. Today* **2004**, *9*, 16.
[33] Wu, X.; Oobata, T.; Khan, Q.; Highshaw, R.A.; De Vere White, R.; Sweeney C. *Br. J. Urol. Int.* **2004**, *93*, 143.
[34] Fujino, H.; Regan, J.W. *Trends Pharmacol. Sci.* **2003**, 24, 335
[35] Hill, M.M.; Hemmings, B.A. *Pharmacol. Therap.* **2002**, 93, 243.
[36] Bedogni, B.; O'Neill, M.S.; Welford, S.M.; Bouley, D.M.; Giaccia, A.J.; Denko, N.C.; Powell M.B. *Cancer Res*. **2004**, *64*, 2552.
[37] Arcaro, A.; Wymann, M.P. *Biochem. J.* **1993**, *296*, 297.
[38] Yano, H.; Nakanishi, S.; Kimura, K.; Hanai, N.; Saitoh, Y.; Fukui, Y.; Nonomura, Y.; Matsuda, Y. *J. Biol. Chem.* **1993**, *268*, 25846.
[39] Ui, M.; Okada, T.; Hazeki, K.; Hazeki, O. *Trends Biochem. Sci.* **1995**, *20*, 303.
[40] Vlahos, C.J.; Matter, W.F.; Hui, K.Y.; Brown, R.F. *J. Biol. Chem.* **1994**, *269*,5241.
[41] Powis, G.; Bonjouklian, R.; Berggren, M.M.; Gallegos, A.; Abraham, R.; Ashendel, C.; Zalkow, L.; Matter, W.F.; Dodge, J.; Grindey, G. *Cancer Res.* **1994**, *54*, 2419.
[42] Schultz, R.M.; Merriman, R.L.; Andis, S.L.; Bonjouklian, R.; Grindey, G.B.; Rutherford, P.G.; Gallegos, A.; Massey, K.; Powis, G. *Anticancer Res.* **1995**, *15*, 1135.
[43] Lindsley, C.W.; Bogusky, M.J.; Leister, W.H.; McClain, R.T.; Robinson, R.G.; Barnett, S.F.; Defeo-Jones, D.; Ross, C.W.; Hartman, G.D. *Tetrahedron Lett.* **2005**, *46*, 2779.
[44] Lindsley, C.W.; Zhao, Z.; Leister, W.H.; Robinson, R.G.; Barnett, S.F.; Defeo-Jones, D.; Jones, R.E.; Hartman, G.D.; Hu, J.R.; Huber, H.E.; Duggan, M.E. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 761.
[45] Varticovski, L.; Lu, Z.R.; Mitchell, K.; de Aos, I.; Kopecek, J. *J. Control. Release* **2001**, *74*, 275.
[46] Creemer, L.C.; Kirst, H.A.; Vlahos, C.J.; Schultz, R.M. *J. Med. Chem.* **1996,** *39,* 5021.
[47] Potter, B.V.L. *Nut. Prod. Rep.* **1990**, *7,* 1.
[48] Kozikowski, A.P.; Fauq, H.; Aksoy, A.; Seewald, M.J.; Powis, G. *J. Am. Chem. Soc.* **1990**, *112*, 7403.
[49] Potter, B.V.L. *Carbohydr. Res.* **1992**, *234*, 107.
[50] Billington, D.C. *The Inositol Phosphates-Chemical Synthesis and Biological Significance,* VCH, Weinheim, New York, [Basel, Cambridge], **1993**.
[51] Kozikowski, A.P.; Ognyanov, V.I.; Fauq, A.H.; Wilcox R.A.; Nahorski S.R. *J. Chem. Soc., Chem. Commun.* **1994**, 599.
[52] Kozikowski, A.P.; Tiickmantel, W.; Powis, G. *Angew. Chem., Int. Ed. Engl.* **1992**, *31,* 1379
[53] Kozikowski, A.P.; Fauq, A.H.; Malaska, M.J.; Tuckmantel, W.; Ognyanov, V.I.; Powis, G. *Curr. Med. Chem.* **1994**, *1*, 1.
[54] Kozikowski, A.P.; Powis, G.; Fauq, A.H.; Tiickmantel, W.; Gallegos, A. *J. Org. Chem.* **1994**, *59,* 963.
[55] Kozikowski, A.P.; Qiao, L.; Tuckmantel, W.; Powis, G. *Tetrahedron* **1997**, 53, 14903.
[56] Qiao, L.; Nan, F.; Kunkel, M.; Gallegos, A.; Powis, G.; Kozikowski, A.P. *J. Med.Chem.* **1998,** *41*, 3303.
[57] Kozikowski, A.P.; Kiddle, J.T.; Frew, T.; Berggren, M.; Powid, G. *J. Med. Chem.*, **1995**, *38*, 1053.

[58] Hu, Y.; Meuillet, E.J.; Berggren, M.; Powis, G.; Kozikowski, A.P. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 173.
[59] Meuillet, E.J.; Mahadevan, D.; Vankayalapati, H.; Berggren, M.; Williams, R.; Coon, A.; Kozikowski, A.P.; Powis G. *Mol. Cancer Ther.* **2003**, *2*, 389.
[60] Hu, Y.; Qiao, L.; Wang, S.; Rong, S.; Meuillet, E.J.; Berggren, M.; Gallegos, A.; Powis, G.; Kozikowski, A.P. *J. Med. Chem.* **2000**, *43*, 3045
[61] Egorin; M.J.; Parise, R.A.; Joseph, E.; Hamburger, D.R.; Lan, J.; Covey, J.M.; Eiseman, J.L. *Proc. Am. Assoc. Cancer Res.* **2002**, *43*, 604.
[62] Sun, H.; Reddy, G. B.; George, C.; Meuillet, E.J.; Berggren, M.; Powisc, G.; Kozikowski, A.P. *Tetrahedron Lett.* **2002**,43, 2835.
[63] Piccolo, E.; Vignati, S.; Maffucci, T.; Innominato, P.F.; Riley, A.M.; Potter, B.V.L.; Pandolfi, P.P.; Broggini, M.; Iacobelli, S.; Innocenti, P.; Falasca, M. *Oncogene* **2004**, *23*, 1754.
[64] Maffucci, T.; Piccolo, E.; Cumashi, A; Iezzi, M.; Riley, A.M.; Saiardi, A.; Godage, H.Y.; Rossi, C.; Broggini, M.; Iacobelli, S.; Potter, B.V.L.; Innocenti, P.; Falasca, M. *Cancer Res*. **2005**, *65*, 8339
[65] Huang, C.; Ma, W.Y.; Hecht, S.S.; Dong, Z. *Cancer Res.* **1997**, *57*, 2873.
[66] Shamsuddin, A.M. *Anticancer Res.* **1999**, *19*, 3733.
[67] Ng, S.S; Tsao, M.S.; Nicklee, T.; Hedley, D.W. *Clin. Cancer Res*. **2001**, *7*, 3269.
[68] Stein, R.C. *Endocr. Relat. Cancer* **2001**, *8*, 237.
[69] Berrie, C.P.; Falasca, M. *FASEB J*. **2000**, *14*, 2618.
[70] Razzini, G.; Berrie, C.P.; Vignati, S.; Broggini, M.; Mascetta, G.; Brancaccio, A.; Falasca, M. *FASEB J*. **2000**, *14*, 1179.
[71] Ferry, S.; Matsuda, M.; Yoshida, H.; Hirata, M. *Carcinogenesis* **2002**, *23*, 2031.
[72] Chrétien, F.; Moitessiera, N.; Roussela, F.; Maugerb, J. -P.; Chapleur Y. *Curr. Org. Chem.* **2000**, *4*, 513.
[73] Castillo, S.S.; Brognard, J.; Petukhov, P.A.; Zhang, C.;Tsurutani, J.; Granville, C.A.; Li, M.; Jung, M.; West, K.A.; Gills, J.G.; Kozikowski, A.P.; Dennis, P.A. *Cancer Res.* **2004**, *64*, 2782.
[74] Tabellini, G.; Tazzari, T.L.; Bortul, R.; Billi, A.M.; Conte, R.; Manzoli, L.; Cocco, L.; Martelli A. *Br. J. Haematol.* **2004**, *126*, 574.
[75] Tropper, F.D.; Andersson, F.O.; Braun, S.; Roy, R. *Synthesis* **1992**, 619.
[76] Garegg, P. J. *Adv. Carbohydr. Chem. Biochem.* **1997**, *52*, 179
[77] De Nooy A.E.J. *Synthesis* **1996**, 1153
[78] Anelli, P.L.; Biffi, C.; Montanari, F.; Quici, S. *J. Org. Chem*. **1987**, 52, 2559
[79] Kannan, T.; Vinodhkumar, S.; Varghese, B.; Loganathanb, D. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2433
[80] Györgydeák, Z.; Thiem, *J. Carbohydr. Res.* **1995**, *286*, 85.
[81] Thomas, C.C.; Deak, M.; Alessi, D.R.; Van Aalten, D.M.F. *Curr. Biol.* **2002**, *12,* 1256.
[82] Morris, G.M.; Goodsell, D.S.; Halliday, R.S.; Huey, R.; Hart, W.W.; Belew R.K.; Olson, A.J. *Automated Docking using a Lamarckian Genetic Algorithm and an Empirical Binding Free Energy Function, Journal of Computational Chemistry*, Vol *19*, **1998**; pp. 1639-1662.

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