Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 909–913

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

LY294002-geldanamycin Heterodimers as Selective Inhibitors of the PI3K and PI3K-related Family

Gabriela Chiosis,* Neal Rosen and Laura Sepp-Lorenzino†

Department of Molecular Oncogenesis, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA

Received 29 September 2000; accepted 6 February 2001

Abstract—Several LY294002-GM heterodimers were synthesized with the intent of modulating their activity in the presence of hsp90 and thereby creating selective inhibitors of PI3K and PI3K-related family.

The specificity and affinity of a ligand–protein interaction can be modulated by borrowing additional surface contacts from another protein.[1] By linking a weak and/or unselective ligand to the ligand of a physiologically abundant protein one could envision increasing the efficacy of the interaction if the created contact among the protein partners is favorable. In this fashion, selective inhibition of proteins that have similar pockets could be achieved.

Phosphoinositol-3 kinase (PI3K) and a newly emerging subfamily called PI3K-related kinases share a similar carboxy-terminus catalytic domain.[2] Given the sequence homology in the kinase domain of these proteins it is expected that they will all be inactivated by the known PI3K inhibitors wortmannin and LY294002.[3] The PI3K kinases consist of enzymes composed of various catalytic subunits of the p110α, p110β, and p110γ and the yeast homologue Vps34p types. The p110α and p110β subtypes form tight heterodimers with a p85 regulatory unit, the assembly being activated by binding to autophosphorylated growth factor receptors or their substrates. Other PI3Ks (e.g., p110γ) seem to propagate signals from seven transmembrane helix receptors as they are activated by G-protein βγ-subunits. The PI3K-related proteins, ataxia-telangiectasia mutated (ATM), ataxia-telangiectasia related (ATR) and DNA-dependent protein kinase (DNA-PK) play important roles in checkpoints that operate to permit cell survival following many forms of DNA damage. Emerging experimental evidence suggests that although these proteins posses similar substrate specificity, they exhibit overlapping but distinct functions in vivo. ATM, ATR, and DNA-PK have been involved in regulating p53, and additionally, ATM has been found to play an important role in the control of the non-receptor tyrosine kinase Abl. Their counterparts in yeast have been identified as rad3p, Mec1p, Tel1p, and Esr1p. Another PI3K-related protein that possesses the kinase domain is FRAP (mTOR). FRAP is homologous to the yeast Tor1p/2p believed to control aspects of several diverse cellular functions, protein stability, translocation and translation as well as glucose metabolism. For example, FRAP controls pathways that are activated by transmembrane receptors, such as regulation of $p70^{s6}$ kinase.

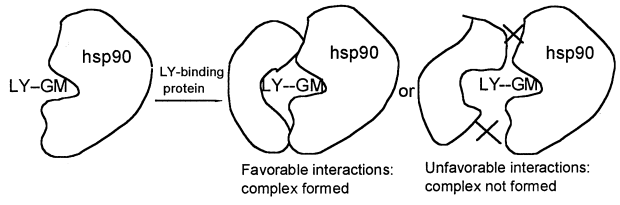


Figure 1. The protein assembly takes place only if favorable interactions among the two partners are allowed by the nature of the heterodimer inhibitor.

Selective inhibitors of the members of this family would constitute important tools for investigating the regulatory functions mediated by each protein. Additionally, due to the radiosensitive phenotypes exhibited by cells defective in ATM, ATR, or DNA-PK, inhibitors of these kinases might enhance the cytotoxic effects of ionizing radiation or DNA damaging cancer chemotherapeutic agents.

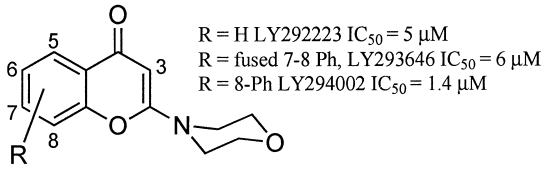
*Corresponding author. Fax: +1-212-717-3627; e-mail: chiosisg@mskmail.mskcc.org

†Present address: Merck & Co., Inc. MRL WP16-327, West Point, PA 19486, USA.

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00099-3

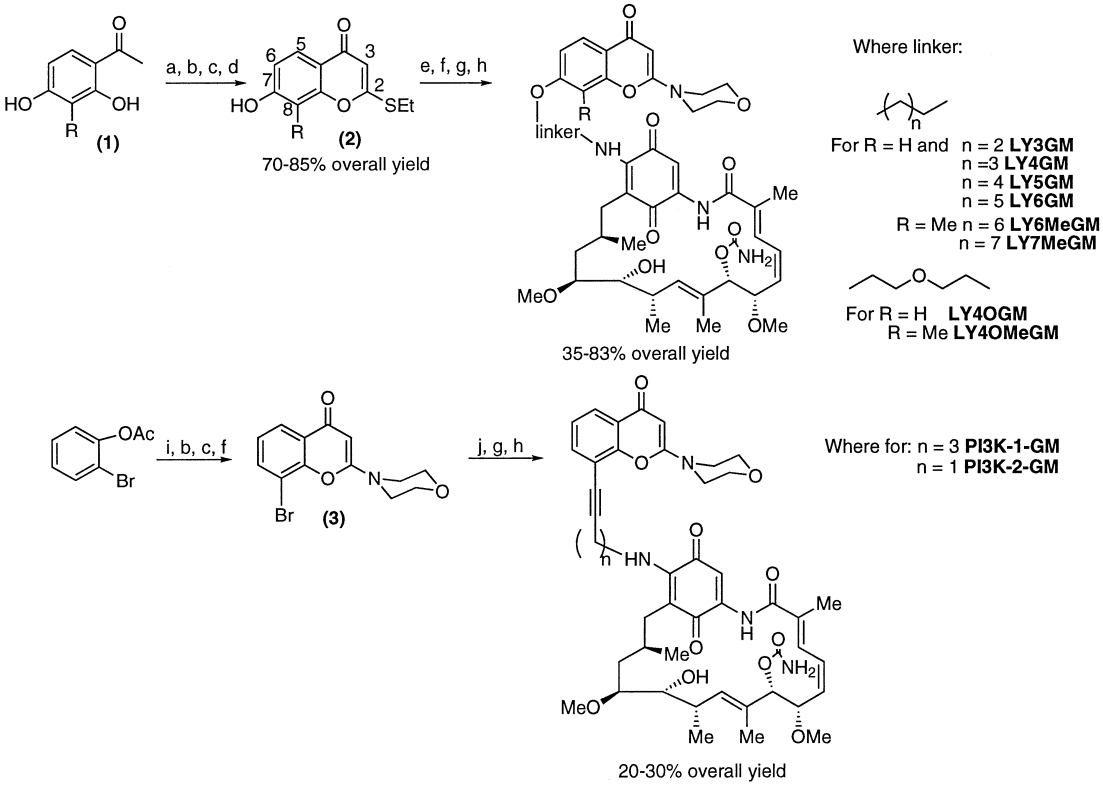
In this paper we describe a new strategy for the synthesis of selective PI3K inhibitors. An LY294002-like molecule was chosen as the kinase domain inhibitor and the hsp90 chaperone[4] as the interaction modulator protein (Fig. 1). The choice of this protein comes from its abundance in the cytosol and the existence of a high affinity binder, geldanamycin (GM).[5] Additionally, hsp90 is expressed at 2- to 10-fold higher levels in tumor cells compared to their normal counterparts. One would expect that given the abundance of hsp90 and the high affinity of GM for the protein, heterodimers of the LY294002-GM type would predominantly bind to hsp90 and be presented to the PI3K family only if the interactions in this tertiary complex are favorable (Fig. 1).

A very important factor in determining the nature of the interaction would be the length, nature, and mode of attachment of the linker. A preferential complex formation would result in selective binding and, therefore, compounds with more restricted cellular effects than the parent LYs.



Due to the relatively similar inhibitory effects of the 7- and 8-substituted derivatives in the LY series, we chose LY292223 as the kinase domain-binding skeleton. OH or Br derivatized 2-hydroxy-3-phenylacetophenones were chosen to be the starting points for the facile reconstruction of such a skeleton (Scheme 1). These functionalities permit the attachment of several linkers through a Mitsunobu or a modified Sagatoshira reaction, respectively. Construction of the LY-skeleton was performed, with the appropriate modifications, based on the method published[6] by the Eli Lilly group. Condensation of the benzyl protected hydroxy-acetophenones (**1**) with carbon disulphide in the presence of potassium *t*-butoxide in benzene gave the corresponding thionocoumarins. These were converted to thioethers by alkylation with iodoethane and potassium carbonate.

If the linkers were to be attached through the Mitsunobu reaction, the benzyl group was first deprotected using boiling trifluoroacetic acid. Alkylation was carried out on the resulting phenols (**2**) with several *N*-Boc protected amino alcohols in a mixture of toluene/dichloromethane. After the attachment of the linker, the 2-sulphinyl moiety was replaced by morpholinyl through a nucleophilic displacement by simple refluxing in morpholine under acetic acid catalysis. Having the LY-skeleton and the linker assembled, GM was introduced through the ability of this molecule to undergo smooth Michael reactions with primary amines.



Scheme 1. Synthesis of the LY-GM heterodimers. (a) BnBr, K_2CO_3, DMF, 60 °C, 16 h; (b) 10 equiv *t*-BuOK, 3 equiv CS_2, benzene, 16 h; (c) EtI, K_2CO_3, acetone, reflux 30 min; (d) TFA, PhSMe, 70 °C, 16 h; (e) 3 equiv HO-linker-NHBoc, 1.3 equiv PPh_3, 1.3 equiv DEAD, toluene/DCM (10:1), 18 h; (f) morpholine, cat. AcOH, 75 °C, 2–3 h; (g) TFA/DCM (1:4), 1 h, rt; (h) GM, 3 equiv TEA, DMF, 15–24 h; (i) $AlCl_3$, 120 °C, 16 h; (j) 1.7 equiv *N*-Boc protected alkyl-ε-ynylamine, $Pd(PPh_3)_4$, PPh_3, CuI, morpholine.

If the attachment mode is the Sagatoshira reaction, the whole LY-skeleton is assembled first to give 2-morpholinyl-8-bromochromone (**3**), followed by addition of the *N*-Boc protected alkyl-ε-ynylamine linker. After standard Boc deprotection, the resulting amine was coupled to GM, giving the derivatives PI3K-1 and PI3K-2.[7]

To assess the effect of the mode of attachment and size of the linker on the activity of these dimers, we tested them for their ability to inhibit PI3K and DNA-PK, and additionally, to compete with solid-phase immobilized GM for hsp90 binding.

Inhibition of PI3-kinase by heterodimers[8]

The PI3K enzyme was immunopurified from insulin stimulated MCF-7 breast cancer cell lines using protein A immobilized anti-p85 antibody. Its ability to catalyze the phosphorylation of phosphatidylinositol by ATP in the presence and absence of drugs was determined. The hybrids LY3-GM, LY4-GM, LY5-GM, LY6-GM, and LY4O-GM showed significantly lower activity than the parent compound LY292223 (Table 1). Surprisingly, LY4OMe-GM, LY6Me-GM, and LY7Me-GM that differ from the above series just by the presence of a methyl group at the 8 position of the LY-skeleton, exhibited potencies comparable to the Lilly derivative, while the derivatives PI3K-1-GM, PI3K-2-GM, and LY6Me-GM were more potent. Free GM (at 500 μM) and DMSO (2.5% v/v) had no effect on the kinase activity of PI3K at the studied concentrations (data not shown).

Inhibition of DNA-PK by heterodimers[9]

A peptidic sequence derived from p53 was used as the substrate to determine the kinase activity of DNA-PK in the presence of the heterodimers. There is a consistent correlation between linker attachment site and potency of inhibition of DNA-PK and PI3-K. All the LYn-GM members are very weak inhibitors of PI3K with IC_{50}s over 300 μM, however, they inhibit DNA-PK at concentrations comparable to the parent Lilly compound. The most selective candidate, LY6-GM is over two orders of magnitude more active against DNA-PK than PI3K. The LYnMe-GM series shows only 1.5- to 16-fold selectivity, while, most surprisingly, the PI3K-n-GM series has the exact potency in inhibiting both proteins. GM (at 40 μM) and DMSO (8% v/v) had no effect on the activity of DNA-PK.

Table 1. Inhibition of PI3K activity and competition for hsp90 binding by the synthesized heterodimers

Compounds	IC_{50}, μM[a] for inhibition of PI3K activity	IC_{50}, μM[a] for inhibition of DNA-PK activity		EC_{50}, μM drug necessary to compete for 50% of Hsp90 a binding
		−hsp90 α	+hsp90 α	
17AAG	>500	>40		1
WTM	ND	0.02		≫50
LY292223	14 (±2)	4.3 (±0.6)		≫50
LY3-GM	>500	28 (±1)		26
LY4-GM	200 (±12)	13.9 (±0.8)		10
LY5-GM	310 (±23)	10 (±0.4)	5 (±0.1)	2.5
LY6-GM	>500	6.1 (±0.2)		4
LY4O-GM	85 (±15)	33 (±1.8)		16
LY4OMe-GM	70 (±13)	12.2 (±1.8)		>50
LY6Me-GM	5 (±1.5)	3.7 (±1)		46
LY7Me-GM	28 (±3)	1.75 (±0.15)		28
PI3K-1-GM	13.5 (±2.5)	13.4 (±1.3)		≫50
PI3K-2-GM	3 (±1)	3 (±0.2)		7

[a]Values are means of two experiments, standard deviation is given in parentheses. 17AAG is a GM derivative with similar potency.

Hsp90 binding assays[10]

GM immobilized on solid phase was utilized to determine the ability of the synthesized heterodimers to bind to the hsp90 α protein. Drugs were pre-incubated with protein and then added to the GM-beads. After an incubation time, the amount of protein left for binding to the solid support was separated and quantified. The ability of drugs to compete with the immobilized GM for 50% of the hsp90 was measured (Table 1). It was expected that the addition of a floppy linker on the GM molecule would diminish somewhat its affinity for hsp90. It is rather unexpected however, that some small variations in the linker substantially affected hsp90 binding. The only difference between LY5-GM and LY4O-GM is the substitution in the linker of one C atom with O, however, the latter is 6 times less active.

Modulation of LY5-GM binding to DNA-PK by hsp90

LY5-GM is the tightest hsp90 α binder and a modest DNA-PK inhibitor. It was, therefore, an attractive candidate to study the influence of hsp90 on kinase activity. Pre-incubation of the heterodimer with hsp90 α increased its inhibition of DNA-PK by 50% (Table 1). No effect on enzyme activity was observed in the samples that hsp90 was pre-incubated with DMSO (not shown).

The study points out how unpredictable and important a small modification in the nature, length, and mode of attachment of the linker can be. The presence of a methyl group at the 8 position of LY292223 seems to have a crucial role in determining the selectivity of the inhibitors. The methylated derivatives are good PI3K and DNA-PK inhibitors and have modest affinity for Hsp90. Meanwhile, compounds without the 8-methyl are good DNA-PK inactivators and maintain good hsp90 binding, however, PI3K is 3 log more resistant to their action. DNA-PK may be important for suppression of apoptosis. Therefore, compounds that selectively affect DNA-PK and not PI3K may be of therapeutic use in cancer and other disorders.

It is noteworthy that PI3K-2-GM has increased PI3-kinase and DNA-PK inhibitory effect compared to the parent Lilly derivative and binding for Hsp90 analogous to 17AAG.

Our study suggests that it is possible to modulate the activity of dual ligands, at least in in vitro systems. Additionally, it implies that the strategy outlined here

can be used to generate a family of inhibitors that differentially inhibit members of the PI3K-family. Screenings in mammalian cell culture systems dependent on various PI3K-related members or in functional yeast hybrid systems will assess the in vivo activity of the heterodimers.

Acknowledgements

The work was funded in part by the Leukemia Research Foundation (GC), the Belfer Foundation and a SPORE grant.

References and Notes

1. Briesewitz, R.; Ray, G. T.; Wandless, T. J.; Crabtree, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1953.
2. Kuruvilla, F. G.; Schreiber, S. L. *Chem. Biol.* **1999**, *6*, R129.
3. Sarkaria, J. N.; Tibbets, R. S.; Busby, E. C.; Kennedy, A. P.; Hill, D. E.; Abraham, R. T. *Cancer Res.* **1998**, *58*, 4375.
4. Buchner, J. *Trends Biochem. Sci.* **1999**, *24*, 136.
5. Whitesell, L.; Mimnaugh, E. G.; De Costa, B.; Myers, C. E.; Neckers, L. M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 8324.
6. Vlahos, C. J.; Matter, W. F.; Hui, K. Y.; Brown, R. F. *J. Biol. Chem.* **1994**, *269*, 5241.
7. LY6-GM: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.18 (s, 1H), 8.03 (d, 1H, J = 8.8 Hz), 7.27 (s, 1H), 6.95 (d, 1H, J = 11.2 Hz), 6.88 (dd, 1H, J = 8.8 Hz, J = 2.2 Hz), 6.73 (d, 1H, J = 2.2 Hz), 6.58 (t, 1H, J = 11.4 Hz), 6.28 (bt, 1H), 5.84 (m, 2H), 5.42 (s, 1H), 5.19 (s, 1H), 4.76 (bs, 2H), 4.28 (m, 2H), 4.03 (t, 2H, J = 5.2 Hz), 3.81 (m, 4H), 3.56 (m, 2H), 3.45 (m, 6H), 3.35 (s, 3H), 3.29 (s, 3H), 2.71 (m, 2H), 2.37 (m, 1H), 2.05 (s, 3H), 1.77 (m, 10H), 1.48 (m, 4H), 1.25 (m, 2H), 1.00 (d, 3H, J = 6.9 Hz), 0.96 (d, 3H, J = 6.5 Hz). MS m/z 897.6 (M + Na).
LY5-GM: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.16 (s, 1H), 8.03 (d, 1H, J = 8.8 Hz), 7.26 (s, 1H), 6.94 (d, 1H, J = 11.7 Hz), 6.88 (dd, 1H, J = 8.8 Hz, J = 2.2 Hz), 6.71 (d, 1H, J = 2.2 Hz), 6.57 (t, 1H, J = 11.4 Hz), 6.28 (bt, 1H), 5.86 (m, 2H), 5.47 (s, 1H), 5.18 (s, 1H), 4.82 (bs, 2H), 4.29 (d, 1H, J = 9.9 Hz), 4.03 (t, 2H, J = 6.0 Hz), 3.81 (m, 4H), 3.56 (m, 2H), 3.50 (m, 5H), 3.34 (s, 3H), 3.25 (s, 3H), 2.68 (m, 2H), 2.37 (m, 1H), 2.02 (s, 3H), 1.77 (m, 2H), 1.76 (s, 3H), 1.68 (m, 6H), 1.25 (m, 2H), 1.00 (d, 3H, J = 6.9 Hz), 0.96 (d, 3H, J = 6.5 Hz). MS m/z 883.8 (M + Na).
LY4-GM: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H), 8.05 (d, 1H, J = 8.8 Hz), 7.27 (s, 1H), 6.94 (d, 1H, J = 12.0 Hz), 6.89 (dd, 1H, J = 8.8 Hz, J = 2.2 Hz), 6.73 (d, 1H, J = 2.2 Hz), 6.57 (t, 1H, J = 11.3 Hz), 6.32 (bt, 1H), 5.86 (m, 2H), 5.42 (s, 1H), 5.19 (s, 1H), 4.76 (bs, 2H), 4.30 (d, 1H, J = 9.9 Hz), 4.24 (bs, 1H), 4.06 (t, 2H, J = 5.0 Hz), 3.80 (m, 4H), 3.53 (m, 3H), 3.48 (m, 5H), 3.31 (s, 3H), 3.26 (s, 3H), 3.08 (m, 2H), 2.67 (m, 2H), 2.39 (m, 1H), 2.02 (s, 3H), 1.99 (m, 4H), 1.78 (s, 3H), 1.68 (m, 4H), 1.25 (m, 2H), 0.99 (d, 3H, J = 6.9 Hz), 0.94 (d, 3H, J = 6.5 Hz). MS m/z 869.8 (M + Na).
LY3-GM: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H), 8.05 (d, 1H, J = 8.8 Hz), 7.27 (s, 1H), 6.92 (m, 2H), 6.82 (d, 1H, J = 2.2 Hz), 6.68 (bt, 1H), 6.56 (t, 1H, J = 11.3 Hz), 5.87 (m, 2H), 5.69 (s, 1H), 5.17 (s, 1H), 4.84 (bs, 2H), 4.29 (d, 1H, J = 9.9 Hz), 4.17 (t, 2H, J = 5.3 Hz), 3.82 (m, 5H), 3.69 (m, 1H), 3.53 (m, 5H), 3.42 (m, 1H), 3.34 (s, 3H), 3.25 (s, 3H), 2.68 (m, 2H), 2.42 (m, 1H), 2.21 (m, 2H), 2.00 (s, 3H), 1.71 (s, 3H), 1.69 (m, 3H), 1.25 (m, 2H), 0.97 (m, 6H). MS m/z 855.8 (M + Na).
LY4OMe-GM: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (s, 1H), 7.96 (d, 1H, = J = 8.7 Hz), 7.25 (s, 1H), 6.94 (d, 1H, J = 11.5 Hz), 6.88 (d, 1H, J = 8.7 Hz), 6.54 (m, 2H), 5.87 (m, 2H), 5.42 (s, 1H), 5.19 (s, 1H), 4.73 (bs, 2H), 4.27 (m, 3H), 3.93 (m, 2H), 3.86 (m, 6H), 3.71 (m, 2H), 3.49 (m, 6H), 3.34 (s, 3H), 3.26 (s, 3H), 2.68 (m, 2H), 2.32 (m, 1H), 2.26 (s, 3H), 1.65 (m, 3H), 0.99 (d, 3H, J = 6.9 Hz), 0.95 (d, 3H, J = 6.6 Hz). MS m/z 877.6 (M + H).
LY6Me-GM: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H), 7.96 (d, 1H, J = 8.7 Hz), 7.25 (s, 1H), 6.94 (d, 1H, J = 12 Hz), 6.88 (d, 1H, J = 8.7 Hz), 6.57 (t, 1H, J = 11.1 Hz), 6.26 (bt, 1H), 5.88 (m, 2H), 5.42 (s, 1H), 5.17 (s, 1H), 4.72 (bs, 2H), 4.28 (d, 1H, J = 10 Hz), 4.07 (m, 2H), 3.83 (m, 4H), 3.55 (m, 7H), 3.32 (s, 3H), 3.25 (s, 3H), 2.69 (m, 2H), 2.39 (m, 1H), 2.24 (s, 3H), 2.01 (s, 3H), 1.84 (m, 2H), 1.79 (s, 3H), 1.74 (m, 4H), 1.57 (m, 4H), 0.99 (d, 3H, J = 6.9 Hz), 0.95 (d, 3H, J = 6.6 Hz). MS m/z 889.6 (M + H).
LY7Me-GM: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.16 (s, 1H), 7.95 (d, 1H, J = 8.5 Hz), 7.26 (s, 1H), 6.94 (d, 1H, J = 12 Hz), 6.86 (d, 1H, J = 8.5 Hz), 6.57 (t, 1H, J = 11.5 Hz), 6.24 (bt, 1H), 5.87 (m, 2H), 5.43 (s, 1H), 5.19 (s, 1H), 4.85 (bs, 2H), 4.30 (d, 1H, J = 9.9 Hz), 4.15 (m, 2H), 3.83 (m, 4H), 3.55 (m, 2H), 3.46 (m, 5H), 3.35 (s, 3H), 3.26 (s, 3H), 2.62 (m, 2H), 2.35 (m, 1H), 2.25 (s, 3H), 2.00 (s, 3H), 1.82 (m, 2H), 1.78 (s, 3H), 1.72 (m, 3H), 1.44 (m, 6H), 0.99 (d, 3H, J = 6.9 Hz), 0.94 (d, 3H, J = 6.6 Hz). MS m/z 902.6 (M + H).
PI3K-1-GM: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H), 8.08 (d, 1H, J = 7.9 Hz), 7.62 (d, 1H, J = 7.5 Hz), 7.27 (m, 2H), 6.93 (d, 1H, J = 11.3 Hz), 6.58 (t, 1H, J = 11.5 Hz), 6.37 (bt, 1H), 5.87 (m, 2H), 5.50 (s, 1H), 5.19 (s, 1H), 4.79 (bs, 2H), 4.29 (d, 1H, J = 9.8 Hz), 4.15 (bs, 1H), 3.85 (m, 4H), 3.72 (m, 2H), 3.54 (m, 5H), 3.33 (m, 1H), 3.27 (s, 3H), 3.26 (s, 3H), 2.66 (m, 4H), 2.41 (m, 1H), 2.08 (s, 3H), 2.00 (m, 1H), 1.78 (m, 3H), 1.67 (m, 5H), 1.01 (d, 3H, J = 6.9 Hz), 0.92 (d, 3H, J = 6.6 Hz). MS m/z 841.7 (M + H).
PI3K-2-GM: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.09 (s, 1H), 8.14 (d, 1H, J = 7.9 Hz), 7.63 (d, 1H, J = 7.5 Hz), 7.33 (s, 1H), 7.31 (dd, 1H, J = 7.9 Hz, J = 7.5 Hz), 6.95 (d, 1H, J = 11.5 Hz), 6.58 (t, 1H, J = 11.7 Hz), 6.37 (bt, 1H), 5.88 (m, 2H), 5.50 (s, 1H), 5.21 (s, 1H), 4.74 (bs, 2H), 4.58 (m, 2H), 4.32 (d, 1H, J = 9.5 Hz), 3.91 (m, 1H), 3.82 (m, 4H), 3.55 (m, 6H), 3.36 (s, 3H), 3.28 (s, 3H), 2.72 (m, 2H), 2.41 (m, 1H), 2.04 (s, 3H), 1.80 (s, 3H), 1.72 (m, 1H), 1.03 (m, 6H). MS m/z 813.7 (M + H).
8. MCF-7 cells were serum starved for 24 h and stimulated with 1 μM insulin. Cell extracts were made in PI3K lysis buffer (137 mM NaCl, 20 mM Tris·HCl, pH 5, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10% v/v glycerol, 1% v/v Triton X-100) and the enzyme was immunoprecipitated using anti-p85 antibody (Upstate Biotechnologies Inc. no. 06-195). The complex was immobilized on Protein A beads (Amersham) and after several washes (3×1% Triton X-100 in PBS, 2×0.1 M Tris·HCl, pH 7.5, 0.5 M LiCl, 1×10 mM Tris·HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA) the immunoprecipitates were subjected to PI3-kinase activity assays. The lipid mix in 10 mM Hepes pH 7, 1 mM EGTA (final concentration 0.5 μM/mL) was incubated with the immobilized enzyme with or without drugs at room temperature for 15 min. The reaction was started upon addition of 10 μL of [γ-^{32}P]ATP (5 μCi/μL) and was allowed to proceed for 20 min at 37 °C after which was quenched by addition of 80 μL 1 M HCl. Lipids were extracted with 160 μL $CHCl_3/CH_3OH$ (1:1). The organic layer was applied to a pre-activated silica gel plate (EM Bioscience) and eluted with *n*-propanol/methanol/2 M AcOH (50:15:35). Plates were visualized by autoradiography and the lipids quantified using BioRad Gel Doc 1000 software.
9. SignaTECT® DNA-PK assay system from Promega was used with small modifications from the manufacturer's instructions. To each drug in 2 μL DMSO or to 2 μL DMSO were added 23 μL reaction mix [250 ng DNA from calf thymus (Sigma#D-3664), 0.2 mM biotinilated peptide substrate, 20 units of DNA-PK (Promega no. V5811), 1 μCi [γ-^{32}P]ATP,

2 μg BSA in 250 mM HEPES, pH 7.5, 500 mM NaCl, 50 mM $MgCl_2$, 1 mM EGTA, 0.5 mM EDTA and 5 mM DTT and the reaction mix was incubated for 10 min at 30 °C. After addition of 12.5 μL guanidine hydrochloride 7.5 M, each reaction was spotted to a SAM$^{2®}$ Biotin capture membrane (Promega no. 2861). After several washes with 2 M NaCl and 1% H_3PO_4 in 2 M NaCl, the samples were quantifies using a scintillation counter. For the interaction modulation assays the drugs were pre-incubated for 17 min on ice with 200 ng hsp90 α in PBS prior to the addition of the kinase mix.

10. GM was immobilized on Affigel 10 resin (BioRad) as described in ref 5. The GM-beads were blocked for 1 h at 4 °C with 0.5% BSA in TEN buffer (50 mM Tris·HCl pH 7.4, 1 mM EDTA, 1% NP-40) prior to use. Hsp90 α protein (Stressgen) was incubated with or without drugs for 20 min on ice. To each sample were added 20 μL GM-beads and the mixtures were rotated at 4 °C for 1 h followed by two washes with 500 μL ice cold TEN each. The GM-beads bound protein was eluted from the solid phase by heating in 35 μL 1×SDS, analyzed by SDS/PAGE and visualized by immunoblotting with Hsp90 α (Stressgen no. SPA-840).