Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 3555–3564

BIOORGANIC & MEDICINAL CHEMISTRY

Development of a Purine-Scaffold Novel Class of Hsp90 Binders that Inhibit the Proliferation of Cancer Cells and Induce the Degradation of Her2 Tyrosine Kinase

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Received 28 February 2002; accepted 23 May 2002

Abstract—The first published synthesis and characterization of a purine-scaffold library of hsp90 inhibitors is presented. The purine-scaffold represents a platform for the creation of easily synthesizable and derivatizable soluble molecules that are amenable for oral administration. The most active compound of the series (**71**) exhibits binding to hsp90 comparable to the natural product derivative 17AAG that is now in Phase I clinical trial as a cancer therapeutic. **71** Induces the degradation of Her2 tyrosine kinase and arrests the MCF-7 breast cancer cell line at low micromolar concentrations ($IC_{50} = 2\ \mu M$).

Introduction

The hsp90 molecular chaperones play a key role in regulating the physiology of cells exposed to environmental stress and in maintaining the malignant phenotype in tumor cells.[1–4] These proteins possess an ATP/ADP binding pocket at their N-terminal that is well-conserved among the four family members: Hsp90 α and Hsp90 β (cytoplasm), Grp94 (endoplasmic reticulum) and Trap-1 (mitochondria).[5–7] Geldanamycin and herbimycin (GM, HA) (Fig. 1) and the unrelated compound radicicol (RD) were found to bind to this N-terminal pocket of Hsp90.[8–10] Addition of these compounds to cells induces the proteasomal degradation of a small subset of proteins involved in signal transduction (i.e., steroid receptors, Raf kinase, Akt, certain transmembrane tyrosine kinases). The met and Her2 tyrosine kinase were shown to be the most sensitive targets described.[11–13] Her2 has oncogenic properties and is overexpressed in many human tumors, including approximately 30% of human breast cancers.[14] In addition, these molecules cause the selective degradation of certain proteins that undergo mutation in cancer, such as *v*-src, bcr-abl and p53, but have little or no effect on their normal counterparts.[15–17] Most cancer cells treated with ansamycins or RD block in G1, phenomenon that is followed by morphological and functional differentiation.[18,19]

The ansamycin derivative 17AAG (Fig. 1) is currently in early clinical trial in cancer patients. 17AAG is a potent anticancer drug, but it is poorly soluble and hard to formulate, and it was shown to induce hepatotoxicity related to the presence of the benzoquinone moiety in its structure.[20] There is no synthetic route available for the construction of this molecule and the drug is obtainable only by fermentation. Radicicol has no activity in animals because of its instability. Oxime-derivatives of RD, like 6-*O*-[2-(2-pyrrolidonyl)-ethyl] radicicol oxime, were found to have potent activity in vitro and in vivo and were extensively studied in Japan.[17,21–23] Unfortunately, the compounds induced severe cataracts in animals, again for reasons unrelated to hsp90 binding.

We have used the structural requirements for Hsp90 α binding and designed a small molecule PU3 (Fig. 1) that exhibited qualitative effects on cellular protein expression, proliferation, and differentiation very similar to those induced by GM and RD. PU3 displayed a relative binding affinity for hsp90 of 15–20 μM. Addition of PU3 to cells caused the degradation of Her2 kinase,

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PII: S0968-0896(02)00253-5

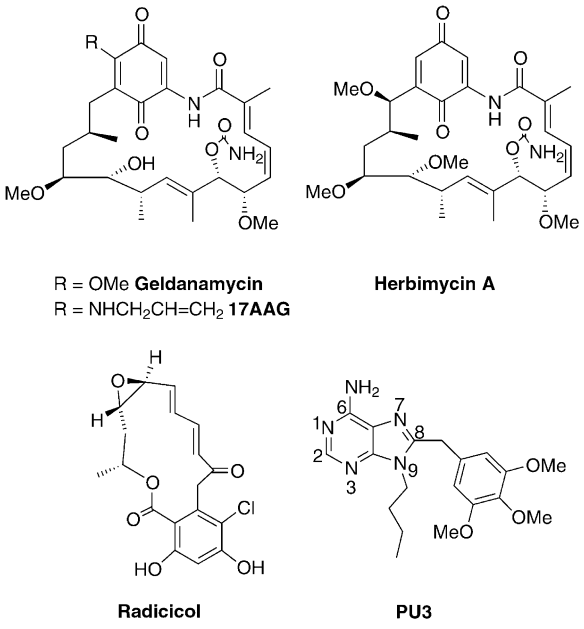


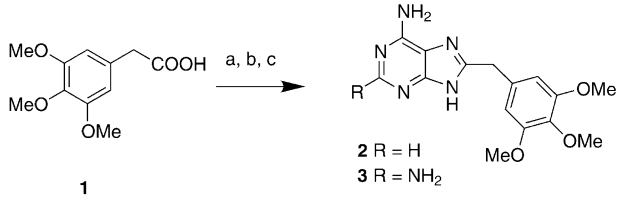
Figure 1. Schematic representation of the known hsp90 binders.

estrogen receptor and Raf kinase. The proteins affected by PU3 were identical to those affected by ansamycins and RD. Exposure of breast cancer cells to PU3 resulted in growth inhibition and profound morphologic changes. Cessation of proliferation was accompanied by induction of a more mature, epithelial morphology consistent with breast differentiation.[24] Although the activity of this molecule is lower compared to the natural products, the structural skeleton of PU3 allows for extensive chemical modifications in the pursuit of compounds with better activity, solubility and therapeutic index. Here we describe the first published synthesis and characterization of a small library of derivatives of PU3 that resulted in a compound with 30-times better cellular effects than our lead, PU3, and which displays a relative binding affinity for hsp90 of 0.55 μM, a value similar to 17AAG.

Results and Discussion

Chemistry

The commercially available 3,4,5-trimethoxyphenylacetic acid (**1**, Scheme 1) was used as the starting point for building a PU3 class library. In general, the carboxylic acid was converted to the acid fluoride and then coupled with 4,5,6-triaminopyrimidine sulfate or 2,4,5,6-tetraaminopyrimidine sulfate to give the corresponding 5-*N*-acylated aminopyrimidines. These were cyclized in NaOMe/MeOH to construct the purine building blocks **2** and **3**, respectively (Scheme 1). Several 9-alkyl-8-benzyl-9*H*-purin-6-ylamines (**4**–**46**) created by 9-*N*-alkylation of **2** are depicted in Fig. 2.[25] The 2-amino group of **3** was initially converted to fluorine via a diazotization reaction in non-aqueous media using *tert*-butyl nitrite (TBN) in HF/pyridine[26] and subsequently alkylated to give the 2-fluoro-9-alkyl-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamines **47**–**59** (Scheme 2). The alkylation was performed using the Mitsunobu reaction, methodology that can accommodate a large array of primary and secondary alcohols. Contrary to alkylation of **2** where the 3-N product was observed as an undesired product, alkylation of the fluoro derivative gave cleanly the 9-N alkyl purine. The 2-fluoro derivatives were further converted to the 2-alkoxy purines **60** and **61** by refluxing in MeOH or EtOH and NaOMe, respectively.[27] Iodine was introduced in that position using isoamyl nitrite in diiodomethane generating **62**.[28,29] The 2-iodo derivative was transformed to the cyano-derivative **63** with tri-*n*-butylcyanostannane and tetrakis(triphenylphosphine)palladium(0) in DMF and to the vinyl-derivative **64** with vinyltributyltin and $(MeCN)_2PdCl_2$.[30]



Scheme 1. Reagents: (a) cyanuric fluoride, pyridine, DCM; (b) 4,5,6-triaminopyrimidine sulfate, DIEA, DMF for **2** and 2,4,5,6-tetraaminopyrimidine sulfate, aqueous NaOH for **3**; (c) NaOMe, MeOH.

The benzyl moiety was enriched in electron-donating groups in order to increase its interaction with the pocket lysine (Scheme 3). Chlorine and bromine were added via a radicalic reaction using *t*-butyl hydroperoxide and the corresponding HX.[31] In the case of chlorine only monosubstitution was observed (**65**), while bromine gave the monosubstituted (**66**) and a small amount of di-bromosubstituted product (**67**).

The nature and length of the bridge between the purine and the phenyl ring were additionally modified (Scheme 4). As starting material we utilized 8-bromoadenine. This was 9-N alkylated and consequently, reacted at high temperature with the aniline-, phenol- or benzyl-derivative to give the corresponding products **68**–**70**.

The assembly of the fully substituted derivatives **71** and **72** commenced with **3** (Scheme 5). Chlorine was added initially via a radicalic reaction to give **73**. Subsequently, the 2-amino group was transformed to fluorine and the resulting purine (**74**) was alkylated via the Mitsunobu reaction.

SAR and Biological Results

All compounds were tested for their ability to compete with geldanamycin for hsp90 binding. To assess their cellular effect we tested the efficacy of the compounds to arrest the growth of the MCF-7 breast cancer cell line and to induce the degradation of the oncogenic Her2 tyrosine kinase. The M logP value was calculated for all compounds and the 'rule of 5' was kept as a guideline in the choice for derivatization.[32–34]

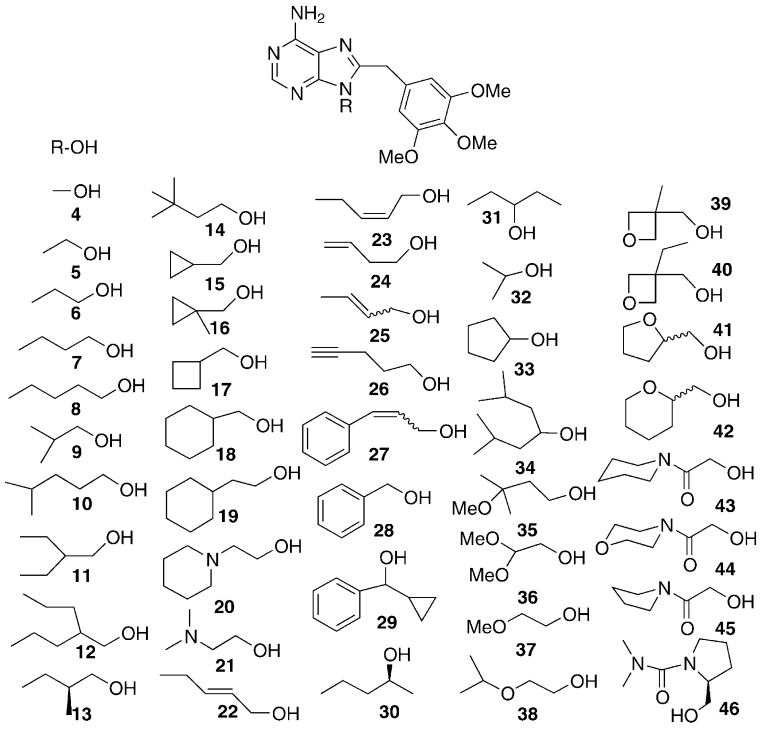
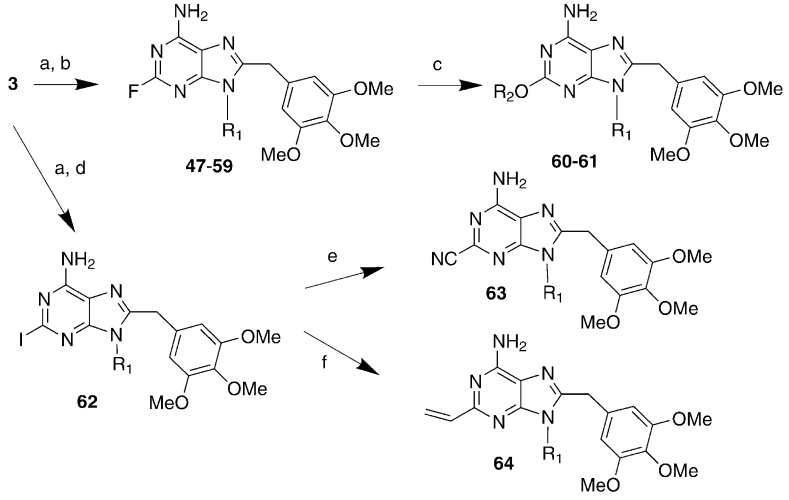
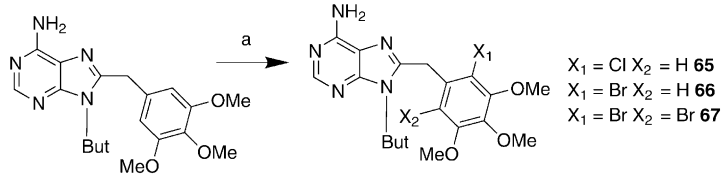


Figure 2. Representative purines obtained by modifying the nature and length of the 9-N butyl chain of PU3.



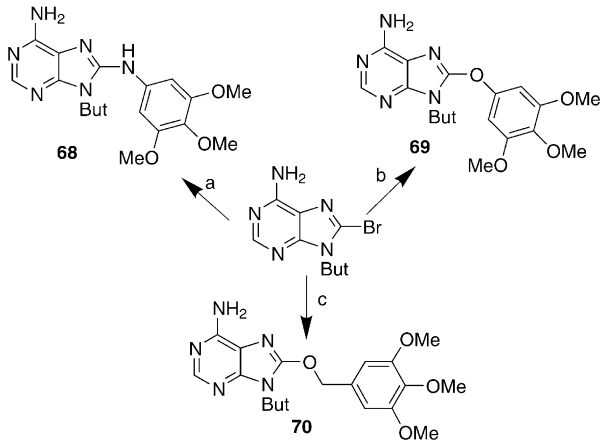
Scheme 2. Reagents: (a) HF, pyridine, *t*-butyl nitrite; (b) R_1OH, PPh_3, diethyl azodicarboxylate or di-*tert*-butyl azodicarboxylate, toluene/DCM; (c) R_2OH, NaOMe; (d) CH_2I_2, isoamyl nitrite; (e) Bu_3SnCN, $Pd[PPh_3]_4$; (f) $(MeCN)_2PdCl_2$, $Bu_3(vinyl)Sn$.



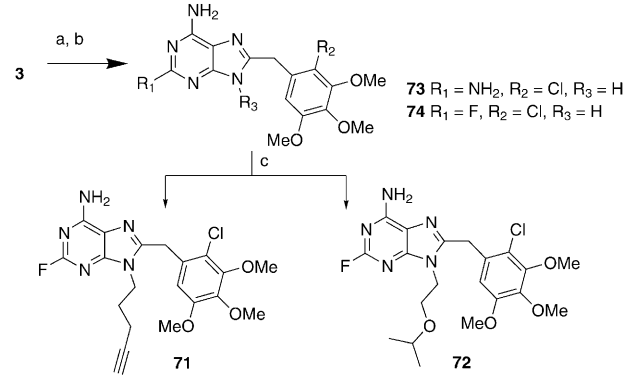
Scheme 3. Reagents: (a) HX, *t*-butylhydroperoxide.

Effect of the 9-alkyl chain on activity

The variety of unbranched and branched, linear and cyclic, saturated and unsaturated primary alcohols and several secondary alcohols available for the 9-*N*-alkylation reaction allowed for the introduction of a high degree of diversity at that position. A first important observation resulted from testing these derivatives is that no compound that has a substituent on C1, such as **29**, **30**, **31**, **33**, and **34** (i.e., resulted by alkylation with secondary alcohols) is active. No binding of these derivatives to hsp90 or any cellular effect is observed at concentrations as high as 250 μM. Secondly, none of the



Scheme 4. Reagents: (a) 3,4,5-trimethoxyaniline; (b) 3,4,5-trimethoxyphenol; (c) 3,4,5-trimethoxybenzyl alcohol.



Scheme 5. Reagents: (a) HCl, *t*-butylhydroperoxide; (b) HF, pyridine, *t*-butyl nitrite; (c) R_3OH, PPh_3, di-*tert*-butyl azodicarboxylate, toluene/DCM.

aromatic moiety containing chains such as in derivatives **27** and **28** allowed for activity. Compounds **43**, **44**, and **45** containing the piperidinyl-, morpholinyl- and pyrrolidinyl-acetamides, respectively, and **39** and **40** containing the oxetane ring-derivatives, have no activity in the binding and cellular assays. Somewhat surprising was the cellular inefficacy of compound **21** that contains a tertiary amine in the 9-N chain. Although it bound hsp90 with a potency comparable to PU3, it shows no effect when added to cancer cells. It is possible that the presence of the tertiary amine limits the cellular uptake of **21**. Compounds **12**, **14**, **19**, **31**, and **46** are insoluble in the assay media and their further characterization was stopped.

Several chains are favorable for activity (Table 1). The greatest gain in activity is obtained by the replacement of the butyl chain of PU3 (**7**) with either a pent-4-ynyl (**26**) or a 2-isopropoxy-ethyl (**38**) chain. These modifications improve binding to hsp90 by almost an order of magnitude.

Effect of the substitution at position 2 of the purine moiety on activity

Addition of cyano, vinyl, iodo, methoxy, ethoxy or amino-functionality at the C2 position of the purine moiety leads to decreased or completely abolished activity. However, fluorine in general increases the potency and water solubility of the 9-alkyl-8-benzyl-9*H*-purin-6-ylamine derivatives (Table 2). Fluorine is a small electron-withdrawing atom and its addition to the purine moiety is very likely to improve the H-donor ability of C6 NH_2.

Table 1.

	EC_{50} hsp90	IC_{50} MCF-7	IC_{50} Her2	M logP
4	NA	70	NA	1.34
5	4.5	80	70	1.59
6	10.8	47	50	1.83
7	13	50	55	2.06
8	11.8	62	50	2.28
9	20.4	98	100	2.06
10	114	69	50	2.51
11	52.1	160	>100	2.51
13	15.7	62	70	2.29
15	16.2	75	70	2.06
16	47.8	111	120	2.29
17	32	46	60	2.29
18	>100	40	50	2.73
22	9.5	47	50	2.21
23	7.5	64	70	2.21
24	10.6	41	30	1.98
25	NA	51	70	1.98
26	1.5	24	20	2.21
36	NA	72	100	0.99
37	5.5	65	85	1.09
38	1.7	39	45	1.55
41	3.4	92	70	1.55

All values are in μM. See Experimental for assay protocols. Standard deviations for binding assays were ±35% of the mean or less, while for growth arrest and Her2 protein degradation of ±20%. The values are the mean of two experiments.
NA, not available.

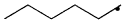
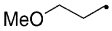
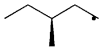
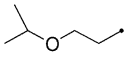
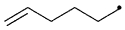
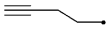
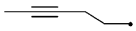
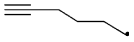
Effect of the addition of halogens to the trimethoxyphenyl moiety

Additional electron-donating functionalities on the trimethoxyphenyl ring should theoretically improve the efficacy of binding to hsp90 by increasing the interaction of this moiety with the Lys residue found at the top of the ADP-binding pocket. We found that both bromine and chlorine improve potency (Table 3). Although bromine is expected to have a greater effect, it is possible that due to its large size it is more difficult to accommodate inside the pocket. If two bromines are added to the molecule, binding to hsp90 in abolished, again probably due to steric effects (**67**). Addition of one Cl to the trimethoxyphenyl moiety improves activity over PU3 by a factor of 2 (**65**).

Effect of the nature and length of the bridge between the purine and trimethoxyphenyl moieties on activity

None of the effected modifications at this position renders active compounds. This observation points to the importance of the angle between the purine and trimethoxyphenyl moieties. It is possible that the replacement of the bridge C with O or N changes the tilt of the trimethoxyphenyl ring and therefore makes the interaction of this moiety with the top Lys of the pocket unfavorable.

Table 2.

		EC_{50} hsp90	IC_{50} MCF-7	IC_{50} Her2	M logP
	**47**	5.3	25	30	1.94
	**48**	3.5	24	25	2.17
	**49**	8.9	36	35	2.40
	**50**	13.8	64	65	1.20
	**51**	15	44	40	2.40
	**52**	1.3	16	15	1.66
	**53**	9.8	33	30	2.09
	**54**	18	45	50	2.32
	**55**	3.5	25	20	2.09
	**56**	10.4	37	45	2.32
	**57**	0.7	11	5	2.32
	**58**	9.2	41	40	2.40
	**59**	6.8	23	20	1.66

All values are in μM. See experimental section for assay protocols. Standard deviations for binding assays were ±35% of the mean or less, while for growth arrest and Her2 protein degradation of ±20%. The values are the mean of two experiments.

Effect of the sum of favorable substitutions on activity

The favorable substitutions made on the 9-alkyl-8-benzyl-9*H*-purin-6-ylamines are additive. Introduction of the best alkyl chains at the position 9-N of purine, of fluorine at position C2 of purine and of chlorine at position 2 of the trimethoxyphenyl ring improves activity over PU3 by a factor of 30 (Table 4). These compounds exhibit a potency of binding to hsp90 equal to the natural product derivative 17AAG[35–38] that is now in Phase I clinical trial. **71** has a molecular weight of 434, the sum of Ns and Os is eight, has one hydrogen bond donor (NH_2) and a calculated M logP[32,33] value of 2.54. Based on these characteristics, it is likely that **71** could have favorable pharmacokinetics as predicted by Lipinski's 'rule of 5'.[34]

Table 3.

	EC_{50} hsp90	IC_{50} MCF-7	IC_{50} Her2	M logP
65	4.6	19	25	2.28
66	11.7	25	50	2.40
67	>100	30	>100	2.73

All values are in μM. See Experimental for assay protocols. Standard deviations for binding assays were ±35% of the mean or less, while for growth arrest and Her2 protein degradation of ±20%. The values are the mean of two experiments.

Table 4.

	EC_{50} hsp90	IC_{50} MCF-7	IC_{50} Her2	M logP
71	0.45	2	2	2.54
72	0.52	5.4	3	1.88

All values are in μM. See Experimental for assay protocols. Standard deviations for binding assays were ±35% of the mean or less, while for growth arrest and Her2 protein degradation of ±20%. The values are the mean of two experiments.

Conclusions

A library of approximately 70 derivatives based on our designed compound PU3 and tailored to meet the 'rule of 5' requirements was synthesized. This is the first biased library of small molecule inhibitors of hsp90 described in literature. Our most potent compound to date, **71**, is 30-fold more active than PU3 and binds hsp90 with affinities comparable to the natural product 17AAG. Addition of **71** to the breast cancer cell line MCF-7 induces growth arrest and leads to the degradation of the oncogenic Her2 tyrosine kinase at low micromolar ($IC_{50} = 2$ μM) concentrations. The skeleton is easily amenable for derivatization and one would predict that sensitive further modifications would bring activity in the nanomolar range. Additionally, the molecules are water soluble at the tested concentrations and are amenable for oral administration. Such molecules could have considerable advantages as drugs including the fact that oral availability would eliminate the inconveniences of prolonged intravenous administration.

Experimental

General

All commercial chemicals and solvents are reagent grade and were used without further purification unless otherwise specified. All reactions except those in aqueous media were carried out with the use of standard techniques for the exclusion of moisture. The Argonaut Quest 210 synthesizer was used for reactions performed in parallel. Reactions were monitored by thin-layer chromatography on 0.25-mm silica gel plates and visualized with UV light or cerium ammonium molybdate in aqueous H_2SO_4.

Final compounds were purified on an ISCO Combi-Flash system and separation of the components was monitored by UV at 254 nm. The identity and purity of each product was characterized by MS, HPLC, TLC

and ^{1}H NMR. ^{1}H NMR spectra were recorded on a Bruker 400 MHz instrument. Splitting patterns are designed as s, singlet; d, doublet; t, triplet; m, multiplet. Low-resolution mass spectra (MS) were recorded in the positive ion mode under electron-spray ionization (ESI).

8-(3,4,5-Trimethoxy-benzyl)-9*H*-purine-2,6-diamine (3). To trimethoxyphenyl acetic acid (1.0 g, 4.4 mmol) in CH_2Cl_2 (20 mL) (under inert atmosphere) was added cyanuric fluoride (371 μL, 4.4 mmol) and pyridine (356 μL, 4.4 mmol). The mixture was stirred for 1 h at room temperature, after which, an additional 30 mL of CH_2Cl_2 was added. The resulting solution was washed once with water (5 mL), and the acid fluoride resulting from the removal of the solvent was taken up in DMF (10 mL) and used in the next step. Separately, 2,4,5,6-tetraaminopyrimidine sulfate (0.9 g, 3.8 mmol) was dissolved in water (40 mL) that contained NaOH (456 mg, 11.4 mmol). The resulting solution was heated to 70 °C and the acid fluoride was added dropwise over a 20 min period. The reaction mixture was stirred for 1.5 h at 70 °C and then concentrated to dryness. To the crude material was added MeOH (16 mL) and a 25% solution of NaOMe in MeOH (16 mL) and the resulting solution was heated at 90 °C for 18 h. Following cooling, the pH of the solution was adjusted to 7 by addition of concentrated HCl. The aqueous solution was removed and the crude taken up in DCM (100 mL) and MeOH (50 mL). The undissolved solids were filtered off and the product (470 mg, 37%) was purified on a silica gel column with EtOAc/CH_2Cl_2/MeOH at 4:4:2. ^{1}H NMR (DMSO-d_6, 400 MHz) 11.90 (b s, 1H), 6.62 (s, 2H), 6.49 (b s, 2H), 5.55 (b s, 2H), 3.91 (s, 2H), 3.73 (s, 6H), 3.55 (s, 3H). MS (ESI) m/z 330.9 (M + 1). R_f (hexane/EtOAc/CH_2Cl_2/MeOH at 10:5:5:2) 0.12.

2-Fluoro-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (FAC). To **3** (500 mg, 1.5 mmol) was slowly added a 70% solution of HF in pyridine (2 mL), pyridine (8 mL), followed by *t*-butyl nitrite (200 μL, 2.0 mmol). The mixture was stirred for 2 h and then quenched overnight with 8 g $CaCO_3$ in water (15 mL) and MeOH (10 mL). The solution was concentrated to dryness and the resulting crude was taken up in MeOH (30 mL) and CH_2Cl_2 (10 mL). The insoluble solids were filtered off and the solvent was removed to give the crude product. This was purified on a silica gel column eluting with hexane/DCM/EtOAc/MeOH at 10:5:5:0.75 (240 mg, 50%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.41 (b s, 2H), 6.46 (s, 2H), 3.86 (s, 2H), 3.57 (s, 6H), 3.23 (s, 3H). MS (ESI) m/z 333.9 (M + 1). R_f (hexane/EtOAc/CH_2Cl_2/MeOH at 10:5:5:2) 0.40.

General method for the 9-*N*-alkylation of purines

The alkylation was performed via a Mitsunobu reaction as described before.[25] Essentially, to **FAC** in toluene/CH_2Cl_2 at 5:1 was added 1.3 equiv of the corresponding alcohol, 2 equiv PPh_3, 3 equiv di-*tert*-butyl azodicarboxylate and the resulting solution was stirred at room temperature for 10 min to 1 h (conversion was monitored by TLC with hexane/EtOAc/CH_2Cl_2/MeOH at 10:5:5:1) to give:

2-Fluoro-9-propyl-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (47). Yield, 66%. R_f 0.38. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.41 (s, 2H), 6.01 (b s, 2H), 4.15 (s, 2H), 3.93 (t, J = 7.6 Hz, 2H), 3.80 (s, 3H), 3.79 (s, 6H), 1.65–1.60 (m, 2H), 0.85 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) 159.6, 157.7, 156.3, 156.1, 153.6, 152.9, 152.8, 151.0, 137.2, 131.0, 116.7, 105.4, 60.9, 56.2, 44.7, 34.9, 22.8, 11.1. MS (ESI) m/z 376.0 (M + 1).

2-Fluoro-9-butyl-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (48). Yield, 60%. R_f 0.38. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.41 (s, 2H), 5.96 (b s, 2H), 4.15 (s, 2H), 3.95 (t, J = 7.7 Hz, 2H), 3.81 (s, 3H), 3.79 (s, 6H), 1.56–1.52 (m, 2H), 1.29–1.23 (m, 2H), 0.85 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) 159.8, 157.7, 156.3, 156.1, 153.6, 152.9, 152.8, 151.0, 137.3, 131.0, 116.7, 105.4, 60.9, 56.2, 43.1, 34.9, 31.5, 19.9, 13.6. MS (ESI) m/z 389.9 (M + 1).

2-Fluoro-9-pentyl-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (49). Yield, 65% R_f 0.39. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.40 (s, 2H), 6.15 (b s, 2H), 4.15 (s, 2H), 3.95 (t, J = 7.9 Hz, 2H), 3.80 (s, 3H), 3.79 (s, 6H), 1.59–1.51 (m, 2H), 1.26–1.18 (m, 4H), 0.81 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) 159.9, 157.7, 156.3, 156.1, 153.6, 152.9, 152.8, 150.9, 137.2, 131.0, 116.7, 105.4, 60.9, 56.1, 43.3, 34.9, 29.2, 28.8, 22.2, 15.3, 13.8. MS (ESI) m/z 404.0 (M + 1).

2-Fluoro-9-(2-methoxy-ethyl)-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (50). Yield, 28%. R_f 0.37. ^{1}H NMR ($CDCl_3$, 400 MHz) δ6.43 (s, 2H), 5.79 (b s, 2H), 4.25 (s, 2H), 4.16 (t, J = 5.1 Hz, 2H), 3.81 (s, 3H), 3.80 (s, 6H), 3.58 (t, J = 5.2 Hz, 2H), 3.26 (s, 3H). MS (ESI) m/z 391.9 (M + 1).

2-Fluoro-9-[(*S*)-2-methyl-butyl]-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (51). Yield, 68%. R_f 0.41. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.38 (s, 2H), 6.23 (b s, 2H), 4.15 (s, 2H), 3.87–3.73 (m, 2H), 3.81 (s, 3H), 3.79 (s, 6H), 1.95–1.85 (m, 1H), 1.29–1.13 (m, 2H), 0.85 (t, J = 7.3 Hz, 3H), 0.78 (d, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) 159.9, 157.7, 156.4, 156.2, 153.7, 153.5, 153.4, 151.3, 137.2, 131.0, 116.7, 105.4, 60.9, 56.1, 48.9, 35.0, 26.8, 16.6, 11.2. MS (ESI) m/z 404.0 (M + 1).

2-Fluoro-9-(2-isopropoxy-ethyl)-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (52). Yield, 86%. R_f 0.42. ^{1}H NMR ($CDCl_3$, 400 MHz) cdd 6.43 (s, 2H), 5.91 (b s, 2H), 4.29 (s, 2H), 4.14 (t, J = 5.2 Hz, 2H), 3.81 (s, 3H), 3.80 (s, 6H), 3.61 (t, J = 5.2 Hz, 2H), 3.48–3.42 (m, 1H), 1.06 (d, J = 6.1 Hz, 6H). MS (ESI) m/z 420.0 (M + 1).

2-Fluoro-9-but-3-enyl-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (53). Yield, 42%. R_f 0.40. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.41 (s, 2H), 5.86 (b s, 2H), 5.69–5.62 (m, 1H), 5.00 (d, J = 9.6 Hz, 1H), 4.91 (dd, J = 9.6, 1.6 Hz, 1H), 4.16 (s, 2H), 4.04 (t, J = 7.4 Hz, 2H), 3.81 (s, 3H), 3.79 (s, 6H), 2.33–2.27 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.6, 157.5, 156.3, 156.1, 153.9, 152.9, 152.8, 151.0, 137.3, 133.7, 131.3, 118.5, 105.8, 61.2, 56.5, 35.4, 33.8. MS (ESI) m/z 388.1 (M + 1).

2-Fluoro-9-pent-4-enyl-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (54). Yield, 86%. R_f 0.41. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.41 (s, 2H), 5.85 (b s, 2H), 5.73–5.63 (m, 1H), 5.00 (d, J = 3.1 Hz, 1H), 4.96 (s, 1H), 4.29 (s, 2H), 3.96 (t, J = 7.7 Hz, 2H), 3.81 (s, 3H), 3.80 (s, 6H), 2.04–1.99 (m, 2H), 1.67 (m, 2H). MS (ESI) m/z 402.1 (M + 1).

2-Fluoro-9-but-3-ynyl-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (55). Yield, 36%. R_f 0.39. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.43 (s, 2H), 6.02 (b s, 2H), 4.25 (s, 2H), 4.15 (t, J = 7.0 Hz, 2H), 3.81 (s, 3H), 3.80 (s, 6H), 2.53 (dd, J = 6.9, 2.6 Hz, 2H), 2.00 (t, J = 2.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.9, 157.7, 156.3, 156.1, 153.7, 152.9, 152.8, 151.3, 137.3, 130.8, 116.7, 105.5, 79.8, 71.2, 60.9, 56.2, 41.6, 34.9, 19.3. MS (ESI) m/z 386.1 (M + 1).

2-Fluoro-9-pent-3-ynyl-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (56). Yield, 25%. R_f 0.41. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.44 (s, 2H), 5.79 (b s, 2H), 4.25 (s, 2H), 4.10 (t, J = 7.0 Hz, 2H), 3.81 (s, 9H), 2.49–2.45 (m, 2H), 1.71 (s, 3H). MS (ESI) m/z 400.0 (M + 1).

2-Fluoro-9-pent-4-ynyl-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (57). Yield, 76%. R_f 0.41. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.45 (s, 2H), 6.00 (b s, 2H), 4.20 (s, 2H), 4.11 (t, J = 7.2 Hz, 2H), 3.81 (s, 3H), 3.80 (s, 6H), 2.21–2.17 (m, 2H), 2.01 (t, J = 2.5 Hz, 1H), 1.92–1.85 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.9, 157.7, 156.2, 156.0, 153.8, 152.9, 152.8, 151.3, 137.2, 130.7, 116.4, 105.2, 82.4, 69.4, 60.6, 55.9, 41.7, 34.4, 27.5, 15.4. MS (ESI) m/z 400.1 (M + 1).

2-Fluoro-9-cyclobutylmethyl-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (58). Yield, 33%. R_f 0.42. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.40 (s, 2H), 5.82 (b s, 2H), 4.15 (s, 2H), 3.99 (d, J = 4.4 Hz, 2H), 3.81 (s, 3H), 3.79 (s, 6H), 2.71–2.67 (m, 1H), 1.94–1.76 (s, 6H). MS (ESI) m/z 402.1 (M + 1).

2-Fluoro-9-(tetrahydrofuran-2-ylmethyl)-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (59). Yield, 53%. R_f 0.36. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.42 (s, 2H), 6.02 (b s, 2H), 4.34–4.23 (m, 2H), 4.19–4.13 (m, 2H), 3.99–3.95 (m, 1H), 3.86–3.79 (m, 10H), 3.74–3.70 (m, 1H), 2.04–2.00 (m, 1H), 1.86–1.76 (m, 2H), 1.61–1.58 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.8, 157.7, 156.3, 156.1, 153.5, 152.9, 152.8, 151.0, 137.3, 131.4, 116.7, 105.7, 68.3, 60.8, 56.2, 46.7, 34.6, 28.9, 25.7. MS (ESI) m/z 417.9 (M + 1).

2-Methoxy-9-butyl-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (60). To a solution of **48** (20 mg, 0.051 mmol) in MeOH (1 mL) was added a 25% solution of NaOMe in MeOH (1.5 mL). The resulting mixture was heated at 85 °C for 2 h. Subsequent to cooling, the mixture was neutralized with 4 N HCl and then concentrated to dryness. The crude was purified on a silica gel column with hexane/EtOAc/DCM/MeOH at 10:5:5:1 to give a solid (11 mg, 51%). R_f 0.25. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.40 (s, 2H), 5.55 (b s, 2H), 4.11 (s, 2H), 3.92–3.90 (m, 5H), 3.80 (s, 3H), 3.78 (s, 6H), 1.58–1.51 (m, 2H), 1.27–1.19 (m, 2H), 0.85 (t, J = 7.2 Hz, 3H). MS (ESI) m/z 402.0 (M + 1).

2-Ethoxy-9-butyl-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (61). To a solution of **48** (20 mg, 0.051 mmol) in EtOH (2 mL) was added NaOMe (15 mg, 0.28 mmol) and the mixture was refluxed for 2 h. After cooling and neutralization with HCl, the solution was concentrated to dryness. The product (12 mg, 56%) was purified as described above. R_f 0.26. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.41 (s, 2H), 6.15 (b s, 2H), 4.40–4.35 (m, 2H), 4.15 (s, 2H), 3.94 (t, J = 7.5 Hz, 2H), 3.80 (s, 3H), 3.79 (s, 6H), 1.56–1.52 (m, 2H), 1.39 (t, J = 7.1 Hz, 3H), 1.27–1.22 (m, 2H), 0.85 (t, J = 7.3 Hz, 3H). MS (ESI) m/z 415.8 (M + 1).

2-Iodo-9-pent-4-ynyl-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (62). To 3 (200 mg, 0.61 mmol) in toluene (20 mL) and CH_2Cl_2 (4 mL) was added PPh_3 (330 mg, 1.3 mmol), 4-pentyne-1-ol (75 μL, 0.8 mmol) and di-*t*-butyl azodicarboxylate (600 mg, 2.5 mmol). The mixture was stirred at room temperature for 2 h. The product was isolated by column chromatography on a silica gel column eluting with hexane/$CHCl_3$/EtOAc/EtOH at 10:8:4:4 (120 mg solid, 49%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.43 (s, 2H), 5.41 (b s, 2H), 4.69 (b s, 2H), 4.15 (s, 2H), 4.11 (t, J = 7.2 Hz, 2H), 3.81 (s, 3H), 3.80 (s, 6H), 2.18–2.14 (m, 2H), 2.00 (t, J = 2.5 Hz, 1H), 1.88–1.81 (m, 2H). MS (ESI) m/z 396.6 (M + 1). To this (50 mg, 0.13 mmol) was added CH_2I_2 (2.5 mL) and isoamyl nitrite (100 μL, 0.78 mmol) and the resulting solution was heated at 80 °C for 1 h. After cooling, the solution was concentrated and then added to a silica gel column. The product was isolated (40 mg, 61%) using eluent hexane:$CHCl_3$:EtOAc:EtOH at 10:4:4:0.75. ^{1}H NMR ($CDCl_3$, 400 MHz) 6.42 (s, 2H), 6.07 (b s, 2H), 4.21 (s, 2H), 4.11 (t, J = 7.3 Hz, 2H), 3.80 (s, 3H), 3.79 (s, 6H), 2.21–2.16 (m, 2H), 2.01 (t, J = 2.5 Hz, 1H), 1.92–1.85 (m, 2H). MS (ESI) m/z 508.0 (M + 1). R_f (hexane/EtOAc/CH_2Cl_2/MeOH at 10:5:5:1) 0.42.

2-Cyano-9-pent-4-ynyl-8-(3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (63). A solution of **62** (20 mg, 0.04 mmol), $Pd(PPh_3)_4$ (7 mg, 6.3×10^{-3} mmol) and tributyltin cyanide (14 mg, 0.043 mmol) in dry DMF (2.5 mL) was heated at 180 °C for 6 h. Following cooling and removal of the solvent, the product (13 mg, 81%) was isolated by column chromatography (hexane/$CHCl_3$/EtOAc/EtOH at 10:8:4:0.75). ^{1}H NMR ($CDCl_3$, 400 MHz) 6.46 (s, 2H), 5.90 (b s, 2H), 4.25 (s, 2H), 4.20 (t, J = 7.4 Hz, 2H), 3.81 (s, 3H), 3.80 (s, 6H), 2.24–2.20 (m, 2H), 2.01 (t, J = 2.5 Hz, 1H), 1.92–1.85 (m, 2H). MS (ESI) m/z 407.2 (M + 1). R_f (hexane/EtOAc/CH_2Cl_2/MeOH at 10:5:5:1) 0.39.

9-Pent-4-ynyl-8-(3,4,5-trimethyl-benzyl)-2-vinyl-9*H*-purin-6-ylamine (64). A solution of **62** (20 mg, 0.04 mmol), $(MeCN)_2PdCl_2$ (0.5 mg, 2×10^{-3} mmol) and tributylvinyltin (12.5 μL, 0.041 mmol) in dry DMF (3 mL) was heated at 100 °C for 1 h. Following cooling and removal of the solvent, the product (15 mg, 92%) was isolated by column chromatography (hexane/$CHCl_3$/EtOAc/EtOH at 10:8:4:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.73 (dd, J = 17.2, 10.5 Hz, 1H), 6.47 (dd, J = 17.2, 1.9 Hz, 1H), 5.56 (dd, J = 10.5, 1.9 Hz, 1H),

5.42 (b s, 2H), 4.23 (s, 2H), 4.16 (t, $J=7.2$ Hz, 2H), 3.81 (s, 3H), 3.79 (s, 6H), 2.21–2.17 (m, 2H), 2.02 (t, $J=2.5$ Hz, 1H), 1.96–1.89 (m, 2H). MS (ESI) m/z 401.8 (M + 1). R_f (hexane/EtOAc/CH_2Cl_2/MeOH at 10:5:5:1) = 0.39.

9-Butyl-8-(2-chloro-3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (65). To **7** (37 mg, 0.1 mmol) in MeOH (3 mL) was added concentrated HCl (33 μL, 0.4 mmol). The solution was cooled to 0 °C and a 90% aqueous solution of *t*-butyl hydroperoxide (44 μL, 0.4 mmol) was added. The resulting solution was refluxed overnight. Following cooling and removal of the solvent, the product (31 mg solid, 72%) was isolated by column chromatography (elute with hexane/EtOAc/CH_2Cl_2/MeOH at 10:5:5:1.5). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.32 (s, 1H), 6.50 (s, 1H), 5.99 (b s, 2H), 4.31 (s, 2H), 4.03 (t, $J=7.5$ Hz, 2H), 3.90 (s, 3H), 3.84 (s, 3H), 3.67 (s, 3H), 1.62–1.54 (m, 2H), 1.33–1.23 (m, 2H), 0.85 (t, $J=7.3$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.3, 150.1, 142.5, 128.9, 119.5, 108.4, 61.1, 56.1, 42.9, 31.8, 31.4, 19.9, 13.6. MS (ESI) m/z 406.0 (M + 1). R_f (hexane/EtOAc/CH_2Cl_2/MeOH at 10:5:5:1) 0.41.

9-Butyl-8-(2-bromo-3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (66) and 9-butyl-8-(2,6-dibromo-3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (67). To **7** (37 mg, 0.1 mmol) in MeOH (3 mL) was added a 48% aqueous solution of HBr (45 μL, 0.4 mmol). The mixture was cooled to 0 °C, and *t*-butyl hydroperoxide (44 μL, 0.4 mmol) was added dropwise. The resulting solution was stirred for 30 min at 0 °C and then refluxed for an additional h. Following cooling and removal of the solvent, the mixture was separated by column chromatography (elute with hexane/EtOAC/CH_2Cl_2/MeOH at 10:5:5:1.5) to give predominantly monobrominated product (31 mg, 69%) and a trace of dibrominated product (2.3 mg, 4.4%). **66:** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.31 (s, 1H), 6.49 (s, 1H), 5.87 (b s, 2H), 4.35 (s, 2H), 4.03 (t, $J=7.3$ Hz, 2H), 3.89 (s, 3H), 3.84 (s, 3H), 3.67 (s, 3H), 1.62–1.54 (m, 2H), 1.34–1.26 (m, 2H), 0.85 (t, $J=7.4$ Hz, 3H). MS (ESI) m/z 452.0 (M + 1). R_f (hexane/EtOAc/CH_2Cl_2/MeOH at 10:5:5:1) 0.42. **67**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.28 (s, 1H), 4.61 (s, 2H), 4.35 (t, $J=7.4$ Hz, 2H), 3.99 (s, 3H), 3.96 (s, 6H), 1.96–1.84 (m, 2H), 1.53–1.46 (m, 2H), 1.05 (t, $J=7.4$ Hz, 3H). MS (ESI) m/z 529.9 (M + 1). R_f (hexane/EtOAc/CH_2Cl_2/MeOH at 10:5:5:1) 0.44.

9-Butyl-*N8*-(3,4,5-trimethoxy-phenyl)-9*H*-purine-6,8-diamine (68).** A mixture of 8-bromo-9-*N*-butyladenine (50 mg, 0.186 mmol) and trimethoxyaniline (120 mg, 0.65 mmol) was heated at 160 °C for 30 min. Following cooling, the solid was taken up in CH_2Cl_2 (20 mL) and MeOH (5 mL) and any insoluble solids were filtered off. The product (57 mg solid, 83%) was purified on a silica gel column eluting with CH_2Cl_2/EtOAc/MeOH at 7:4:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.20 (s, 1H), 6.82 (s, 2H), 6.48 (b s, 1H), 5.42 (b s, 2H), 4.06 (t, $J=7.3$ Hz, 2H), 3.83 (s, 6H), 3.80 (s, 3H), 1.80–1.73 (m, 2H), 1.40–1.30 (m, 2H), 0.92 (t, $J=7.4$ Hz, 3H). MS (ESI) m/z 373.3 (M + 1).

9-Butyl-8-(3,4,5-trimethoxy-phenoxy)-9*H*-purin-6-ylamine (69). A mixture of trimethoxyphenol (75 mg, 0.4 mmol), *t*-BuOK (34 mg, 0.3 mmol), Cu powder (10 mg, 0.64 mmol) and 8-bromo-9-*N*-butyladenine (26 mg, 0.1 mmol) was heated for 1 h at 140 °C. The product (13 mg solid, 35%) was isolated by column chromatography (hexane/CH_2Cl_2/EtOAc/MeOH at 10:5:5:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.29 (s, 1H), 6.58 (s, 2H), 5.25 (b s, 2H), 4.16 (t, $J=7.3$ Hz, 2H), 3.85 (s, 6H), 3.84 (s, 3H), 1.90–1.82 (m, 2H), 1.46–1.37 (m, 2H), 0.95 (t, $J=7.4$ Hz, 3H). MS (ESI) m/z 374.1 (M + 1).

9-Butyl-8-(3,4,5-trimethoxy-benzyloxy)-9*H*-purin-6-ylamine (70). A solution of benzyl alcohol (250 μL, 2.5 mmol) in 25% NaOMe in MeOH (50 mL) was stirred for 5 min. Following the removal of methanol, 8-bromo-9-*N*-butyladenine (26 mg, 0.096 mmol) and Cu powder (10 mg, 0.16 mmol) was added, and the mixture was heated for 2 min at 180 °C. The product was purified on silica gel column with hexane/CH_2Cl_2/EtOAc/MeOH at 10:5:5:1 (5.5 mg,14%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.25 (s, 1H), 6.69 (s, 2H), 5.45 (s, 2H), 5.36 (b s, 2H), 4.03 (t, $J=7.3$ Hz, 2H), 3.86 (s, 6H), 3.85 (s, 3H), 1.79–1.72 (m, 2H), 1.41–1.23 (m, 2H), 0.98 (t, $J=7.4$ Hz, 3H). MS (ESI) m/z 388.3 (M + 1).

8-(2-Chloro-3,4,5-trimethoxy-benzyl)-9*H*-purin-2,6-diamine (73). To a slurry of 3 (1 g, 3.0 mmol) in MeOH (50 mL) was added concentrated HCl (1.5 mL, 18 mmol). The resulting solution was cooled to 0 °C and a 70% aqueous solution of *t*-butyl hydroperoxide (2. 5 mL, 8 mmol) was slowly added. The mixture was stirred for 30 min at 0 °C and then refluxed for 20 h. The solvent was removed under high vacuum to give clean product (1.09 g, 98%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.62 (b s, 2H), 8.36 (b s, 2H), 4.14 (s, 2H), 3.73 (s, 3H), 3.71 (s, 3H), 3.68 (s, 3H). MS (ESI) m/z 364.8 (M + 1).

8-(2-Chloro-3,4,5-trimethoxy-benzyl)-2-fluoro-9*H*-purin-6-ylamine (74). To a 70% solution of HF in pyridine (6.5 mL) was added (under inert atmosphere) pyridine (13.5 mL), followed by **73** (1 g, 2.7 mmol). The mixture was stirred for 5 min and consequently, *t*-butyl nitrite (450 μL, 3.5 mmol) was slowly added. Stirring continued for another 30 min. The reaction was quenched by addition of $CaCO_3$ (16.5 g) in water (10 mL): MeOH (10 mL) and stirring for 2 h. The solution was concentrated and the resulting slurry was taken up in CH_2Cl_2 (50 mL)/MeOH (50 mL). The insoluble solids were filtered off and washed with CH_2Cl_2/MeOH at 1:1 (2×25 mL). Following solvent removal, the product was purified on a silica gel column eluting with hexane:EtOAc/CH_2Cl_2/MeOH at 10:5:5:1 (370 mg solid, 37%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.9 (s, 1H), 7.61 (b s, 2H), 7.03 (s, 1H), 4.23 (s, 2H), 3.85 (s, 6H), 3.82 (s, 3H). MS (ESI) m/z 367.7 (M + 1).

2-Fluoro-9-pent-4-ynyl-8-(2-chloro-3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (71). (308 mg, 76%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.46 (s, 1H), 6.17 (b s, 2H), 4.31 (s, 2H), 4.12 (t, $J=7.3$ Hz, 2H), 3.91 (s, 3H), 3.86 (s, 3H), 3.72 (s, 3H), 2.19 (dd, $J=6.65$, 2.5 Hz, 2H), 1.97 (t,

$J = 2.5$ Hz, 1H), 1.94–1.87 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.8, 157.7, 156.2, 152.8, 152.4, 150.5, 150.2, 142.6, 128.7, 119.7, 116.8, 108.5, 82.2, 69.8, 61.1, 56.2, 42.1, 31.5, 28.1, 15.7. MS (ESI) m/z 434.1 (M + 1). R_f (hexane/EtOAc/CH_2Cl_2/MeOH at 10:5:5:1) 0.42.

2-Fluoro-9-(2-isopropoxy-ethyl)-8-(2-chloro-3,4,5-trimethoxy-benzyl)-9*H*-purin-6-ylamine (72). ^1H NMR ($CDCl_3$, 400 MHz) δ 6.49 (s, 2H), 6.02 (b s, 2H), 4.40 (s, 2H), 4.22 (t, $J = 5.2$ Hz, 2H), 3.88 (s, 3H), 3.85 (s, 6H), 3.67 (t, $J = 5.2$ Hz, 2H), 3.53–3.45 (m, 1H), 1.06 (d, $J = 6.1$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.7, 157.7, 156.1, 152.7, 152.4, 150.5, 150.2, 142.6, 128.5, 119.7, 108.5, 82.2, 72.5, 66.6, 61.1, 56.4, 43.6, 34.9, 34.3, 22.2. MS (ESI) m/z 454.2 (M + 1). R_f (hexane/EtOAc/CH_2Cl_2/MeOH at 10:5:5:1) 0.43.

Cell culture. The human cancer cell line MCF-7 was obtained from the American Type Culture Collection (Manassas, VA, USA) and maintained in 1:1 mixture of DME:F12 supplemented with 2 mM glutamine, 50 u/mL penicillin, 50 u/mL streptomycin and 5% heat inactivated fetal bovine serum (Gemini Bioproducts) and incubated at 37 °C in 5% CO_2.

Protein assays. Cells were grown to 60–70% confluence and exposed to drugs or DMSO vehicle for the indicated time periods. Lysates were prepared using 50 mM Tris pH 7.4, 2% SDS and 10% glycerol lysis buffer. Protein concentration was determined using the BCA kit (Pierce Chemical Co.), according to the manufacturers instructions. Clarified protein lysates (20–50 μg) were electrophoretically resolved on denaturing SDS-PAGE, transferred to nitrocellulose and probed with the anti-Her2 (C-18) primary antibody (Santa Cruz Biotechnology) and the corresponding HRP-linked secondary antibody. Blots were visualized by autoradiography and the protein quantified using BioRad Gel Doc 1000 software. The IC_{50} was calculated as the drug concentration needed to degrade 50% of the total Her2 protein.

Growth arrest studies

Growth inhibition studies were performed using the sulforhodamine B assay described.[39] Stock cultures were grown in T-175's flask containing 30 mL of DME (HG, F-12, non-essential amino acids, and penicillin and streptomycin), with glutamine, and 10% FBS. Cells were dissociated with 0.05% trypsin and 0.02% EDTA in PBS without calcium and magnesium. Experimental cultures were plated in microtiter plates (Nunc) in 100 μL of growth medium at densities of 1000 MCF-7 cells per well. One column of wells was left without cells to serve as the blank control. Cells were allowed to attach overnight. The following day, an additional 100 μL of growth medium was added to each well. Stock drug or DMSO was dissolved in growth medium at twice the desired initial concentration. Drug or DMSO was serially diluted at a 1:1 ratio in the microtiter plate and added to duplicate wells. After 72 h of growth, the cell number in treated versus control wells was estimated after treatment with 10% trichloroacetic acid and staining with 0.4% sulforhodamine B in 1% acetic acid. The IC_{50} is calculated as the drug concentration that inhibits cell growth by 50% compared with control growth.

Binding studies: solid-phase competition assays. GM was immobilized on Affigel 10 resin (BioRad) as described.[15,31] The GM-beads were washed with TEN buffer (50 mM Tris–HCl pH 7.4, 1 mM EDTA, 1% NP-40) containing protease inhibitors and then blocked for 45 min at 4 °C with 0.5% BSA in TEN buffer. Hsp90 protein from Stressgen (SPP-770) was incubated with or without drugs for 17 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 three washes with 500 μL ice cold TEN each. The GM-beads bound protein was eluted from the solid phase by heating in 65 μL 1×SDS. A 40 μL aliquot was applied to the SDS/PAGE gel and visualized by immunoblotting with anti-hsp90 (Neo-Markers#RB-118) and the corresponding HRP-linked secondary antibody. Blots were visualized by autoradiography and the protein quantified using BioRad Gel Doc 1000 software. The EC_{50} was calculated as the drug concentration needed to compete with the immobilized GM for 50% of the hsp90 protein.

M logP values were calculated using the algorithm implemented in Sybyl 6.7.

Acknowledgements

This work was supported by the Susan G. Komen Foundation (G.C.), NIH/NCI (1U01 CA91178-01) (G.C. and N.R.) and a generous donation by the Taub Foundation.

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