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# Design, synthesis and biological evaluation of substituted pyrrolo[2,3-*d*]pyrimidines as multiple receptor tyrosine kinase inhibitors and antiangiogenic agents

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**Abstract**—Direct and indirect involvement of receptor tyrosine kinases (RTKs) in tumor growth and metastasis makes them ideal targets for anticancer therapy. A paradigm shift from inhibition of single RTK to inhibition of multiple RTKs has been recently demonstrated. We designed and synthesized eight $N^4$-phenylsubstituted-6-(2-phenylethylsubstituted)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamines as homologated series of our previously published RTK inhibitors. We reasoned that increased flexibility of the side chain, which determines potency and selectivity, would improve the spectrum of RTK inhibition. These compounds were synthesized using a bis-electrophilic cyclization to afford substituted pyrrolo[2,3-*d*]pyrimidines followed by chlorination and substitution at the 4-position with various anilines. Five additional compounds of this series were previously reported by Gangjee et al.[1] with activities against IGFR only. Their synthesis, characterization and biological activities against a variety of other RTKs are reported in this study for the first time. The biological evaluation, in whole cell assays, showed several analogs had remarkable inhibitory activity against epithelial growth factor receptor (EGFR), vascular endothelial growth factor receptor-1 (VEGFR-1), platelet-derived growth factor receptor-β (PDGFR-β), the growth of A431 cells in culture, and in the chicken embryo chorioallantoic membrane (CAM) angiogenesis assay. The inhibitory data against the RTKs in this study demonstrate that variation of the 6-ethylaryl substituents as well as the $N^4$-phenyl substituents of these analogs does indeed control both the potency and specificity of inhibitory activity against RTKs. In addition, homologation of the chain length of the 6-substituent from a methylene to an ethyl increases the spectrum of RTK inhibition. New multi-RTK inhibitors (**8**, **12**) and potent inhibitors of angiogenesis (**15**, **19**) were identified with the best compound, $N^4$-(3-trifluromethylphenyl)-6-(2-phenylethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (**15**), with an $IC_{50}$ value of 30 nM in the CAM angiogenesis inhibition assay.


## 1. Introduction

Receptor tyrosine kinases (RTKs) are a subfamily of protein tyrosine kinases, which play key roles in tumor growth, survival, and dissemination. A variety of growth factors particularly vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), platelet derived growth factor (PDGF), and their receptors are overexpressed in several tumors. These growth factors and their receptors are thought to be directly as well as indirectly involved in the growth of tumors and metastases.[2]

Angiogenesis, the formation of new blood vessels from existing vasculature, is essential for both physiological and pathological processes. It is a complex cascade that is tightly regulated by proangiogenic and antiangiogenic factors.[3] VEGF is the predominant stimulator of angiogenesis.[4,5] The expression of VEGF is efficiently controlled under physiologic conditions. However, VEGF is secreted by a large number of tumor cells, leading to angiogenesis that allows the tumor to grow and metastasize. In addition to VEGF, several external factors are also involved in stimulating tumor angiogenesis. Some of the principal proangiogenic regulators include growth factors EGF, PDGF, fibroblast growth factor (FGF), insulin-like growth factor-1 (IGF-1), transforming

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growth factors (TGFα and β), and tumor necrosis factor α (TNF-α) among others.[6] The newly sprouted blood vessels provide an additional supply of nutrients to the tumor to grow beyond a certain size (1–2 $mm^3$). Angiogenesis is a pivotal step in the transition of some solid tumors from a dormant state to a malignant state; it also provides metastatic pathways for solid tumors.[7] In addition, angiogenesis contributes to the development of hematologic malignancies, particularly multiple myeloma, leukemia, and lymphoma. However, the role of angiogenesis has not been clearly defined in hematologic malignancies.[8–10] Angiogenesis has been described as one of the hallmarks of cancer.[11] Thus inhibition of tumor angiogenesis affords attractive targets for the development of novel antitumor agents. Antiangiogenic therapy is targeted to non-tumor cells (endothelial cells) which are expected to have less ability to mutate in order to produce resistance compared with tumor cells. Thus, antiangiogenic agents have afforded a new paradigm for the treatment of cancer.[11]

There has been considerable discussion in the literature regarding the use of RTK inhibitors as monotherapy for cancer or the combination of multiple RTK inhibitors either as single agents[12–15] or in combination with other chemotherapeutic agents.[6–18] The majority of earlier reports underlined the development of targeted therapy against single RTK by small molecules, some of which afforded clinical agents such as gefitinib **1** (specific EGFR inhibitor; approved for limited use for the treatment of non-small cell lung cancer)[19] (Fig. 1) and erlotinib **2** (specific EGFR inhibitor; approved for the treatment of non-small cell lung cancer).[19] However, tumors have redundant signaling pathways for angiogenesis and often develop resistance to agents that target one specific pathway.[20] Recently, crosstalk has been implicated between EGFR and other growth factor receptors involved in tumorigenesis.[6] Hence, a multifaceted approach that targets multiple signaling pathways has been shown to be more effective than the inhibition of a single target.[6,16] The most important consequence of inhibiting multiple RTKs would be to retard tumor resistance by blocking potential 'escape routes.'[21] Since RTKs are present in endothelial cells (VEGFR, PDGFR), tumor cells (FGFR, PDGFR), and pericytes/smooth muscle cells (FGFR, PDGFR), inhibition of more than one RTK does provide synergistic inhibitory effects against solid tumors.[20] Recently, VEGFR-2 and PDGFR-β have been implicated in controlling angiogenesis at two different stages of the angiogenic process, and it has been shown that inhibition of VEGFR-2 and PDGFR-β with two separate inhibitors produces a synergistic effect in early stage as well as late stage pancreatic islet cancer in mouse models by attacking the angiogenic process at two different sites.[17,22]

A flood of reports on the design of multikinase inhibitors have appeared in the past few years,[2,6,17,22–24] some of which have led to clinically approved agents such as imatinib **3** [inhibits Abelson Tyrosine Kinase (Abl), c-kit protein (CD117), and PDGFR; approved for chronic myelogenous leukemia (CML),[25] gastrointestinal stromal tumors (GISTs), and a number of other malignancies], sunitinib **4** [inhibits VEGFR-2, PDGFR-β, c-kit, and FLT3 (FMS-like tyrosine kinase 3); approved for the treatment of renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumor)],[26] sorafenib **5** [inhibits Raf kinase and VEGFR-2, PDGFR-β and c-kit; approved for the treatment of advanced renal cell carcinoma],[27] and lapatinib **6** [inhibits EGFR and ErbB-2 (erythroblastic leukemia viral oncogene homolog 2); approved for advanced metastatic breast cancer in conjunction with the chemotherapy].[28] In 2003, Gangjee et al.[29] demonstrated that fine-tuning of the molecular substitution pattern on a pyrrolo[2,3-*d*]pyrimidine scaffold affords multikinase inhibitors with potencies equivalent to or better than standard agents. Since substitutions on the phenyl ring of the 6-arylmethyl group in **7** dictated both the inhibitory potencies as well as the selectivity against RTKs, it was of interest to determine the effect of side chain homologation of the previously reported compounds of general structure **7**, on the potency and spectrum of RTK inhibitory activity.

## 2. Design of inhibitors

Since the aim of this study was to design multikinase inhibitors, a general RTK pharmacophore model was utilized rather than X-ray crystal structures of specific RTKs. On the basis of this general pharmacophore model depicted in Figure 2, we reasoned that the addition of a second carbon in the bridge at the 6-position of the pyrrolo[2,3-*d*]pyrimidine **7** would afford greater conformational flexibility and allow the phenyl ring to adopt multiple low energy-binding orientations such that it could perhaps bind to multiple RTKs providing multi-RTK inhibitors. Such agents could expand the spectrum of RTK inhibition compared to **7**. Thus we embarked on the homologation of our previously reported RTK inhibitors using the pyrrolo[2,3-*d*]pyrimidine scaffold with a 2-$NH_2$ moiety as in compounds **8–12** (Fig. 4). The 2-$NH_2$ group in our compounds provides a third H-bonding moiety in the Hinge Region of RTKs, as shown in the pharmacophore model in Figure 1, and should increase binding and consequently potency of our scaffold, as compared to the majority of the pyrimidine-containing RTK inhibitors which do not contain the 2-$NH_2$ group.[30] The 4-anilino ring with a 3-bromo substitution has provided potent inhibition of RTKs and is accommodated either in the Hydrophobic Region I, in the Sugar Pocket, or in the Phosphate Binding Region (Fig. 2) depending on the mode of attachment for our proposed pyrrolo[2,3-*d*]pyrimidines.[29] As the first iteration of our previous series of RTK inhibitors,[29] we elected to keep these two substitutions [i.e., the 2-$NH_2$ group and the 4-amino-(3′-bromophenyl)] constant. The purpose being to determine if variations in the phenyl ring of a 6-phenylethyl moiety appended to the pyrrolo[2,3-*d*]pyrimidine scaffold would allow for inhibition of multiple RTKs and also dictate the potency and selectivity as reported for **7**.

Molecular modeling, using the SEARCH option in SYBYL 7.3,[31] of the homologated ethyl side chain

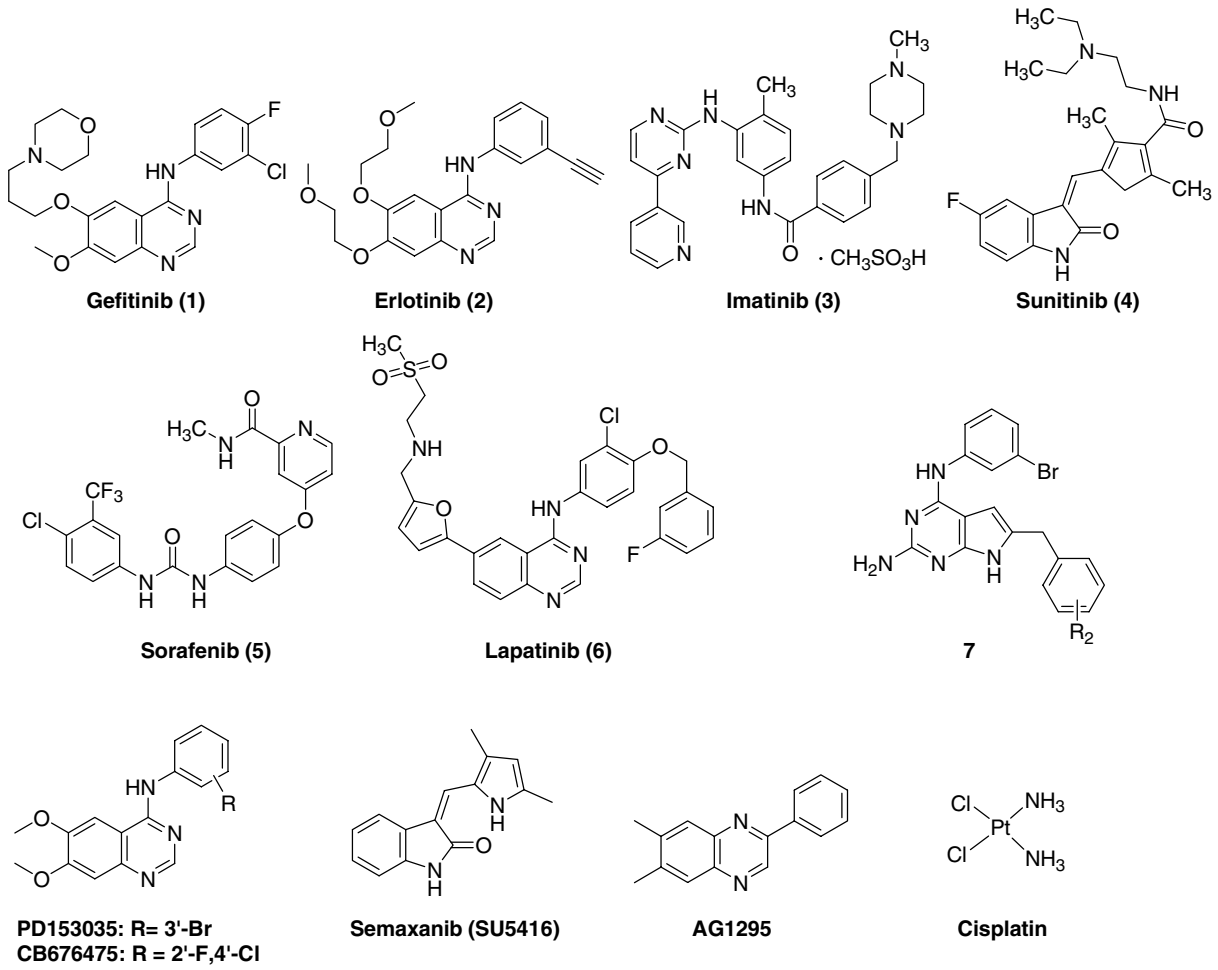



**Figure 1.** Structures of RTK inhibitors.

analog **8** using the five rotatable bonds through 30° increments afforded 97875 conformations (compared to 3174 for **7**). The lowest energy conformation and conformations within 2 kcal/mol generate side chain orientations of the anilino and the 6-phenylethyl side chain that place these side chains in multiple positions that include the Hydrophobic Region 1, the Sugar Pocket, and the Phosphate Binding Region for the 6-phenylethyl moiety when in Mode 1 (Fig. 2). The anilino moiety can be oriented in the Hydrophobic Region 1 in Mode 1 and in the Sugar Pocket in Mode 2. The conformations within 1 kcal/mol of the least energy conformation also show multiple orientations of both side chains on the pyrrolo[2,3-*d*]pyrimidine scaffold. Three low energy conformations of **8** (within 0.5 kcal/mol of the lowest energy conformation) are depicted in Figure 3 and show the wide variations of the orientations of the 4-anilino group (green) and the 6-phenylethyl substituents (yellow). Thus molecular modeling lends credence to the idea that adding an additional carbon in the side chain significantly increases the flexibility and allows for multiple low energy orientations of the 6-side chain phenyl ring to perhaps allow for multiple low energy binding orientations to target different RTKs. Thus we synthesized compounds **8**–**12** which contain a 2-amino group, a 4-meta-bromo aniline and a phenylethyl substitution at the 6-position. Compounds **8**–**11** were previously disclosed without synthetic details, and were found inactive against IGF-1R.[1] The synthesis, characterization, and activities of **8**–**11** against EGFR, VEGFR-1, VEGFR-2, PDGFR-β, A431 cells in culture, and the CAM assay are reported for the first time in this study.

In addition, as proposed in the pharmacophore model in Figure 2, the 4-anilino ring could bind in the Hydrophobic Pocket 1 (Binding mode 1 and Binding mode 3) or in the Sugar Binding Pocket (Binding mode 2). Molecular modeling indicates that the anilino moiety adopts a variety of low energy conformations similar to the 6-substituent. Hence, variations on the 4-anilino ring (**13**–**20**) were also synthesized to probe these binding areas and to determine whether selectivity and/or potency can be influenced by substitutions on the 4-anilino moiety with the 6-phenylethyl substituent constant. Compound **8**, the phenyl unsubstituted analog, was the most potent and this side chain at position-6 was maintained in compounds **13**–**20**. Several substituents on the 4-position that were reported in the literature to provide potent RTK inhibitors[19,32,33] were incorporated in target compounds **13**–**20** . Compound **20** was designed to influence and perhaps improve the cellular activity of the compound as reported for a quinazoline series with similar bicyclic 4-substituents.[34,35]

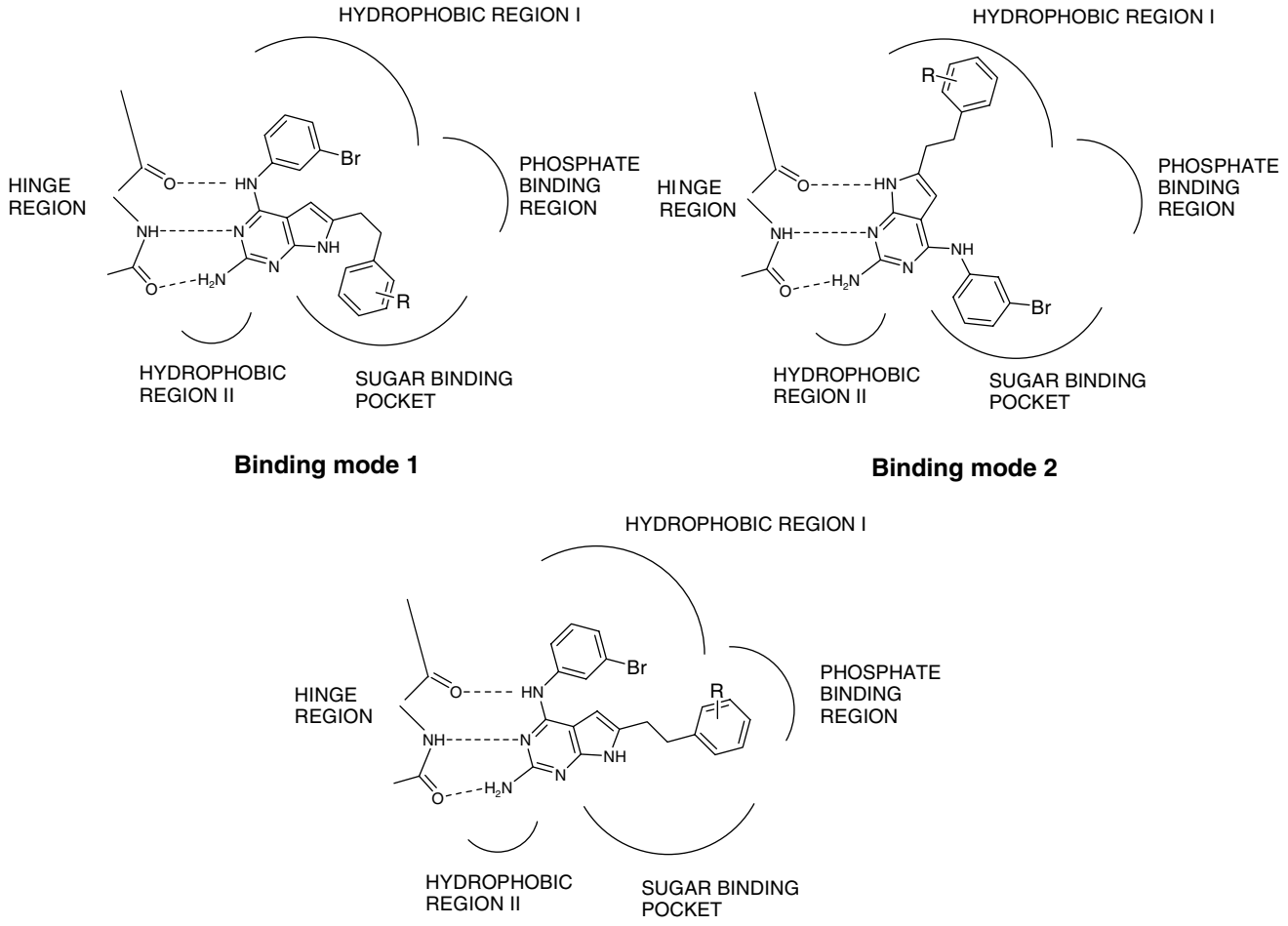



**Figure 2.** General pharmacophore model of pyrrolo[2,3-*d*]pyrimidines.

## 3. Chemistry

The synthesis of compounds **8**–**12** is shown in Scheme 1. Gangjee et al.[36] previously reported **21a**–**e** via the palladium-catalyzed Sonogashira coupling followed by catalytic reduction of the triple bond with 5% Pd on charcoal. Chlorination of **21a**–**e** with phosphorus oxychloride afforded **22a**–**e**. Compounds **22a**–**e** were reacted with 3-bromoaniline **23** in isopropanol at reflux in the presence of two drops of concd HCl for 1–3 h afforded **24a**–**e**. Depivalation of **24a**–**e** with 1 N NaOH in methanol followed by chromatographic purification afforded **8**–**12**.

The synthesis of target compounds **13**–**20** is shown in Scheme 2. 3-Phenylpropanoic acid **25** was converted to 3-phenylpropanoyl chloride **26** with thionyl chloride in benzene at reflux. Compound **26** in diethyl ether was added dropwise to a solution (in diethyl ether) of freshly generated diazomethane[37] (from *N*-methyl-*N*-nitrosourea) in an ice-salt bath; maintaining the temperature between 0 and 5 °C. The resulting yellow solution was warmed to room temperature and stirred for an additional 30 min. The diazo intermediate **27** was treated with 48.5% HBr solution and refluxed to form the α-bromoketone **28**. Stirring **28** with 2,4-diamino-6-hydroxypyrimidine **29** in DMF in the presence of molecular sieves (13×; 16–2.5 mm beads) afforded **30**.[29] The presence of a singlet at $\delta$5.85 in $^1$H NMR corresponding to C5-C*H* of the pyrrolo[2,3-*d*]pyrimidine confirmed the cyclization to **30**. Chlorination of **30** with phosphorous oxychloride at reflux afforded **31**. Nucleophilic displacement of the 4-chloro group in **31** with various substituted anilines gave the target compounds **13**–**20**.

## 4. Results and discussion

RTK inhibitory activity of the compounds **8**–**20** was evaluated using human tumor cells known to express high levels of EGFR, VEGFR-1, VEGFR-2, PDFGR-β, and FGFR1 using a phosphotyrosine ELISA cytoblot.[38,39] Compounds known to inhibit a particular RTK were used as positive controls for these assays. Whole cell assays were used for RTK inhibitory activity since these assays afford more meaningful results for translation to *in vivo* studies. The effect of compounds on cell proliferation was measured using inhibition of the growth of A431 cancer cells known to overexpress EGFR. EGFR has been shown to play a role in the

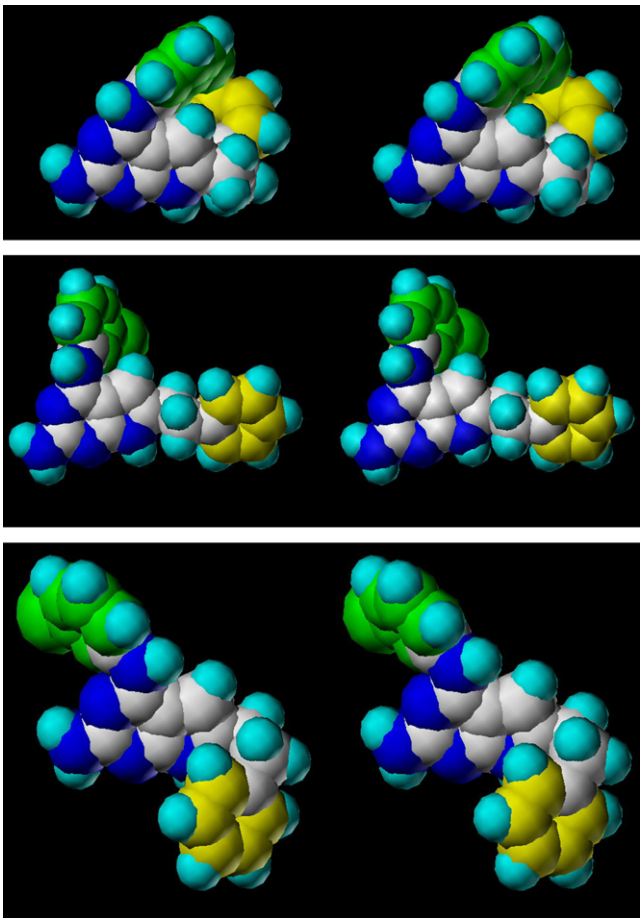


**Figure 3.** Stereoview of low energy conformations of 8 using SYBYL 7.3.[31] 4-Anilino group (green); 6-phenylethyl substituent (yellow). The conformations within 1 kcal/mol of the least energy conformation show multiple orientations of both side chains on the pyrrolo[2,3-*d*]pyrimidine scaffold.

overall survival of the cells.[40] Cell proliferation was assessed using CYQUANT®, a DNA intercalating dye that has been shown to give a linear approximation of cell number.[41] Finally, the effect of selected compounds on blood vessel formation was assessed using the chicken embryo chorioallantoic membrane (CAM) assay, a standard test for angiogenesis.[42] In this assay, purified angiogenic growth factors are placed locally on a vascularized membrane of a developing chicken embryo together with possible inhibitors. Digitized images of the vasculature are taken at 48 h after growth factor administration and the number of vessels per unit area evaluated as a measure of vascular density.

Since the $IC_{50}$ values of RTK inhibitors vary under different assay conditions, we used a standard (control) compound in each of the evaluations. For VEGFR-2 the standard was SU5416; for VEGFR-1 the standard was CB676475 (4-chloro-2-fluoro-phenyl)-(6,7-dimethoxy-quinazolin-4-yl)-amine; for EGFR the standard was PD153035; for PDGFR-β the standard was AG1295; for the cytotoxicity study against the growth of A431 cells in culture the standard was cisplatin. SU5416 was also used as the standard for the antiangiogenic CAM assay.

### 4.1. EGFR inhibition

Compound **8** with an unsubstituted phenyl side chain was the most potent compound and was similar in potency to the standard analog PD153035. Compounds **9**–**12** showed single-digit micromolar inhibition of EGFR, and were about 10- to 20-fold less than the standard PD153035. Electron-donating group ($OCH_3$, compound **9**) in the 4′-position and withdrawing groups (Cl, compound **10**) in the 2′-position on the phenyl ring were detrimental to activity. Both compounds **11** and **12** with 2,3- and 3,4-di substitution with hydrophobic carbon atoms and bulkier side chains also showed a decrease in potency by about 16-fold as compared with the 6-phenyl compound **8**. Thus any substitution of a 4′-$OCH_3$, 2′-Cl, 2,3-, and 3,4-carbon substitution is detrimental to EGFR inhibition. Homologation from one to two carbon chain improved EGFR inhibitory activity for

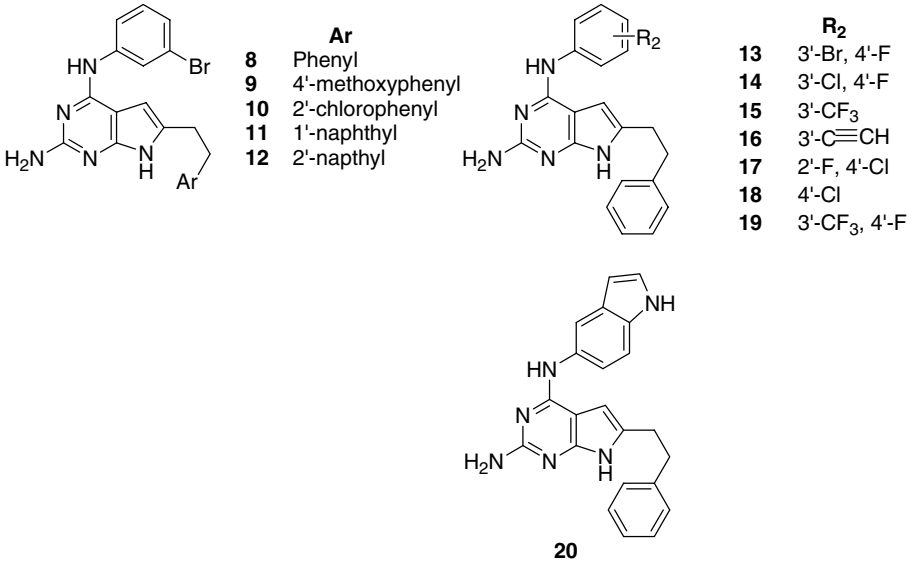
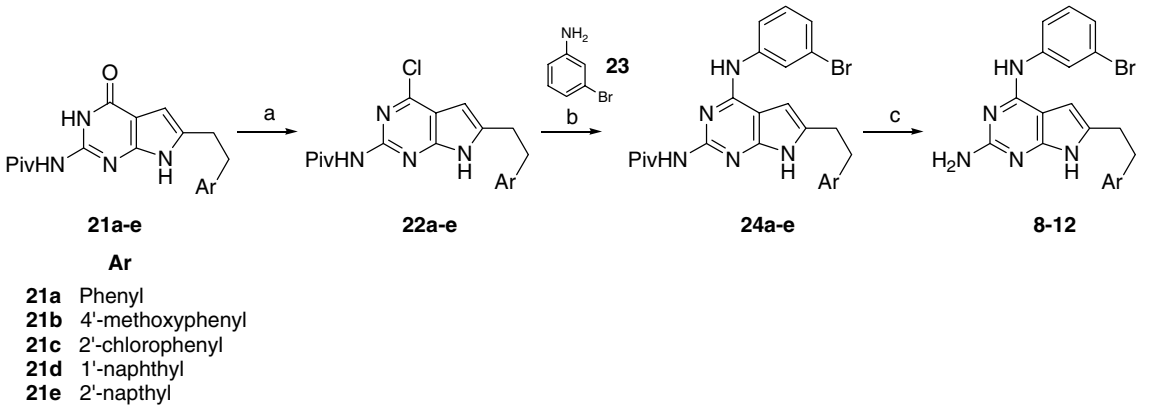
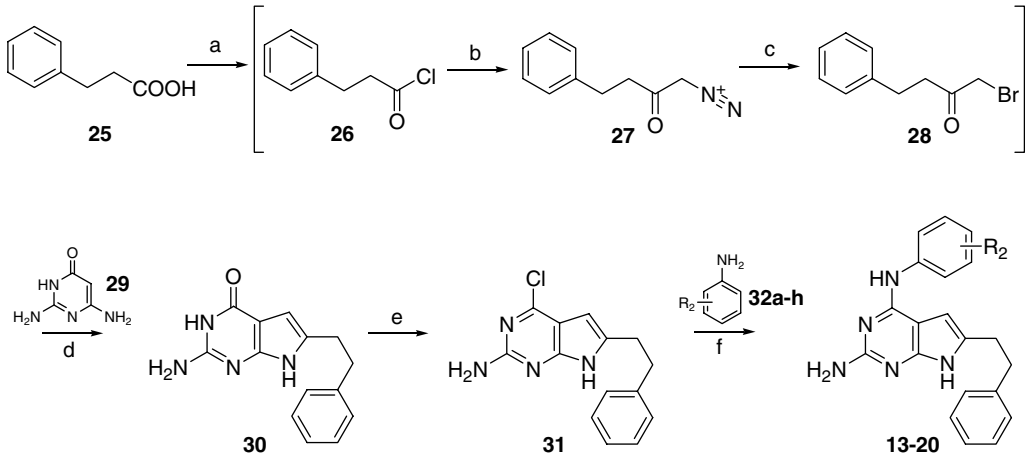



**Figure 4.** Homologated analogs.




**Scheme 1.** Synthesis of **8**–**12**. Reagents and conditions: (a) $POCl_3$, reflux, 2 h; (b) 3-bromoaniline, *i*-PrOH, 2 drops of concd HCl, reflux, 1–3 h; (c) 1 N NaOH, methanol, 70 °C, 10 h.




**Scheme 2.** Synthesis of **13**–**20**. Reagents and conditions: (a) Thionyl chloride, benzene, reflux, 1.5 h; (b) diazomethane, ether, 0–5 °C 15 min; rt 30 min.; (c) 47.5% aq. HBr, ether, reflux, 1 h; (d) dimethyl formamide, MS 13×, rt, 3d; (e) phosphorus oxychloride, *N*,*N*′-dimethylaniline, reflux, 4 h; (f) isopropanol, 2–3 drops concd HCl, reflux 4 h (for **17**, no concd HCl).

**8**, **10**, and **11** and only in **12** was there a decrease in activity compared to the single atom bridge. For the four analogs for which direct comparison is available there was an increase in inhibitory activity in three of the four.

### 4.2. VEGFR-1 inhibition

Compounds **8**–**10** exhibited two-digit micromolar inhibition of VEGFR-1, which is about 2- to 2.5-fold less than the standard CB676475. Compounds **11** and **12**, the naphthyl analogs, showed better inhibitory activity than the standard compound against VEGFR-1. Electron-withdrawing or electron-donating groups did not have much influence on VEGFR-1 inhibition. Against VEGFR-1, the bulk on the phenyl ring affords better inhibition compared to the phenyl unsubstituted analog **8**. In this regard the activities against VEGFR-1 are in contrast to the inhibitory activity against EGFR, where bulk was detrimental to activity. Once again, homologation from one to two carbon atoms in the chain improved inhibitory activity (VEGFR-1) in three (**8**, **11**, and **12**) of the four compounds.

### 4.3. VEGFR-2 inhibition

Compound **12** was the most potent inhibitor of VEGFR-2 and was only 1.5-fold less than the standard SU5416. Compounds **8** and **9** were inactive at 50 μM and compound **10** was about one fourth as potent as SU5416. Homologation improved the activity in **12** but decreased the activity for **10**. Thus no clear-cut trends were apperent for VEGFR-2 inhibition for this series.

### 4.4. PDGFR-β inhibition

Compounds **8**, **11**, and **12** showed 2-digit micromolar inhibition of PDGFR-β. Compound **11** with a 1-naphthyl side chain was the best in this series and was only 1.7-fold less potent than the standard AG1295. Homologation from a one to a two-carbon chain provides for PDGFR-β inhibition in three (**8**, **11**, and **12**) of the five analogs synthesized. The corresponding single atom-bridged analogs were all devoid of PDGFR-β inhibitory activity. These results suggest that increasing the chain length of the bridge from one to two-carbon atoms does provide for additional RTK inhibitory activity. It is

interesting to note that only the 6-phenyl and 6-naphthyl analogs (hydrocarbons) had PDGFR-β activity. The electron-donating 4′-methoxy and electron withdrawing 2′-chloro analogs **9** and **10**, respectively, were not active against PDGFR-β.

### 4.5. A431 cytotoxicity

Compound **11** was equipotent with the standard cisplatin but was more than 2-fold better than SU5416, and was also better than the other standard PD153035. Compound **10** was a reasonably potent inhibitor comparable to PD153035. However, the correlation with EGFR inhibitory potency did not always translate to high A431 inhibitory activity. This may reflect transport and/or other pharmacokinetic differences of analogs between the two cell systems. The EGFR inhibitory activity of **10** and **11** does translate to A431 cytotoxicity. However, for **9** and **12** the EGFR inhibitory activity is similar to **10** and **11** but the A431 inhibition is not. Similar discrepancies have been noted in several literature reports[43–50] and may reflect differences in transport or other factors.

### 4.6. CAM angiogenesis

All the compounds **8**–**12** showed $IC_{50}$s at two-digit micromolar concentration in the CAM assay. The best compound is **10**. However, this is about 118-fold less potent than the standard SU5416, but could reflect differences in transport or other pharmacokinetic differences of the analogs.

Taken together the inhibitory activities against RTKs (Table 1) show that the spectrum of RTK inhibition for three of the four homologated analogs **8**, **11**, and **12** includes PDGFR-β which was distinctly absent for the corresponding 6-methylphenyl compounds[29], and lends support to our hypothesis that homologation and greater conformational flexibility do indeed provide for the inhibition of an additional RTK and depend on the nature of the substitution on the 6-phenyl side chain.

Compounds **13**–**20** were synthesized to determine the effect of the different substitutions on the aniline phenyl ring and were chosen, as mentioned above, on the basis of the activities these substitutions provided against RTK with other scaffolds.[19,32,33] These analogs were also evaluated against RTKs, A431 cells in culture, and in the CAM assay and the results are listed in Table 2.

### 4.7. EGFR inhibition

The best compound in this series against EGFR is **17**, with a 2′-fluoro, 4′-chloroaniline substituents. It is, however, 6.6-fold less active than the standard PD153035. Similarly, **18** with a 4′-chloro substituent on the aniline exhibited the next best inhibitory activity against EGFR. However, both compounds were less potent than the *meta*-Br analog **8**. Thus substitution on the aniline ring affords significant variability in EGFR inhibition. Compounds **13**, **15**, **16**, and **20** were inactive against EGFR.

**Table 1.** $IC_{50}$ values (μM) of kinase inhibition, A431 cytotoxicity, and inhibition of the CAM assay[a]

| Compound | EGFR inhibition | VEGFR-1 inhibition | VEGFR-2 inhibition | PDGFR-β inhibition | A431 cytotoxicity | CAM angiogenesis inhibition |
|---|---|---|---|---|---|---|
| **8** | 0.3 ± 0.042 (1.67 ± 0.31)[a] | 45.1 ± 8.9 (>50) | >50 (>50) | 11.8 ± 3.7 (>50) | 28.6 ± 8.2 (31.8) | 19.5 ± 5.1 (ND) |
| **9** | 2.2 ± 0.09 | 31.8 ± 7.1 | >50 | >50 | >50 | 21.1 ± 3.6 |
| **10** | 3.4 ± 0.41 (4.31 ± 0.68) | 32.7 ± 5.1 (26.8 ± 0.) | 42.3 ± 2.8 (5.58 ± 1.5) | >50 (>50) | 13.2 ± 1.9 (>50) | 10.08 ± 3.7 (1.7 ± 0.3) |
| **11** | 4.8 ± 1.0 (>50) | 11.2 ± 2.3 (19.2) | ND (5.08) | 11 ± 2.1 (>50) | 8.2 ± 1.6 (>50) | 15.2 ± 1.4 (0.053) |
| **12** | 4.7 ± 1.9 (1.24 ± 0.12) | 7.8 ± 0.9 (15.2 ± 0.21) | 16.8 ± 2.1 (>50) | 13.7 ± 2.3 (>50) | >50 (33.2 ± 4.1) | 18.9 ± 5.1 (ND) |
| PD153035 | 0.2 ± 0.04 | | | | 12.6 ± 2.9 | |
| CB676475 | | 17.7 ± 5.5 | | | | |
| SU5416 | | | 10.6 ± 2.7 | | 19.2 ± 1.1 | 0.085 ± 0.0031 |
| AG1295 | | | | 6.2 ± 1.3 | | |
| Cisplatin | | | | | 7.65 ± 1.4 | |

[a] Numbers in parenthesis are the $IC_{50}$ values (μM) of the corresponding 6-methylphenyl analogs from Ref. 21.

**Table 2.** $IC_{50}$ values (μM) of kinase inhibition, A431 cytotoxicity, and inhibition of the CAM assay

| Compound | EGFR inhibition | VEGFR-1 inhibition | VEGFR-2 inhibition | PDGFR-β inhibition | A431 cytotoxicity | CAM angiogenesis inhibition |
|---|---|---|---|---|---|---|
| **8** | 0.3 ± 0.042 | 45.1 ± 8.9 | >50 | 11.8 ± 3.7 | 28.6 ± 8.2 | 19.5 ± 5.1 |
| **13** | >200 | >200 | 198.3 ± 24.3 | >500 | 3.4 ± 0.41 | 6.1 ± 0.83 |
| **14** | 22.8 ± 4.7 | >200 | 132.1 ± 16.7 | >500 | 6.8 ± 0.71 | 5.9 ± 0.7 |
| **15** | >200 | >200 | >200 | >500 | 4.6 ± 0.67 | 0.03 ± 0.004 |
| **16** | >200 | >200 | >200 | 100.4 ± 22.1 | 8.5 ± 1.1 | 1.1 ± 0.07 |
| **17** | 1.32 ± 0.09 | >200 | >200 | >500 | 1.4 ± 0.12 | 8.6 ± 0.76 |
| **18** | 3.18 ± 0.41 | >200 | >200 | >500 | 1.6 ± 0.06 | 3.3 ± 0.82 |
| **19** | 122 ± 30.1 | >200 | >200 | 90.0 ± 17.8 | 9.8 ± 1.0 | 0.12 ± 0.012 |
| **20** | >200 | >200 | 24.9 ± 4.1 | 77.4 ± 8.9 | 5.5 ± 0.92 | 50.6 ± 7.1 |
| PD153035 | 0.2 ± 0.04 | | | | 12.6 ± 2.9 | |
| CB676475 | | 17.7 ± 5.5 | | | | |
| SU5416 | | | 10.6 ± 2.7 | | 19.2 ± 1.1 | 0.085 ± 0.0031 |
| AG1295 | | | | 6.2 ± 1.3 | | |
| Cisplatin | | | | | 7.65 ± 1.4 | |

### 4.8. VEGFR-1 inhibition

All the compounds **13**–**20** in this series were inactive against VEGFR-1 indicating a lack of tolerance for VEGFR-1 inhibitory activity with substituents other than a *meta*-Br.

### 4.9. VEGFR-2 inhibition

Compound **20**, with 5-aminoindole substituents, is the best inhibitor of VEGFR-2 and is only 2.3-fold less potent than the standard SU5416.

### 4.10. PDGFR-β inhibition

All the compounds showed diminished activity as compared to **8**. Only compounds **19** and **20** exhibited 2-digit micromolar $IC_{50}$ values, however, this was more than 12-fold less potent than the standard AG1295 and about 7-fold less potent than **8**. Clearly the 3′-Br substituents (**8**) are the most potent, among the substituents evaluated, for PDGFR-β inhibition. This indicates that, as for the other RTKs, both the aniline and the 6-substituent determines the inhibitory activity.

### 4.11. A431 cytotoxicity

Interestingly, all the compounds **13**–**20** showed good inhibition (single-digit micromolar) as compared to **8** and the standards PD153035 and cisplatin (except **16** and **19**). Compound **17** is the most potent and is 9- and 14-fold more potent than PD153035 and SU5416, respectively, and about 5.5-fold more potent than cisplatin. Compound **18** showed similar activity as **17**. Compounds **17** and **18** were the most potent EGFR inhibitors and this rank order translates into A431 cytotoxicity. None of the other analogs **13**–**16** and **19**–**20** showed any significant EGFR inhibitory activity and yet were reasonably potent against the growth of A431 cells in culture. This could be attributed to differences in the transport and/or inhibition of other targets. As mentioned for compounds **8**–**12** (Table 1), the literature[43–50] contains several reports of similar activities where EGFR inhibitory activity does not translate to A431 cell inhibitory activity.

### 4.12. CAM angiogenesis

Compounds **13**–**19** showed improved inhibition of angiogenesis as compared with **8**. Compound **15** with 3′-trifluoromethylaniline group was the best and was about 2.8 times more potent than the standard SU5416 in the inhibition of angiogenesis. Similarly **19**, with 3′-trifluoromethyl, 4′-fluoroaniline substitution, showed significant antiangiogenic effects. However, for both these compounds, the excellent potency against angiogenesis could not be explained with the limited number of RTKs evaluated. This raises the possibility of alternate antiangiogenic mechanisms for these compounds and is currently under investigation.

## 5. Summary

Thirteen $N^4$-phenylsubstituted-6-(2-phenylethylsubstituted)-7*H*-pyrrolo[2,3-*d*]pyrimidine 2,4-diamines **8**–**20** were designed and synthesized as RTK inhibitors. The biological evaluation showed several analogs had remarkable inhibitory activity against EGFR, VEGFR-1, A431 proliferation, and in the CAM angiogenesis assay. Of the analogs evaluated, 11 compounds (compounds **8** and **11**–**20**) were equipotent or more potent as compared to the standard compounds tested. Eight analogs, **9**, **13**–**18**, and **20**, showed potent cytotoxic effects against the growth of A431 cells in culture and two analogs, **15** and **19**, demonstrated high antiangiogenic activity in the CAM assay. In addition, one analog (compound **8**) showed dual inhibitory activity against EGFR and PDGFR-β; while another analog (compound **12**) inhibits three of the kinases (VEGFR-1, VEGFR-2, and PDGFR-β) evaluated. The inhibitory data against the RTKs in this study demonstrate that variation of the 6-ethylaryl substituents as well as the $N^4$-phenyl substituents of these $N^4$-phenylsubstituted-6-(2-phenylethylsubstituted)-7*H*- pyrrolo[2,3-*d*]pyrimidine-2,4-diamines does indeed control both the potency and specificity of inhibitory activity against RTKs. In addition, homologation of the 6-chain length does increase the spectrum of RTK inhibition to include PDGFR-β that was absent for the unhomologated analogs. Using the analogs described in this study as leads, a

structure-based design and synthesis of further RTK inhibitors are currently in progress and will be the subject of future reports.

## 6. Experimental

All evaporations were carried out in vacuo with a rotary evaporator. Analytical samples were dried in vacuo (0.2 mm Hg) in a CHEM-DRY drying apparatus over $P_2O_5$ at 70 °C. Melting points were determined on a MEL-TEMP II melting point apparatus with FLUKE 51 K/J electronic thermometer and are uncorrected. Nuclear magnetic resonance spectra for proton ($^1$H NMR) were recorded on a Bruker WH-300 (300 MHz) spectrometer. The chemical shift values are expressed in ppm (parts per million) relative to tetramethylsilane as an internal standard: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad singlet. The relative integrals of peak areas agreed with those expected for the assigned structures. Thin-layer chromatography (TLC) was performed on WHATMAN UV254 silica gel plates with a fluorescent indicator, and the spots were visualized under 254 and/or 365 nm illumination. Proportions of solvents used for TLC are by volume. Column chromatography was performed on a 230–400 mesh silica gel purchased from Fisher Scientific. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Element compositions are within 0.4% of the calculated values. Fractional moles of water or organic solvents frequently found in some analytical samples of compounds in this report related to the ones like antifolates,[1,29,51] could not be prevented in spite of 24–48 h of drying in vacuo and were confirmed where possible by their presence in the $^1$H NMR spectra. All solvents and chemicals were purchased from Aldrich Chemical Co. or Fisher Scientific and were used as received.

### 6.1. General procedure for the synthesis of compounds 22a–e

To a round-bottomed flask were added **21a**–**e** suspended in phosphorous oxychloride (20 mL). The reaction was continued at reflux for 4 h. After cooling to room temperature, the solvent was evaporated under reduced pressure and the residue was treated with ice water (20 mL) in ice bath. The pH of the suspension was adjusted to 4 with ammonium hydroxide. The precipitate was filtered, air-dried and then dissolved in chloroform/methanol (1:1, 25 mL). Silica gel (1 g) was added to the solution which was then evaporated to dryness to form a plug. The silica gel plug obtained was loaded onto a silica gel column and eluted with 20% ethyl acetate in hexanes. Fractions corresponding to the product (TLC) were pooled and evaporated to dryness under reduced pressure to afford the product.

**6.1.1 *N*-[4-Chloro-6-(2-phenylethyl)-7*H*-pyrrolo[2,3-d]pyrimidin-2-yl]-2,2-dimethylpropanamide (22a).** Compound **21a** (0.3 g, 1 mmol) using a general procedure described above, gave **22a** (0.25 g; 68%) as a white solid: TLC $R_f$ 0.60 (MeOH/$CHCl_3$, 1:19); mp: 230–231 °C; $^1$H NMR (DMSO-$d_6$): $\delta$ 1.22 (s, 9H, C(C$H_3$)$_3$), 3.02 (s, 4H, C$H_2$C$H_2$), 6.23(s, 1H, C5-C*H*), 7.24 (m, 5H, $C_6H_5$), 9.99 (s, 1H, 2-N*H*Piv, exch), and 12.32 (s, 1H, 7-N*H*, exch). Anal. Calcd for $C_{19}H_{21}N_4OCl$: C, 63.95; H, 5.93; N, 15.70; Cl, 9.93. Found C, 63.84; H, 5.96; N, 15.64; Cl, 10.04.

**6.1.2. *N*-{4-Chloro-6-[2-(4-methoxyphenyl)ethyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl}-2,2-dimethylpropanamide (22b ).** Compound **21b** (0.3 g, 0.80 mmol) using the general procedure described above, afforded **22 b** (0.25 g; 79%) as a white solid: TLC $R_f$ 0.5 (MeOH/$CHCl_3$, 1:19); mp: 204.5–206 °C; $^1$H NMR (DMSO-$d_6$): $\delta$ 1.2 (s, 9H, C(C$H_3$)$_3$), 2.97 (s, 4 H, C$H_2$C$H_2$), 3.7 (s, 3H, OC$H_3$), 6.22 (s, 1 H, C5-C*H*), 6.82 (d, 2H, $C_6H_4$, $J$ = 8.5 Hz), 7.13 (d, 2H, $C_6H_4$, $J$ = 8.5 Hz), 9.97 (s, 1H, 2-N*H*Piv, exch), and 12.29 (s, 1H, 7-N*H*, exch). Anal. Calcd for $C_{20}H_{23}N_4O_2Cl$: C, 62.09; H, 5.99; N, 14.48; Cl, 9.16. Found C, 61.91; H, 6.01; N, 14.54; Cl, 9.33.

**6.1.3. *N*-{4-Chloro-6-[2-(2-chlorophenyl)ethyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl}-2,2-dimethylpropanamide (22c).** Compound **21c** (0.23 g, 0.6 mmol) using the general procedure described above, gave **22c** (0.16 g; 64%) as a pale yellow solid: TLC $R_f$ 0.45 (MeOH/$CHCl_3$, 1:19); mp 195–197 °C; $^1$H NMR (DMSO-$d_6$): $\delta$ 1.22 (s, 9H, C(C$H_3$)$_3$), 3.03 (t, 2H, C$H_2$C$H_2$, $J$ = 7.2 Hz), 3.16 (t, 2H, C$H_2$C$H_2$, $J$ = 7.2 Hz), 6.24 (s, 1H, C5-C*H*), 7.26 (m, 4H, $C_6H_4$), 9.98 (s, 1H, 2-N*H*Piv), and 12.14 (s, 1H, 7-N*H*, exch).

**6.1.4 . *N*-{4-[Chloro]-6-[2-(1-naphthyl)ethyl]-7*H*-pyrrolo-[2,3-*d*]pyrimidin-2-yl}-2,2-dimethylpropanamide (22d).** Compound **21d** (0.5 g, 1.29 mmol) using the general procedure described above, gave **22d** (0.27 g; 52%) as a pale yellow foam: TLC $R_f$ 0.52 (MeOH/$CHCl_3$, 1:19); $^1$H NMR (DMSO-$d_6$): $\delta$ 1.24 (s, 9H, C(C$H_3$)$_3$), 3.1(t, 2H, C$H_2$CH$_2$), 3.52 (t, 2H, CH$_2$C$H_2$), 6.33 (s, 1H, C5-C*H*), 7.36–7.85 (m, 4H, $C_{10}H_7$), 7.95 (d, 1H, $C_{10}H_7$, $J$ = 9 Hz), 8.24 (d, 1H, $C_{10}H_7$, $J$ = 9 Hz), 10.23 (s, 1H, 2-N*H*Piv), and 12.44 (s, 1H, 7-N*H*, exch).

**6.1.5. *N*-{4-[Chloro]-6-[2-(2-naphthyl)ethyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl}-2,2-dimethylpropanamide (22e).** Compound **21e** (0.12 g, 0.31 mmol) using the general procedure described above, gave **22 d** (0.08 g; 64%) as a pale yellow solid: TLC $R_f$ 0.45 (MeOH/$CHCl_3$, 1:19); mp 217.5–220 °C; $^1$H NMR (DMSO-$d_6$): $\delta$ 1.2 (s, 9H, C(C$H_3$)$_3$), 3.22 (m, 4H, C$H_2$CH$_2$), 6.27 (s, 1H, C5-C*H*), 7.47 (m, 3H, $C_{10}H_7$), 7.5 (s, 1H, $C_{10}H_7$), 7.85 (s, 1H, $C_{10}H_7$), 9.99 (s, 1H, 2-N*H*Piv), and 12.36 (s, 1H, 7-N*H*, exch). Anal. Calcd for $C_{23}H_{23}N_4OCl \cdot 0.2\ H_2O$: C, 67.29; H, 5.75; N, 13.65; Cl, 8.64. Found C, 67.22; H, 5.65; N, 13.38; Cl, 8.92.

### 6.2. General procedure for the synthesis of compounds 24a–e

To a round-bottomed flask were added **22a**–**e** and 3-bromoaniline **23** in isopropanol (10 mL), followed by the addition of two drops of concd HCl. The reaction was continued at reflux for 1–3 h at the end of which the solvent was evaporated under reduced pressure. The

residue was dissolved in methanol (10 mL), and silica gel (10 g) was added to the solution which was then evaporated to dryness to form a plug. The silica gel plug obtained was loaded onto a silica gel column and eluted with 1.5% methanol in chloroform. Fractions corresponding to the product (TLC) were pooled and evaporated to dryness under reduced pressure to afford the product.

**6.2.1. *N*-{4-[(3-Bromophenyl)amino]-6-(2-phenylethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl}-2,2-dimethylpropanamide (24a).** Reaction of **22a** (0.07 g, 0.19 mmol) and 3-bromoaniline **23** (0.13 g, 0.76 mmol), using a general procedure described above, gave **24a** (0.1 g; 100%) as a brown solid: TLC $R_f$ 0.40 (MeOH/$CHCl_3$, 1:19); mp: 93–95 °C; $^1$H NMR (DMSO-$d_6$): $\delta$ 1.25 (s, 9H, C($CH_3$)$_3$), 2.99–3.15 (m, 4H, $CH_2CH_2$), 6.45(s, 1H, C5-C*H*), 7.08–7.44 (m, 7 H, $C_6H_5$ and $C_6H_4$), 8.02 (d, 1H, $C_6H_4$), 8.51 (s, 1H, $C_6H_4$), 9.31 (s, 1H, 2-N*H*Piv or 4-N*H*, exch), 9.42 (s, 1H, 2-N*H*Piv or 4-N*H*, exch), and 11.55 (s, 1H, 7-N*H*, exch). HRMS (EI) calcd for $C_{25}H_{26}N_5OBr$: 491.1320, found 491.1301.

**6.2.2. *N*-{4-[(3-Bromophenyl)amino]-6-[2-(4-methoxyphenyl)ethyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl}-2,2-dimethylpropanamide (24b).** Reaction of **22 b** (0.13 g, 0.32 mmol) and 3-bromoaniline **23** (0.22 g, 1.3 mmol), using the general procedure described above, afforded **24 b** (0.16 g; 92%) as a pale yellow solid: TLC $R_f$ 0.30 (MeOH/$CHCl_3$, 1:19); mp: 97.5–99.5 °C; $^1$H NMR (DMSO-$d_6$): $\delta$ 1.25 (s, 9H, C($CH_3$)$_3$), 2.94 (m, 4H, $CH_2CH_2$), 3.70 (s, 3H, O$CH_3$), 6.42 (s, 1H, C5-C*H*), 6.82–7.25 (m, 6H, $C_6H_4$ and $C_6H_4$), 8.04 (d, 1H, $C_6H_4$), 8.50 (s, 1H, $C_6H_4$), 9.29 (s, 1H, 2-N*H*Piv or 4-N*H*, exch), 9.39 (s, 1H, 2-N*H*Piv or 4-N*H*, exch), and 11.49 (s, 1H, 7-N*H*, exch). HRMS (EI) calcd for $C_{26}H_{28}N_5O_2Br$: 521.1426, found 521.1431.

**6.2.3. *N*-{4-[(3-Bromophenyl)amino]-6-[2-(2-chlorophenyl)ethyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl}-2,2-dimethylpropanamide (24c).** Reaction of **22c** (0.06 g, 0.15 mmol) and 3-bromoaniline **23** (0.10 g, 0.60 mmol), using the general procedure described above, gave **24 c** (0.07 g; 95%) as a yellow foam: TLC $R_f$ 0.46 (MeOH/$CHCl_3$, 1:19); $^1$H NMR (DMSO-$d_6$): $\delta$ 1.25 (s, 9H, C($CH_3$)$_3$), 2.99–3.14 (m, 4H, $CH_2CH_2$), 6.45 (s, 1H, C5-C*H*), 7.09–7.45 (m, 6H, $C_6H_4$ and $C_6H_4$), 8.02 (d, 1H, $C_6H_4$), 8.50 (s, 1H, $C_6H_4$), 9.29 (s, 1H, 2-N*H*Piv or 4-N*H*, exch), 9.39 (s, 1H, 2-N*H*Piv or 4-N*H*, exch), and 11.54 (s, 1H, 7-N*H*, exch). HRMS (EI) calcd for $C_{25}H_{25}N_5OBrCl$: 525.0931, found 525.0917.

**6.2.4. *N*-{4-[(3-Bromophenyl)amino]-6-[2-(1-naphthyl)ethyl]-7*H*-pyrrolo[2,3- *d*]pyrimidin-2-yl}-2,2-dimethylpropanamide (24d).** Reaction of **22d** (0.11 g, 0.27 mmol) and 3-bromoaniline **23** (0.19 g, 1.08 mmol), using the general procedure described above, gave **24 d** (0.13 g; 90%) as a pale yellow foam: TLC $R_f$ 0.42 (MeOH/$CHCl_3$, 1:19); $^1$H NMR (DMSO-$d_6$): $\delta$ 1.25 (s, 9H, C($CH_3$)$_3$), 3.07 (t, 2H, J = 9.0 Hz, $CH_2CH_2$), 3.48 (t, 2H, J = 9.0 Hz, $CH_2CH_2$), 6.47 (s, 1H, C5-C*H*), 7.11–8.38 (m, 10H, $C_{10}H_7$ and $C_6H_4$), 8.50 (s, 1H, $C_6H_4$), 9.29 (s, 1H, 2-N*H*Piv or 4-N*H*, exch), 9.39 (s, 1H, 2-N*H*Piv or 3-N*H*, exch), and 11.63 (s, 1H, 7-N*H*, exch). HRMS (EI) calcd for $C_{29}H_{28}N_5OBr$: 541.1477, found 541.1477.

**6.2.5. *N*-{4-[(3-Bromophenyl)amino]-6-[2-(2-naphthyl)ethyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl}-2,2-dimethylpropanamide (24e).** Reaction of **22e** (0.12 g, 0.28 mmol) and 3-bromoaniline **23** (0.19 g, 1.12 mmol), using the general procedure described above, gave **24e** (0.08 g; 53%) as a yellow foam: TLC $R_f$ 0.43 (MeOH/$CHCl_3$, 1:19); $^1$H NMR (DMSO-$d_6$): $\delta$ 1.25 (s, 9H, C($CH_3$)$_3$), 3.16 (m, 4H, $CH_2CH_2$), 6.45 (s,1H, C5-C*H*), 7.08–8.00 (m, 9H, $C_{10}H_7$ and $C_6H_4$), 8.02 (d, 1H, $C_6H_4$), 8.49 (s, 1H, $C_6H_4$), 9.31 (s, 1H, 2-N*H*Piv or 4-N*H*, exch), 9.41 (s, 1H, 2-N*H*Piv or 4-N*H*, exch), and 11.56 (s, 1H, 7-N*H*, exch). HRMS (EI) calcd for $C_{29}H_{28}N_5OBr$: 541.1477, found 541.1491.

### 6.3. General procedure for the synthesis of compounds 8–12

Into a round-bottomed flask was added **24a**–**e** in methanol (10 mL), followed by the addition of 1 N NaOH (2 mL). The reaction was completed after heating at 70 °C for 10 h. The solvent was evaporated under reduced pressure. The residue was dissolved in methanol (10 mL), and silica gel (1 g) was added to the solution which was then evaporated to dryness to form a plug. The silica gel plug obtained was loaded onto a silica gel column and eluted with 1.5% methanol in chloroform. Fractions corresponding to the product (TLC) were pooled and evaporated to dryness under reduced pressure to afford the product.

**6.3.1. $N^4$-(3-Bromophenyl)-6-(2-phenylethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (8).** Using the general procedure described above, reaction of **24a** (0.08 g, 0.19 mmol) afforded **8** (0.04 g; 53%) as a pale yellow solid: TLC $R_f$ 0.20 (MeOH/$CHCl_3$, 1:19); mp: 185–187 °C; $^1$H NMR (DMSO-$d_6$): $\delta$ 2.88–3.07 (m, 4H, $CH_2CH_2$), 5.74 (br, 2H, 2-N$H_2$, exch), 6.27 (s, 1H, C5-C*H*), 7.07–8.13 (m, 9H, $C_6H_5$ and $C_6H_4$), 8.91 (s, 1H, 4-N*H*, exch), and 10.92 (br, 1H, 7-N*H*, exch). HRMS (EI) calcd for $C_{20}H_{18}N_5Br$ 407.0746, found 407.0732.

**6.3.2 $N^4$-(3-Bromophenyl)-6-[2-(4-methoxyphenyl)ethyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (9).** Using the general procedure described above, reaction of **24b** (0.12 g, 0.23 mmol) afforded **9** (0.04 g; 35%) as a off-white solid: TLC $R_f$ 0.21 (MeOH/$CHCl_3$, 1:19); mp: 196.5–198.5 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.86 (m, 4H, $CH_2CH_2$), 3.70 (s, 3H, O$CH_3$), 5.79 (br, 2H, 2-N$H_2$, exch), 6.23 (s, 1H, C5-C*H*), 6.82–7.24 (m, 6H, $C_6H_4$ and $C_6H_4$), 8.00 (d, 1H, $C_6H_4$), 8.12 (s, 1H, $C_6H_4$), 8.95 (s, 1H, 4-N*H*, exch), 10.91 (br, 1H, 7-N*H*, exch). HRMS (EI) calcd for $C_{21}H_{20}N_5BrO$ 437.0851, found 437.0837.

**6.3.3. $N^4$-(3-Bromophenyl)-6-[2-(2-chlorophenyl)ethyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (10).** Using the general procedure described above, reaction of **24c** (0.06 g, 0.11 mmol) afforded **10** (0.03 g; 56%) as an orange solid: TLC $R_f$ 0.17 (MeOH/$CHCl_3$, 1:19); mp: 178.5–180.5 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.88–3.07

(m, 4H, $CH_2CH_2$), 5.79 (br, 2H, 2-N$H_2$, exch), 6.26 (s, 1H, C5-C*H*), 7.07–7.42 (m, 6H, $C_6H_4$ and $C_6H_4$), 8.01 (d, 1 H $C_6H_4$), 8.12 (s, 1H, $C_6H_4$), 8.94 (s, 1H, 4-N*H*, exch), 10.95 (br, 1H, 7-N*H*, exch). HRMS (EI) calcd for $C_{20}H_{17}N_5ClBr$ 441.0356, found 441.0356.

**6.3.4. $N^4$-(3-Bromophenyl)-6-[2-(1-naphthyl)ethyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (11).** Using the general procedure described above, reaction of **24d** (0.10 g, 0.18 mmol) afforded **11** (0.02 g; 18%) as a pale yellow solid: TLC $R_f$ 0.23 (MeOH/$CHCl_3$, 1:19); mp: 120–122 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.98–3.15 (m, 4H, $CH_2CH_2$), 5.92 (br, 2H, 2-N$H_2$, exch), 6.31 (s, 1H, C5-C*H*), 7.10–8.20 (m, 11H, $C_{10}H_7$ and $C_6H_4$), 9.01 (s, 1H, 4-N*H*, exch), and 11.12 (br, 1H, 7-N*H*, exch). HRMS (EI) calcd for $C_{24}H_{20}N_5Br$ 457.0902, found 457.0881.

**6.3.5. $N^4$-(3-Bromophenyl)-6-[2-(2-naphthyl)ethyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine ( 12).** Using the general procedure described above, reaction of **24e** (0.06 g, 0.11 mmol) afforded **12** (0.04 g; 75%) as a yellow solid: TLC $R_f$ 0.22 (MeOH/$CHCl_3$, 1:19); mp: 97.5–99.5 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.97–3.14 (m, 4H, $CH_2CH_2$), 5.71 (br, 2H, 2-N$H_2$, exch), 6.24 (s, 1H, C5-C*H*), 7.07–8.11 (m, 11H, $C_{10}H_7$ and $C_6H_4$), 8.88 (s, 1H, 4-N*H*, exch), and 10.92 (br, 1H, 7-N*H*, exch). HRMS (EI) calcd for $C_{24}H_{20}N_5Br$ 457.0902, found 457.0885.

### 6.4. Synthesis of 2-amino-6-(2-phenylethyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (30)

3-Phenyl propanoic acid **25** (2 g, 13.3 mmol) in dry benzene (10 mL) was reacted with thionyl chloride (10 mL, excess) at reflux for 1 h. Evaporation of the reaction mixture, followed by washings with toluene (thrice, to remove excess thionyl chloride) gave 3-propanoyl chloride **26** (crude; 2 g). Compound **26** was dissolved in anhydrous ether (15 mL) and added dropwise with stirring to a solution of diazomethane in ether (50 mL; about 30 mmol, prepared from 50 mmol of *N*-methyl-*N*-nitroso urea)[37] maintaining the temperature between 0 and 5 °C. The resulting yellow solution was warmed to room temperature, stirred for 1 h, then 47.5% aqueous HBr (15 mL) was added dropwise to the solution and the resulting yellow mixture was heated at 70–80 °C (oil bath) for 1 h. The reaction was cooled to room temperature and the ether layer was separated, washed with water (25 mL × 1), saturated $NaHCO_3$ aqueous solution (25 mL × 1), and brine (25 mL × 1) and dried (anhydrous $Na_2SO_4$). The solution was evaporated to afford 1-bromo-4-phenylbutan-2-one **28** (2.43 g; crude) as a light yellow liquid, which was used directly in the next step without purification. Compound **28** (2.43 g, 10.7 mmol) was placed in a 100-mL flask and an equivalent amount of 2,4-diamino-6-hydroxy-pyrimidine **29** (1.35 g, 10.7 mmol), molecular sieves (13×; 16–2.5 mm beads; 100 mg), and anhydrous DMF (15 mL) was added. The slurry was stirred at room temperature for 3 days. The color of the slurry changed from yellow to orange on the second day. After 3 days, silica gel (5 g) was added to the reaction mixture and the solvent was evaporated in vacuo to afford a dry plug. This plug was placed on the top of silica gel column (20 cm × 3 cm) and eluted with chloroform/methanol (a gradient elution, 2% methanol in chloroform, and 5% methanol in chloroform). Fractions containing the product (TLC) were pooled and evaporated to obtain a light pink residue, which was suspended in acetone (10 mL) and filtered to afford the pure 2-amino-6-(2-phenylethyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one **30** (1.22 g, 45%) as a light pink solid. TLC $R_f$ 0.56 (MeOH/$CHCl_3$, 1:5); mp: 145°C; $^1$HNMR (DMSO-$d_6$): $\delta$ 2.77 (t, 2H, $CH_2$), 2.88 (t, 2H, $CH_2$), 5.85 (s, 1H, C5-C*H*), 5.96(s, 2H, N$H_2$, exch), 7.22 (m, 5H, $C_6H_5$), 10.12( s, 1H, N*H*, exch), and 10.87( s, 1H, N*H*, exch). Anal. Calcd for $C_{14}H_{14}N_4O{\cdot}0.2741\ C_3H_6O$: C, 65.88; H, 5.83; N, 20.73. Found C, 66.23; H, 5.86; N, 20.67.

### 6.5. Synthesis of 4-chloro-6-(2-phenylethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (31)

A mixture of **30** (2.54 g; 10 mmol) and *N*,*N*-dimethylaniline (0.13 ml, 1 mmol) was kept at reflux with freshly distilled phosphorous oxychloride (25 mL, excess) for 4 h. At the end of the reaction (monitored by TLC), the solvent was evaporated under reduced pressure. The resulting brown residue was cooled in ice-bath. Crushed ice (10 g) was added to the residue followed by dropwise addition of ammonium hydroxide to adjust the pH to 7–8. The resulting olive green sticky residue was extracted with chloroform (50 mL × 3). The extracts were dried (anhydrous $MgSO_4$), evaporated under reduced pressure followed by addition of silica gel (5 g) to make a silica gel plug which was placed on top of a silica gel column (15 cm × 3 cm) and eluted with 2% methanol in chloroform. Fractions containing the product were evaporated to obtain **31** (0.9 g; 33%) as a dull white solid. TLC $R_f$ 0.76 (MeOH/$CHCl_3$, 1:5); mp 212.5–213.5 °C; $^1$HNMR (DMSO-$d_6$): $\delta$ 2.91 (t, 2H, $CH_2$), 2.94 (t, 2H, $CH_2$), 5.95 (s, 1H, C5-C*H*), 6.38 (br, 2H, N$H_2$, exch), 7.22–7.9 (m, 5H, $C_6H_5$), 11.41(br, 1H, 7-N*H*). Anal. Calcd for $C_{14}H_{13}N_4Cl{\cdot}0.10H_2O$: C, 61.25; H, 4.85; N, 20.41; Cl, 12.91. Found: C, 61.05; H, 4.79; N, 20.25; Cl, 12.7.

### 6.6. General method for synthesis of $N^4$-phenylsubstituted-6-(2-phenylethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamines (13–20)

Into a 50-mL round-bottomed flask was added 4-chloro-6-(2-phenylethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine **31**, substituted aniline, and isopropanol (10 mL) followed by 2–3 drops of concd HCl. The mixture was heated at reflux for 4 h. At the end of the reaction, mixture was cooled to room temperature and pH was adjusted to 11–12 using ammonium hydroxide followed by evaporation in vacuo. The residue was dissolved in methanol and silica gel (0.5 g) was added followed by evaporation in vacuo to afford a silica gel plug, which was loaded on top of silica gel column (10 cm × 1 cm; pre-treated with 1% ammonia–methanol in chloroform). Column was eluted with 2% methanol in chloroform. Fractions containing the product were pooled and evaporated under reduced pressure to afford a residue, which

was analytically pure (**13**, **15**–**17**). Compounds that showed trace impurities were washed with acetone and hexanes (**14**); triturated with diethyl ether (**18**); recrystallized from hexanes-ethyl acetate (**19**) or from chloroform (**20**) to afford analytically pure $N^4$-phenylsubstituted-6-(2-phenylethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamines.

**6.6.1. $N^4$-(3-bromo-4-fluorophenyl)-6-(2-phenylethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (13).** Using the general procedure described above, reaction of **31** (0.1 g, 0.36 mmol) and 3-bromo-4-fluoroaniline **32a** (0.102 g, 0.54 mmol) afforded **13** (0.095 g, 61%) as an off-white solid. TLC $R_f$ 0.73 (MeOH/$CHCl_3$, 1:5); mp: 222–224 °C. $^1$HNMR (DMSO-$d_6$): $\delta$ 2.89–3.00 (m, 4H, $CH_2CH_2$), 5.70 (br,2H, 2-N$H_2$, exch), 6.20 (s, 1H, C5-C*H*), 7.17–7.30 (m, 5H, $C_6H_5$), 7.96–8.26 (m, 3H, $C_6H_3$), 8.87 (br, 1H, 4-N*H*, exch), 10.86 (br, 1H, 7-N*H*, exch). Anal. Calcd for $C_{20}$ $H_{17}N_5BrF{\cdot}0.2H_2O$: C, 55.88; H, 4.08; N, 16.29; Br, 18.59; F, 4.42. Found: C, 55.68; H, 3.91; N, 16.08; Br, 18.21; F, 4.38.

**6.6.2. $N^4$-(3-Chloro-4-fluorophenyl)-6-(2-phenylethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (14).** Using the general procedure described above, reaction of **31** (0.1 g, 0.36 mmol) and 3-chloro-4-fluoroaniline **32 b** (0.078 g, 0.54 mmol) afforded a dark brown residue which was warmed with acetone (1 mL) and filtered followed by washing with hexanes (10 mL) to afford **14** (0.107 g, 80%) as light brown solid. TLC $R_f$ 0.606 (MeOH/$CHCl_3$, 1:5); mp: 214.5–216.5 °C. $^1$HNMR (DMSO-$d_6$): $\delta$ 2.89 (t, 2H, $CH_2$), 2.93 (t, 2H, $CH_2$), 5.80 (br, 2H, 2-N$H_2$, exch), 6.21(s, 1H, C5-C*H*), 7.17–8.23 (m, 8H, $C_6H_5$ & $C_6H_3$), 8.96 (br, 1H, 4-N*H*, exch), 10.91(br, 1H, 7-N*H*, exch). Anal. Calcd for $C_{20}H_{17}N_5ClF{\cdot}0.1299$ $C_6H_{14}$: C, 63.50; H, 4.82; N, 17.81; Cl, 9.02; F, 4.83. Found: C, 63.18; H, 4.66; N, 17.48; Cl, 8.88; F, 4.54.

**6.6.3. $N^4$-(3-Trifluromethylphenyl)-6-(2-phenylethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (15).** Using the general procedure described above reaction of **31** (0.06 g, 0.21 mmol) and 3-trifluoromethylaniline **32c** (0.051 g, 0.32 mmol) afforded **15** (0.076 g, 89%) as a light brown solid. TLC $R_f$ 0.59 (MeOH/$CHCl_3$, 1:5); mp: 189–192 °C. $^1$HNMR (DMSO-$d_6$): $\delta$ 2.93 (t, 2H, $CH_2$), 2.98 (t, 2H, $CH_2$), 5.72 (br, 2H, 2-N$H_2$, exch), 6.24 (s, 1H, C5-C*H*), 7.24–8.44 (m, 9H, $C_6H_5$& $C_6H_4$), 9.08 (s, 1H, 4-N*H*, exch), and 10.90(s, 1H, 7-N*H*, exch). Anal. Calcd for $C_{21}H_{18}N_5F_3$: C, 63.47; H, 4.56; N, 17.62; F, 14.34. Found: C, 63.22; H, 4.68; N, 17.43; F, 14.41.

**6.6.4. $N^4$-(3-Ethynylphenyl)-6-(2-phenylethyl)-7*H*-pyrrolo[2,3-*d* ]pyrimidine-2,4-diamine (16).** Using the general procedure described above, reaction of **31** (0.08 g, 0.29 mmol) and 3-ethynylaniline **32d** (0.052 g, 0.43 mmol) afforded **16** (0.093 g, 90%) as a lustrous light brown solid. TLC $R_f$ 0.566 (MeOH/$CHCl_3$, 1:5); mp: 163–167 °C. $^1$HNMR (DMSO-$d_6$): $\delta$ 2.89 (t, 2H, $CH_2$), 2.93 (t, 2H, $CH_2$), 4.12 (s, 1H, $C_2H$), 5.69 (br, 2H, 2-N$H_2$, exch), 6.23 (s, 1H, C5-C*H*), 7.03–8.09 (m, 9H, $C_6$ $H_5$& $C_6H_4$), 8.83 (s, 1H, 4-N*H*, exch), and 10.86 (s, 1H, 7-N*H*, exch). Anal. Calcd for $C_{22}H_{19}N_5{\cdot}0.$ $4H_2O$: C, 73.27; H, 5.53; N, 19.42. Found C, 73.63; H, 5.77; N, 19.04.

**6.6.5. $N^4$-(4-chloro-2-fluorophenyl)-6-(2-phenylethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (17).** Using the general procedure described above, reaction of 31 (0.08 g, 0.29 mmol) and 4-chloro-2-fluoroaniline **32 e** (0.064 g, 0.43 mmol) gave **17** (0.095 g, 85%) as light brown solid. TLC $R_f$ 0.66 (MeOH/$CHCl_3$, 1:5); mp: 160–164 °C. $^1$HNMR (DMSO-$d_6$): $\delta$ 2.853–2.869 (t, 2H, $CH_2$), 2.91–2.938 (t, 2H, $CH_2$), 5.6 (br,2H, 2-N$H_2$, exch), 6.119 (s, 1H, C5-C*H*), 7.176–7.303 (m, 5H, $C_6$ $H_5$), 7.422–7.96 (m, 3H, $C_6$ $H_3$), 8.569 (br, 1H, 4-N*H*, exch), 10.855 (br, 1H, 7-N*H*, exch). Anal. Calcd for $C_{20}H_{17}ClFN_5{\cdot}0.$ $1H_2O$: C, 62.62; H, 4.52; N, 18.25; Cl, 9.24; F,4.95. Found: C, 62.4; H, 4.58; N, 18.12; Cl, 9.44; F, 4.69.

**6.6.6 . $N^4$-(4-Chlorophenyl)-6-(2-phenylethyl)-7*H*-pyrrolo[2,3-*d* ]pyrimidine-2,4-diamine (18).** Using the general procedure described above, reaction of **31** (0.08 g, 0.29 mmol) and 4-chloroaniline **32f** (0.055 g, 0.43 mmol) gave a semisolid; which was triturated with diethyl ether to afford a solid which was filtered and washed with diethyl ether (5 mL) to get **18** (0.09 g, 85% yield) as a light brown solid. TLC $R_f$ 0.57 (MeOH/$CHCl_3$, 1:5); mp: 165–168 °C. $^1$HNMR (DMSO-$d_6$): $\delta$ 2.88 (t, 2H, $CH_2$), 2.94 (t, 2H, $CH_2$), 5.7 (bs, 2H, 2-N$H_2$, exch), 6.23 (s, 1H, C5-C*H*), 7.24–7.28 (m, 7H, $C_6H_5$ & C $_6H_4$), 7.96–7.99 (d, 2H, $C_6H_4$), 8.88 (br, 1H, 4-N*H*, exch), and 10.85 (s, 1H, 7-N*H*, exch). Anal. Calcd for $C_{20}H_{18}ClN_5{\cdot}0.46$ $C_4H_{10}O$: C, 65.91; H, 5.72; N,17.59; Cl, 8.9. Found C, 66; H, 5.48; N, 17.52; Cl, 8.82.

**6.6.7. $N^4$-[4-Fluoro-3-(trifluoromethyl)phenyl]-6-(2-phenylethyl)-7*H* -pyrrolo[2,3- *d*]pyrimidine-2,4-diamine (19).** Using the general procedure described above, reaction of **31** (0.07 g, 0.255 mmol) and 3-ethynylaniline **32 g** (0.068 g, 0.38 mmol) afforded a pale yellow solid which was recrystallized from hexanes/ethyl acetate (1:1) to get **19** (0.087 g, 82%) as an off-white solid. TLC $R_f$ 0.61 (MeOH/$CHCl_3$, 1:5); mp: 164–166 °C. $^1$HNMR (DMSO-$d_6$): $\delta$ 2.93 (t, 2H, $CH_2$), 2.97 (t, 2H, $CH_2$), 5.70 (br,2H, 2-N$H_2$, exch), 6.20(s, 1H, C5-C*H*), 7.24–8.42 (m, 8H, $C_6$ $H_5$ &$C_6H_3$), 9.07(s, 1H, 4-N*H*, exch), and 10.89 (br, 1H, 7-NH, exch). Anal. Calcd for $C_{21}H_{17}N_5F_4{\cdot}0.41$ $C_6H_{14}$: C, 61.19; H, 4.37; N, 16.52; F, 17.92. Found: C, 61.13; H, 4.20; N, 16.54; F, 17.97.

**6.6.8. $N^4$-1*H*-indol-5-yl-6-(2-phenylethyl)-7*H*-pyrrolo [2,3-*d*]pyrimidine-2,4-diamine (20).** Initial attempts to synthesize **20** using the general procedure described above failed. The reaction mixture turned black almost instantaneously. Use of excess **32h** did not lead to any improvement. However, avoiding the addition of concd HCl and using 5-aminoindole **32h** in excess (20-fold), the reaction went to completion. Using the general procedure described above for chromatographic purification afforded light brown solid, which was recrystallized from chloroform to obtain **20** (50 mg, 61% yield) as a buff colored solid. TLC $R_f$ 0.52 (MeOH/$CHCl_3$, 1:10 + 1 drop of $NH_4OH$); mp: 154–156.6 °C. $^1$HNMR (DMSO-$d_6$): $\delta$ 2.84–2.9 (m, 4H, $CH_2CH_2$), 5.46 (s, 2H,

2-N$H_2$, exch), 6.05 (s, 1H, C5-C$H$), 6.35 (s, 1H, $C_8H_6$N), 7.23–7.34 (m, 7H, $C_6$ $H_5$ and $C_8H_6$N), 8.01 (s, 1H, $C_8H_6$N), 8.5 (s, 1H, 4-N$H$, exch), 10.7 (br, 1H, N$H$, exch), 10.9 (br, 1H, N$H$, exch). Anal. Calcd for $C_{22}H_{20}N_6$·0. 547$CHCl_3$: C, 62.43; H, 4.77; N, 19.37. Found: C, 62.52; H, 4.89; N, 19.12.

HRMS (ES) calcd for $C_{22}H_{21}N_6$ $(M+H)^+$: 369.1828, found 369.1830.

### 6.7. Biological evaluation

**6.7.1. Cells.** All cells were maintained at 37 °C in a humidified environment containing 5% $CO_2$ using media from Mediatech (Hemden, NJ). A-431 cells were from the American Type Tissue Collection (Manassas, VA).

**6.7.2. Chemicals.** All growth factors (bFGF, VEGF, EGF, and PDGF-β) were purchased from Peprotech (Rocky Hill, NJ). PD153035, SU5416, AG1295, and CB676475 (4-[(4′-chloro-2′-fluoro)phenylamino]-6,7-dimethoxyquinazoline) were purchased from Calbiochem (San Diego, CA). The CYQUANT cell proliferation assay was from Molecular Probes (Eugene, OR). All other chemicals were from Sigma Chemical unless otherwise noted.

**6.7.3. Antibodies.** The PY-HRP antibody was from BD Transduction Laboratories (Franklin Lakes, NJ). Antibodies against EGFR, PDGFR-β, FGFR-1, Flk-1, and Flt-1 were purchased from Upstate Biotech (Framingham, MA).

**6.7.4. Phosphotyrosine ELISA.** Cells used were tumor cell lines naturally expressing high levels of EGFR (A431), Flk-1 (U251), Flt-1 (A498), PDGFR-β (SF-539), and FGFR-1 (NIH OVCAR-8). Expression levels at the RNA level were derived from the NCI Developmental Therapeutics Program (NCI-DTP) web site public molecular target information (http://www.dtp.nci.nih.gov/mtargets/mt_index.html). Briefly, cells at 60–75% confluence were placed in serum-free medium for 18 h to reduce the background of phosphorylation. Cells were always >98% viable by Trypan blue exclusion. Cells were then pretreated for 60 min with 10, 3.33, 1.11, 0.37, and 0.12 μM compounds followed by 100 ng/ml EGF, VEGF, PDGF-BB, or bFGF for 10 min. The reaction was stopped and cells permeabilized by quickly removing the media from the cells and adding ice-cold Tris-buffered saline (TBS) containing 0.05% Triton X-100, protease inhibitor cocktail, and tyrosine phosphatase inhibitor cocktail. The TBS solution was then removed and cells fixed to the plate for 30 min at 60 °C and further incubation in 70% ethanol for an additional 30 min. Cells were further exposed to block (TBS with 1% BSA) for 1 h, washed, and then a horseradish peroxidase (HRP)-conjugated phosphotyrosine (PY) antibody added overnight. The antibody was removed, cells were washed again in TBS, exposed to an enhanced luminal ELISA substrate (Pierce Chemical, Rockford, IL), and light emission measured using a UV product (Upland, CA) BioChemi digital darkroom. The known RTK-specific kinase inhibitor, PD153035, was used as a positive control compound for EGFR kinase inhibition; SU5416 for Flk1 kinase inhibition; AG1295 for PDGFR-β kinase inhibition; and CB676475 (4-[(4′-chloro-2′-fluoro)phenylamino]-6,7-dimethoxyquinazoline) was used as a positive control for both Flt1 and Flk1 kinase inhibition. Data were graphed as a percent of cells receiving growth factor alone and $IC_{50}$ values were estimated from two to three separate experiments ($n$ = 8–24) using hand-drawn probit plots. In each case, the activity of a positive control inhibitor did not deviate more than 10% from the $IC_{50}$ values listed in the text.

**6.7.5. CYQUANT cell proliferation assay.** As a measure of cell proliferation, the CYQUANT cell counting/proliferation assay was used as previously described.[41] Briefly, cells are first treated with compounds for 12 h and then allowed to grow for an additional 36 h. The cells are then lysed and the CYQUANT dye, which intercalates into the DNA of cells, is added and after 5 min the fluorescence of each well measured using a UV product BioChemi digital darkroom. A positive control used for cytotoxicity in each experiment was cisplatin, with an apparent average $IC_{50}$ value of 8.2 ± 0.65 μM. Data are graphed as a percent of cells receiving growth factor alone and $IC_{50}$ values estimated from two to three separate experiments ($n$ = 6–15) using probit plots.

**6.7.6. Chorioallantoic membrane assay of angiogenesis.** The chorioallantoic membrane (CAM) assay is a standard assay for testing antiangiogenic agents.[42] The CAM assay used in these studies was modified from a procedure by Sheu[52] and Brooks[53] and as published previously.[54] Briefly, fertile leghorn chicken eggs (CBT Farms, Chestertown, MD) are allowed to grow until 10 days of incubation. The proangiogenic factors, human VEGF-165 and bFGF (100 ng each) are then added at saturation to a 3-mm microbial testing disk (BBL, Cockeysville, MD) and placed onto the CAM by breaking a small hole in the superior surface of the egg. Antiangiogenic compounds are added 8 h after the VEGF/bFGF at saturation to the same microbial testing disk and embryos allowed to incubate for an additional 40 h. After 48 h, the CAMs are perfused with 2% paraformaldehyde/3% glutaraldehyde containing 0.025% Triton X-100 for 20 s, excised around the area of treatment, fixed again in 2% paraformaldehyde/3% glutaraldehyde for 30 min, placed on petri dishes, and a digitized image taken using a dissecting microscope (Wild M400; Bannockburn, IL) at 7.5× and Retiga enhanced digital imaging system (QImaging, Burnaby, BC, Canada). A grid is then added to the digital CAM images and the average number of vessels within 5–7 grids counted as a measure of vascularity. AGM-1470 (a kind gift of the NIH Developmental Therapeutics Program) and SU5416 are used as a positive control for antiangiogenic activity. Data are graphed as a percent of CAMs receiving bFGF/VEGF and $IC_{50}$ values estimated from two to three separate experiments ($n$ = 5–11) using nonlinear regres-

sion analysis and a Sigmoidal Dose–response analysis using Prism 4.0 (GraphPad Software, San Diego, CA).

**6.7.7. Statistics.** All analysis was done using Prism 4.0. (GraphPad Software, San Diego, CA).

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## Appendix A

| | Formula | Calculated (%) | | | | | | Found (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | H | N | Cl | Br | F | C | H | N | Cl | Br | F |
| **22a** | $C_{19}H_{21}N_4OCl$ | 63.95 | 5.93 | 15.7 | 9.93 | | | 63.84 | 5.96 | 15.64 | 10.04 | | |
| **22b** | $C_{19}H_{21}N_4O_2Cl$ | 62.09 | 5.99 | 14.48 | 9.16 | | | 61.91 | 6.01 | 14.54 | 9.33 | | |
| **22e** | $C_{23}H_{23}N_4OCl{\cdot}0.2H_2O$ | 67.29 | 5.75 | 13.65 | 8.64 | | | 67.22 | 5.65 | 13.38 | 8.92 | | |
| **30** | $C_{14}H_{14}N_4O{\cdot}$ $0.2741C_3H_6O$ | 65.88 | 5.83 | 20.73 | | | | 66.23 | 5.86 | 20.67 | | | |
| **31** | $C_{14}H_{13}N_4Cl{\cdot}0.10\ H_2O$ | 61.25 | 4.85 | 20.41 | 12.91 | | | 61.05 | 4.79 | 20.25 | 12.7 | | |
| **13** | $C_{20}\ H_{17}N_5BrF{\cdot}0.2\ H_2O$ | 55.88 | 4.08 | 16.29 | | 18.59 | 4.42 | 55.68 | 3.91 | 16.08 | | 18.21 | 4.38 |
| **14** | $C_{20}H_{17}N_5ClF{\cdot}0.3$ $CH_3COCH_3$ | 62.87 | 4.76 | 17.49 | 8.85 | | 4.74 | 63.18 | 4.66 | 17.48 | 8.88 | | 4.54 |
| **15** | $C_{21}H_{18}N_5F_3$ | 63.47 | 4.56 | 17.62 | | | 14.34 | 63.22 | 4.68 | 17.43 | | | 14.41 |
| **16** | $C_{22}H_{19}N_5{\cdot}0.4\ H_2O$ | 73.27 | 5.53 | 19.42 | | | | 73.63 | 5.77 | 19.04 | | | |
| **17** | $C_{20}H_{17}ClFN_5{\cdot}0.1\ H_2O$ | 62.62 | 4.52 | 18.25 | 9.24 | | 4.95 | 62.4 | 4.58 | 18.12 | 9.44 | | 4.69 |
| **18** | $C_{20}H_{18}ClN_5{\cdot}0.6$ $CH_3COCH_3$ | 65.67 | 5.46 | 17.55 | 8.89 | | | 66.0 | 5.48 | 17.52 | 8.82 | | |
| **19** | $C_{21}H_{17}N_5F_4{\cdot}0.13$ $CH_3COCH_3$ | 60.74 | 4.24 | 16.54 | | | 17.95 | 61.13 | 4.20 | 16.54 | | | 17.97 |

High-resolution mass spectra (**HRMS**) (**EI**)

| Compound | Formula | Calcd. mass | Found mass |
|---|---|---|---|
| **24a** | $C_{25}H_{26}N_5OBr$ | 491.1320 | 491.1301 |
| **24b** | $C_{26}H_{28}N_5O_2Br$ | 521.1426 | 521.1431 |
| **24c** | $C_{25}H_{25}N_5OBrCl$ | 525.0931 | 525.0917 |
| **24d** | $C_{29}H_{28}N_5OBr$ | 541.1477 | 541.1477 |
| **24e** | $C_{29}H_{28}N_5OBr$ | 541.1477 | 541.1491 |
| **8** | $C_{20}H_{18}N_5Br$ | 407.0746 | 407.0732 |
| **9** | $C_{21}H_{20}N_5BrO$ | 437.0851 | 437.0837 |
| **10** | $C_{20}H_{17}N_5ClBr$ | 441.0356 | 441.0356 |
| **11** | $C_{24}H_{20}N_5Br$ | 457.0902 | 457.0881 |
| **12** | $C_{24}H_{20}N_5Br$ | 457.0902 | 457.0885 |
| **20** | $C_{22}H_{21}N_6$ | 369.1828 | 369.1830 |

## References and notes

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