Tyrosine Kinase Inhibitors. 16. 6,5,6-Tricyclic Benzothieno[3,2-*d*]pyrimidines and Pyrimido[5,4-*b*]- and -[4,5-*b*]indoles as Potent Inhibitors of the Epidermal Growth Factor Receptor Tyrosine Kinase

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Several elaborations of the fundamental anilinopyrimidine pharmacophore have been reported as potent and selective inhibitors of the epidermal growth factor receptor (EGFr) tyrosine kinase. This paper reports on a series of inhibitors whereby some 6,5-bicyclic heteroaromatic systems were fused through their C-2 and C-3 positions to this anilinopyrimidine pharmacophore. Although the resulting tricycles did not produce the enormous potency of some of the (5/6),6,6-bicyclic systems, the best of them had IC_{50}s for the EGFr TK around 1 nM. Investigation of 4-position side chains in the indolopyrimidines confirmed that *m*-bromoaniline was an optimal substituent for potency. Investigation of substitution within the C-(benzo)ring of benzothienopyrimidines confirmed that introduction of an extra ring can change sharply the effects of substituents when compared to similar bicyclic nuclei, and only two substituents were found which even moderately enhanced inhibitory activity over the parent compound for this series.

Introduction

Among the most common oncogenes that are overexpressed in such solid tumors as colon, breast, gastric, prostatic, and head and neck cancers are those of the type I receptor tyrosine kinase (RTK) family. These consist of the epidermal growth factor receptor (EGFr), erbB-2, erbB-3, and erbB-4 tyrosine kinases.[1−6] As the ability to induce tyrosine phosphorylation is essential to the oncogenic potential of this family, there has been an intensive search for inhibitors of its members.[5,7−10] Although this field started slowly, the discovery of highly selective, ATP-competitive inhibitors based on diarylamines[11] has led to the development of agents with great pharmaceutical potential. The development of such compounds has led to many compounds showing oral activity in xenograft models, and at least two agents have gone through phase I clinical trials.[12] The most explored pharmacophore is a 4-(*meta*-substituted anilino)pyrimidine, which can be modeled into the ATP-binding site of EGFr in such a way that the pyrimidine ring orients into the hydrophobic adenine-binding site to form two backbone H-bonds in the same manner as adenine.[13−15] In the Parke-Davis model[13] the aniline ring forms a considerable dihedral angle to the pyrimidine and occupies a hydrophobic cleft toward the back of the ATP-binding site, which is unoccupied by substrate. In accord with the model, some anilinopyrimidines such as **1**[16] (Chart 1) show low nanomolar IC_{50}s toward the EGFr TK. In the model, the 5,6-bond of the pyrimidine faces toward the outside of the catalytic cleft and there is no particular encumbrance in that region. Consistent with this, fusing a second aromatic ring to the 5,6-positions of the pyrimidine leads to compounds which can have good (∼50 nM)[17] to truly outstanding (<10 pM)[18] IC_{50}s for the enzyme as exemplified by compounds **2**−**6** in Table 1, with examples **3** and **4**[5] illustrating that a five-membered heteroaromatic ring can be as effective as a phenyl ring.[19] For the 6,6-bicycles the 6- and 7-position substituents are often highly activating[18,20] (see **5** and **6**). Furthermore, the model suggests that bulky 6- and 7-substituents can protrude from the binding site into the solvent and that there is room for a further ring fused to the 6- and 7-positions of the bicycle. Such tricycles have been made and can also be extremely potent and selective inhibitors, as exemplified by the imidazoquinazoline **7a** (8 pM),[21] the pyrroloquinazoline **7b** (440 pM), and the pyrazoloquinazoline **7c** (440 pM).[21] Nonlinear tricycles are considerably less potent than their linear isomers, e.g., **8a,b** and **9,**[21] in accord with the bicyclic SAR, which demonstrated that 6- and 7-position substitution can be beneficial whereas 5- and 8-substitutions are deactivating.[22]

In this paper we wish to report on the SAR of a series of 6,5,6-fused tricycles as EGFr inhibitors. Such compounds have an inherent bend in the tricyclic nucleus, but less than that seen in angularly fused (5/6),6,6-tricycles. In accordance with the previous SAR,[13,21] these inhibitors tend to have inhibitory potencies intermediate between the linear and angularly fused *X*,6,6-tricycles. However, the effects of substitution on the C-benzenoid ring tend to be quite different from their effects on the B-phenyl ring of quinazolines.

Chemistry

4-Amino-substituted pyrimido[5,4-*b*]indole analogues were prepared as detailed in Scheme 1. Condensation of 4-chloropyrimido[5,4-*b*]indole[23] (**11**) was carried out

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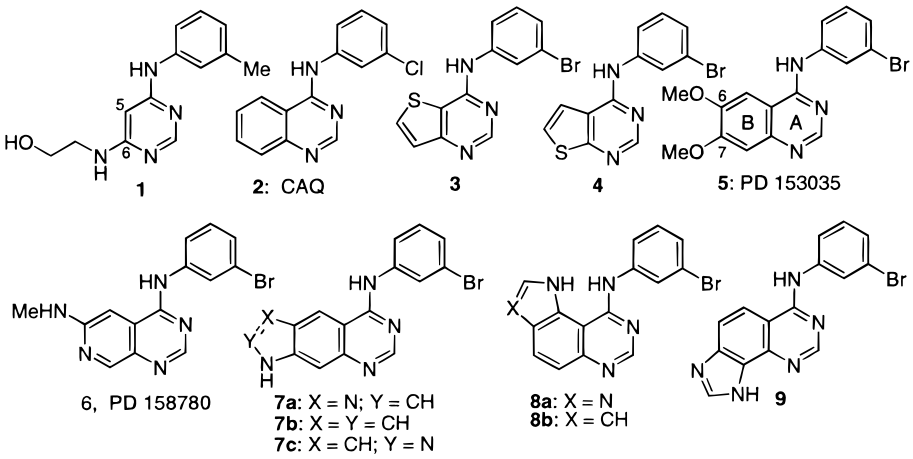
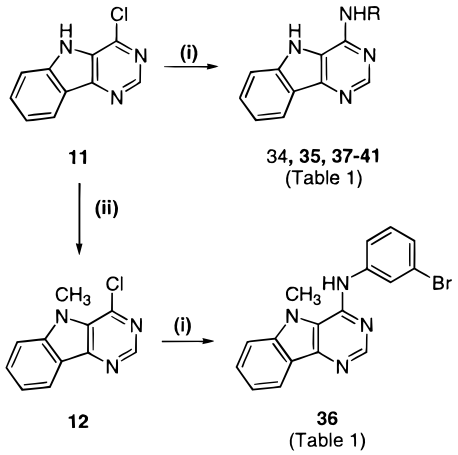
Chart 1



Scheme 1[a]

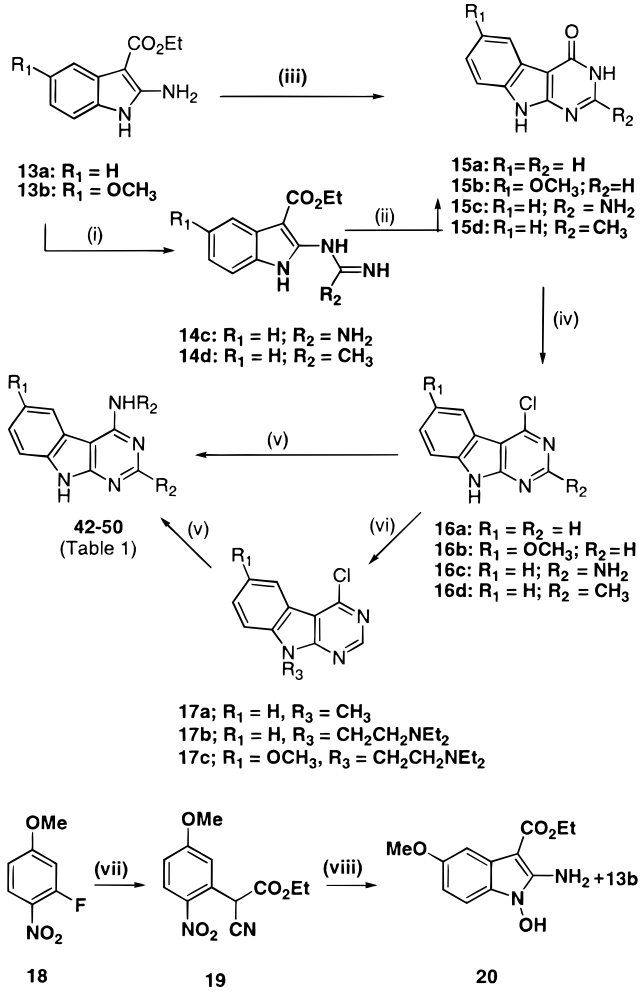


[a] (i) RNH_2 in an alcohol/cat. HCl (procedure A) or neat RNH_2/heat (procedure B); (ii) Cs_2CO_3, dimethyl sulfate, refluxing acetone, 3A sieves.

with a variety of aliphatic and aromatic amines to give target compounds **34**, **35**, and **37**−**41** listed in Table 1. The preferred conditions were to carry out the reaction in a refluxing lower alcohol (and on occasion DMA) with a catalytic amount of HCl (procedure A). In a few instances, the reaction was run with neat amine at 150 °C (procedure B), although yields tended to be poorer. Methylation of the N-5 position of **11** with cesium carbonate in refluxing acetone followed by the addition of dimethyl sulfate gave the known compound **12**.[24] Condensation of **12** with 3-bromoaniline then provided the target analogue **36** of Table 1.

The preparation of a number of 4-amino-substituted pyrimido[4,5-*b*]indole analogues is detailed in Scheme 2. Ring closure of either the known 2-amino-1*H*-indole-3-carboxylic acid ethyl ester (**13a**)[25] or the 6-methoxy congener **13b** to the corresponding pyrimido[4,5-*b*]indol-4-ones **15a,b**, respectively, was performed in hot formamide with sodium methoxide catalysis as previously described.[26] The synthesis of analogues bearing amino or methyl substituents at the C-2 position was carried out utilizing literature conditions described to make similar congeners of intermediates **15c,d**.[27] Thus, condensation of **13a** with cyanamide under acidic conditions provided the guanidine **14c**. Reaction of **14c** with refluxing aqueous sodium hydroxide resulted in ring closure to 2-amino-1,9-dihydro-4*H*-pyrimido[4,5-*b*]indol-4-one (**15c**). Condensation of **13a** with acetonitrile to give amidine **14d** followed by ring closure to **15d** proceeded similarly. Reaction of **15a**−**d** with $POCl_3$ either neat or in refluxing *p*-dioxane then provided the

Scheme 2[a]



[a] (i) NH_2CN, concd HCl, *p*-dioxane, reflux (for **14c**), CH_3CN, dry HCl, reflux (for **14d**); (ii) aq NaOH, reflux; (iii) formamide, NaOMe, 220 °C (for **15a,b**); (iv) $POCl_3$; (v) RNH_2 in an alcohol/cat. HCl or DMA/cat. HCl/120 °C (procedure A) or neat RNH_2/heat (procedure B); (vi) Cs_2CO_3, refluxing acetone or 2:1 acetone:DMF, 3A or 4A sieves, dimethyl sulfate (for **17a**), $Cl(CH_2)_2NEt_2$·HCl (for **17b,c**); (vii) $CNCH_2CO_2Et$, *t*-BuOK, THF, reflux; (viii) Zn dust, acetic acid, 55 °C.

known 4-chloropyrimido[4,5-*b*]indole (**16a**)[26] and **16b**−**d**, respectively. Chloropyrimido[4,5-*b*]indoles **16a**−**d** were then condensed with selected amines by the procedures described above to give the target 4-amino-substituted analogues (compounds **42**−**44** and **47**−**49** in Table 1). Condensation of **16b** with 3-bromoaniline proceeded poorly in refluxing 2-propanol/HCl, primarily due to its lowered reactivity relative to **16a**. However, upon reaction in DMA/HCl at 120 °C, a modest yield of target compound **49** (Table 1) was obtained. The 4-chloropyrimido[4,5-*b*]indole (**16a**) was also *N*-methylated under the conditions described above for isomeric compound **11** to give the known **17a**.[28]

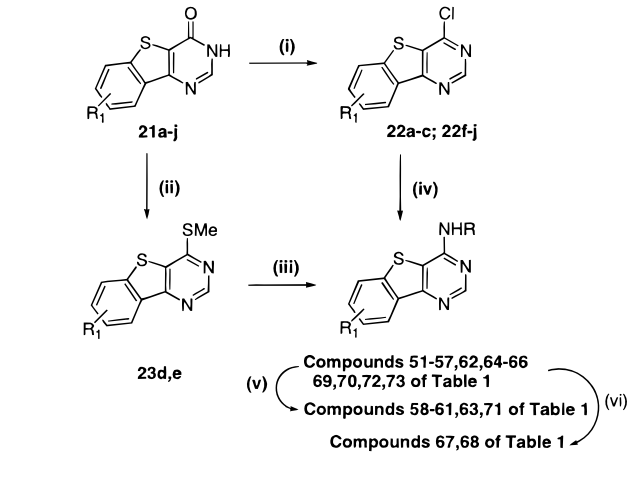
To introduce aqueous solubilizing functionality onto the *N*-9 position, chloro intermediates **16a,b** were alkylated with 2-diethylaminoethyl chloride, either in refluxing acetone or in 2:1 acetone:DMF, using cesium carbonate as base to provide the corresponding *N*-alkylated compounds **17b,c**. Compounds **17a**−**c** were then condensed with 3-bromoaniline in refluxing 2-propanol catalyzed with HCl (procedure A) to provide target compounds **45**, **46**, and **50** of Table 1 in excellent yield.

The synthesis of 2-amino-5-methoxy-1*H*-indole-3-carboxylic acid ethyl ester (**13b**) was carried out utilizing the same procedure as its 6-protio congener[25] and is outlined in Scheme 2. Condensation of 3-fluoro-4-nitroanisole (**18**) with the potassium salt of ethyl cyanoacetate gave adduct **19** in quantitative yield. Zinc dust reduction of **19**, utilizing the literature conditions,[26] gave a chromatographically separable 1:1 mixture of **13b** and the *N*-hydroxy side product **20** resulting from incomplete reduction.

The synthesis of benzothieno[3,2-*d*]pyrimidone **21a**,[29,30] and its various phenyl ring-substituted derivatives (Scheme 3), has been described elsewhere.[31] Generally these were converted to the corresponding 4-chlorides **22** in good yields with Vilsmeier's reagent (oxalyl chloride/DMF), and the chlorides were then displaced by amines in a refluxing lower alcohol to give the final products (procedure A). The two amine-containing tricyclic pyrimidones **21d**,**e** did not react successfully with a variety of chlorinating agents. They were activated by conversion into the corresponding 4-thiones with P_2S_5 or Lawesson's reagent[32] in diglyme at 115 °C and were then converted in situ into the corresponding thioethers **23d**,**e** with methyl iodide and Hunig's base. The thioethers were surprisingly unreactive and could only be displaced slowly with 3-bromoaniline under quite drastic conditions in hot ethylene glycol in the presence of excess aniline hydrochloride[21] to give inhibitors **64** and **65** (procedure A). All of the other amine-containing benzothienopyrimidines were made by Raney nickel reductions of the corresponding nitro compounds as the final step of the synthesis (procedure C), which allowed the side chains to be introduced via the corresponding chlorides without any problems. Methylation of the 8-amino functionality of **61** was carried out under Eschweiler−Clarke conditions[33] (procedure D) and led to a mixture of mono- and dimethylation products, **67** and **68**, which were separated chromatographically.

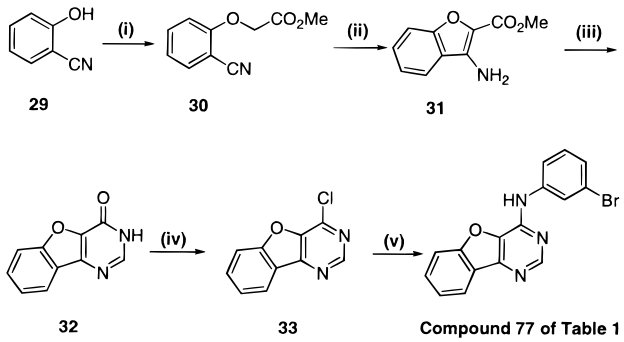
Both the benzofuranopyrimidone **77** (Scheme 4) and the pyrido[3,2′:4,5]thieno[3,2-*d*]pyrimidones **74** and **75** (Scheme 3) were made by a process analogous to the benzothienopyrimidones. Alkylation of 2-cyanophenol

Scheme 3[a]



[a] (i) DMF/oxalyl chloride, 1,2-dichloroethane; (ii) P_2S_5 or Lawesson's reagent, diglyme, 115 °C, then $(i\text{-Pr})_2$NEt, MeI; (iii) RNH_2 in ethylene glycol/cat. HCl, 140 °C (procedure A); (iv) RNH_2 in an alcohol/cat. HCl (procedure A); (v) H_2, Raney Ni (procedure C); (vi) HCO_2H, aq HCHO, 90 °C (procedure D); (vii) ethyl thioglycolate, NaH, DMSO; (viii) formamide, 190 °C.

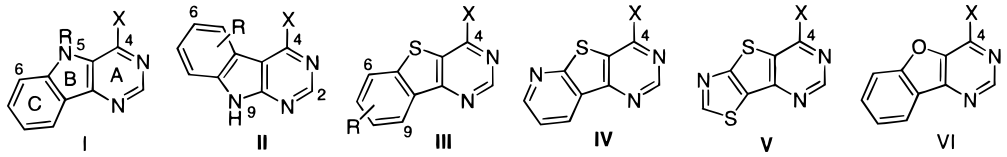
Scheme 4[a]



[a] (i) Methyl bromoacetate, K_2CO_3, acetone; (ii) NaH, DMSO; (iii) formamide, 170 °Cl (iv) DMF/oxalyl chloride, 1,2-dichloroethane; (v) 3-bromoaniline, 2-ethoxyethanol, 135 °C (procedure A).

(**29**) with methyl bromoacetate followed by treatment with sodium hydride and then cyclization in DMSO gave the benzofuran **31** in moderate yield. Niemetowski

Table 1. Physicochemical and Enzyme Inhibitory Data for 6,5,6-Tricyclic Benzothienopyrimidines and Pyrimidoindoles



I II III IV V VI

no.	form	4-X	R	procedure	formula	analysis[a]	IC_{50}[b]
				Reference Compounds			
1		see Figure 1			ref 16		1
2		see Figure 1			ref 17		23
3		see Figure 1			ref 5		11
4		see Figure 1			ref 5		35
5		see Figure 1			ref 18		0.025
6		see Figure 1			ref 20		0.008
7a		see Figure 1			ref 21		0.008
7b		see Figure 1			ref 21		0.39
7c		see Figure 1			ref 21		0.44
8a		see Figure 1			ref 21		29
8b		see Figure 1			ref 21		1.24
9		see Figure 1			ref 21		272
				Pyrimido[5,4-*b*]indoles			
34	I	NHPh	H	A	$C_{16}H_{12}N_4 \cdot 1.1HCl$	C, H, N	2110
35	I	NH(3-BrPh)	H	A	$C_{16}H_{11}BrN_4 \cdot 1.1HCl$	C, H, N	72
36	I	NH(3-BrPh)	5-Me	A	$C_{17}H_{13}BrN_4 \cdot HCl$	C, H, N	132
37	I	NHBn	H	B	$C_{17}H_{14}N_4$	C, H, N	460
38	I	NH(*R*)CH(Me)Ph	H	B	$C_{18}H_{16}N_4 \cdot 0.8HCl$	C, H, N	419
39	I	NH(*S*)CH(Me)Ph	H	B	$C_{18}H_{16}N_4 \cdot 0.1HCl$	C, H, N	$>10^5$
40	I	NH-*c*-C_6H_{11}	H	B	$C_{16}H_{18}N_4 \cdot 0.2H_2O$[c]	C, H, N	$>10^5$
41	I	$NH(CH_2)_2NMe_2$	H	B	$C_{14}H_{17}N_5 \cdot 0.2HCl$	C, H, N	$>10^5$
				Pyrimido[4,5-*b*]indoles			
42	II	$NH(CH_2)_2NMe_2$	H	B	$C_{14}H_{17}N_5 \cdot 0.1EtOAc$	C, H, N	$>10^4$
43	II	NHPh	H	A	$C_{16}H_{12}N_4$	C, H, N	264
44	II	NH(3-BrPh)	H	A	$C_{16}H_{11}BrN_4$	C, H, N	31
45	II	NH(3-BrPh)	9-Me	A	$C_{17}H_{13}BrN_4 \cdot 0.7H_2O$	C, H, N	742
46	II	NH(3-BrPh)	9-$(CH_2)_2NEt_2$	A	$C_{22}H_{24}BrN_5 \cdot 2HCl \cdot 1.5H_2O$	C, H, N	4100
47	II	NH(3-BrPh)	2-Me	A	$C_{17}H_{13}BrN_4 \cdot 1.1HCl$	C, H, N	$>10^4$
48	II	NH(3-BrPh)	2-NH_2	A	$C_{16}H_{12}BrN_5 \cdot 0.2H_2O$	C, H, N	147
49	II	NH(3-BrPh)	6-OMe	A	$C_{17}H_{13}BrN_4O \cdot 0.7H_2O$	C, H, N	1.2
50	II	NH(3-BrPh)	9-$(CH_2)_2NEt_2$ 6-OMe	A	$C_{23}H_{26}BrN_5O \cdot 2.1HCl \cdot 0.9H_2O$	C, H, N	4270
				Benzothieno[3,2-*d*]pyrimidines			
51	III	NHBn	H	A	$C_{17}H_{13}N_3S$	C, H, N	191
52	III	NH(*R*)CH(Me)Ph	H	A	$C_{18}H_{15}N_3S$	C, H, N	538
53	III	NH(3-BrPh)	H	A	$C_{16}H_{10}N_3S$	C, H, N	1.8
54	III	NHPh	8-NO_2	A	$C_{16}H_{10}N_4O_2S \cdot HCl$	C, H, N	58
55	III	NH(3-MePh)	8-NO_2	A	$C_{17}H_{12}N_4O_2S \cdot 0.5HCl$	C, H, N	11
56	III	NH(3-CF_3Ph)	8-NO_2	A	$C_{17}H_9F_3N_4O_2S \cdot 0.9HCl$	C, H, N	460
57	III	NH(3-BrPh)	8-NO_2	A	$C_{16}H_{10}N_4O_2S \cdot HCl$	C, H, N	12.3
58	III	NHPh	8-NH_2	C	$C_{16}H_{12}N_4S \cdot 0.1HCl \cdot 0.2EtOH$	C, H, N	9.4
59	III	NH(3-MePh)	8-NH_2	C	$C_{17}H_{14}N_4S \cdot 0.1H_2O$	C, H, N	2.1
60	III	NH(3-CF_3Ph)	8-NH_2	C	$C_{17}H_{11}F_3N_4S \cdot 0.3H_2O$	C, H, N	47
61	III	NH(3-BrPh)	8-NH_2	C	$C_{16}H_{11}BrN_4S$	C, H, N	0.27
62	III	NH(3-BrPh)	7-NO_2	A	$C_{16}H_9BrN_4O_2S \cdot HCl$	C, H, N	48
63	III	NH(3-BrPh)	7-NH_2	C	$C_{16}H_{11}BrN_4S \cdot 0.5HCl$	C, H, N	0.47
64	III	NH(3-BrPh)	7-NH_2-8-F	A	$C_{16}H_{10}BrFN_4S$	C, H, N	1.0
65	III	NH(3-BrPh)	7-NHEt-8-F	A	$C_{18}H_{14}BrFN_4S \cdot 0.3EtOH$	C, H, N	7.9
66	III	NH(3-BrPh)	7-OMe	A	$C_{17}H_{12}BrN_3OS$	C, H, N	80
67	III	NH(3-BrPh)	8-NHMe	D	$C_{17}H_{13}BrN_4S \cdot 0.5EtOH$	C, H, N	1.1
68	III	NH(3-BrPh)	8-NMe_2	D	$C_{18}H_{15}BrN_4S$	C, H, N	19.8
69	III	NH(3-BrPh)	6-OMe	A	$C_{17}H_{12}BrN_3OS \cdot HCl$	C, H, N	12.6
70	III	NH(3-BrPh)	6-NO_2	A	$C_{16}H_9BrN_4O_2S \cdot HCl$	C, H, N	158
71	III	NH(3-BrPh)	6-NH_2	C	$C_{16}H_{11}BrN_4S \cdot 0.3HCl \cdot 0.7MeOH$	C, H, N	2.7
72	III	NH(3-BrPh)	9-OMe	A	$C_{17}H_{12}BrN_3OS \cdot HCl$	C, H, N	~20[d]
73	III	NH(3-BrPh)	7,8-$(OMe)_2$	A	$C_{18}H_{14}BrN_3O_2S \cdot HCl$	C, H, N	444
				Pyridothieno[3,2-*d*]pyrimidines			
74	IV	NH(3-ClPh)	H	A	$C_{15}H_9ClN_4S$	C, H, N	$>10^3$
75	IV	NH(3-BrPh)	H	A	$C_{15}H_9ClN_4S$	C, H, N	732
				Thiazolothieno[3,2-*d*]pyrimidines			
76	V	NH(3-BrPh)	H	A	$C_{13}H_7BrN_4S_2 \cdot 0.1CHCl_3 \cdot 0.2MeOH$	C, H, N	40
				Benzofurano[3,2-*d*]pyrimidines			
77	VI	NH(3-BrPh)	H	A	$C_{16}H_{10}BrN_3O$	C, H, N	740

[a] The analyses were within ±0.4% of the theoretical values. [b] IC_{50}: concentration of compound (nM) to inhibit the phosphorylation of a 14-residue fragment of phospholipase C-γ1 by EGFr (prepared from human A431 carcinoma cell vesicles by immunoaffinity chromatography). See Experimental Section for details. Values are the averages from at least two independent dose−response curves; variation was generally ±15%. [c] N: calcd, 20.75%; found, 20.30%. [d] The low solubility of this compound precluded an accurate IC_{50} determination.

synthesis made the desired tricycle **32** in good yield (Scheme 4). Similarly, treatment of 2-chloronicotinonitrile (**24**) with sodium ethyl thioglycolate followed by formamide cyclization gave **26**. Both were chlorinated with the oxalyl chloride-derived Vilsmeier reagent and displaced with 3-bromoaniline without difficulty.

One 5,5,6-tricycle was prepared. 4-Chlorothiazolo-[2′,3′:4,5]thieno[3,2-*d*]pyrimidine **(28)** was prepared by the method of Iddon,[34] and the 4-chloro substituent was readily displaced with 3-bromoaniline to give the desired tricycle **76** (Scheme 3).

Results and Discussion

The structures and physicochemical properties of the compounds prepared are given in Table 1. All of the analogues were evaluated for their ability to inhibit tyrosine phosphorylation of a polypeptide (a portion of phospholipase C-γ) by EGF-stimulated full-length EGFr enzyme isolated from A431 cells. Full dose−response curves were determined for each compound, and the resulting IC_{50}s listed in Table 1 are the average of at least two such determinations.

As can be seen in Table 1 many potent inhibitors of the EGFr TK can be found in these 6,5,6-tricycles, and at a first level the SAR is clearly parallel to the previous series that we have examined.[22] Aromatic amine side chains at the 4-position usually produce sub-micromolar inhibitors, with many of the better ones being low nanomolar. Nonaromatic amine side chains lead, as anticipated, to very poor inhibitors, and in the limited SAR done *m*-bromoanilino appears to be the best substituent, followed by *m*-methylanilino. The two *m*-bromoaniline derivatives with IC_{50}s greater than 1 μM, **47** and **50**, contain a 2-methyl and a bulky 9-substituent (equivalent to the 8-position in a quinazoline), respectively, neither of which would be tolerated in the corresponding bicyclic SARs. Thus, from what can be predicted from previous SARs, including the other tricyclic SAR examined,[21] tolerance at these key positions and the beneficial effect of small lipophilic *m*-anilino substituents seem to hold up in these compounds as well, suggesting that they may well bind in a similar mode to the well-studied bicyclic compounds. This would be in accord with molecular modeling, which suggests that the same binding mode is tolerated for these tricycles, the *X*,6,6-tricycles, and the bicycles. However, there are some differences from the bicyclic SARs, which will be discussed with the individual tricyclic nuclei below.

Looking at the aromatic nuclei, the benzothieno[3,2-*d*]pyrimidine nucleus is clearly the best of those in this study. Considered as a bare "parent" nucleus, the benzothiophene **53** is among the most potent that we observed and is 40-fold more potent than the corresponding indolo[3,2-*d*]pyrimidine **35** and surprisingly 400-fold more potent than the corresponding benzofuran **77**. As previous SAR studies had shown that the enzyme appears to tolerate electron richness better than electron deficiency in the B/C-ring region, and 6/7-substitution better than 5/8-substitution, the potency of the benzothienopyrimidines is unremarkable. The lack of potency of the more electron-deficient pyridothienopyrimidines **74** and **75** was not unexpected (see below), and the reasonable potency of the thiazolothienopyrimidine **76** was also unsurprising. The isomeric indolo[2,3-*d*]-pyrimidine **44** is twice as potent as **35**, suggesting that this ring fusion may be the more advantageous, which would be consistent both with the demonstrated greater tolerance of substituents at the 5- rather than the 8-position in quinazolines and in the angular imidazoquinazolines studied previously. Unfortunately, attempts at developing an adaptable synthesis of benzo ring-substituted benzothieno[2,3-*d*]pyrimidines[35] were unsuccessful, thus not permitting meaningful exploration of that SAR.

A few compounds confirmed that substitutions in the A- and B-rings were similar to the quinazolines.[22] Substitutions at the 2-position of the indolo[2,3-*d*]-pyrimidines **47** and **48** were detrimental with methyl leading to at least a 300-fold loss of activity and amino a more modest 5-fold loss. On the same nucleus 9-*N* substitution, corresponding to the highly disfavored 8-position of quinazolines, with a methyl group (**45**) or a solubilizing diethylaminoethyl side chain **(50)** led to 15- and 138-fold losses of potency with respect to the corresponding 9-protio compound **44**. In the indolo[3,2-*d*]pyrimidine series, *N*-methylation of the 5-position of compound **36** led to only a 2-fold loss of activity with respect to the 5-protio compound **35**. In the quinazolines methoxylation of this position led to about a 25-fold loss of potency.

A brief examination of the 4-amino substituent confirmed that these compounds fit the SAR of the previously examined anilinopyrimidines. In the indolo[3,2-*d*]pyrimidines, two nonaromatic side chains, **40** and **41**, showed no activity at all, whereas a benzyl side chain, compound **37**, gives moderate activity (460 nM). In this case the (*R*)-methyl substituent (compound **38**) is not significantly better, but the (*S*)-methyl substituent (compound **39**) is strongly deactivating, as had been seen for the quinazolines.[36] A simple phenyl substituent (compound **34**) was surprisingly poor in this series (2.11 μM), but the *m*-bromo substituent (compound **35**) produced the usual activation and, with an IC_{50} of 72 nM, was the most potent member of this series. In the isomeric indolo[2,3-*d*]pyrimidine series, similar trends were seen. The *m*-bromoanilino derivative **44** was about 8.5-fold more potent than the corresponding phenyl compound **43**, and the nonaromatic side chain (compound **42**) had no activity. In the benzothieno[3,2-*d*]-pyrimidine series, benzyl (compound **51**) was a mediocre inhibitor, and here the (*R*)-methyl substituent (compound **52**) was about 3-fold detrimental, as opposed to moderately activating in the quinazolines. Here, the *m*-bromoanilino side chain of **53** led to excellent activity (1.8 nM). With the deactivating 8-nitro and the activating 8-amino substituents on this nucleus, a small series of *m*-anilino substitutions were examined and once again showed similarity with previously described SARs. The less activated nitro series showed $CH_3 \sim Br > H > CF_3$ (compounds **54**−**57**) and the more activated amino series $Br > CH_3 > H > CF_3$ (compounds **58**−**61**), which is very similar to the parent quinazoline series.

In the benzothieno[3,2-*d*]pyrimidine series, the effects of C-ring (benzo) substitution were examined to see if they are similar to the bicyclic series where electron-donating substituents in the B-ring are potentiating at

the 6- and 7-positions. As these heterocycles are both longer than the bicycles and have a bent geometry, which was strongly disfavored in the 5,6,6-tricyclic series, there was no guarantee that substituent effects would be similar although it was anticipated that the usual preference for electron-donating substituents would be found. A series of methoxy substitutions were introduced on the phenyl ring, all with the *m*-bromoanilino side chain. Both 6- and 9-methoxy-substituted analogues **69** and **72** showed a moderate loss (about 10-fold) in potency compared to **53**, but the 7-substituted compound **66** surprisingly showed a 40-fold loss. The 7,8-dimethoxy derivative **73** showed an even larger loss of affinity (250-fold). Nitro and amino substitutions at the 6-, 7-, and 8-positions were also examined, as they have large effects in the bicyclic series. The 6-nitro compound **70** was strongly deactivated (88-fold with respect to **53**), the 7-nitro isomer **62** was less so (32-fold), and the 8-isomer **57** was only 7-fold deactivated. The same trend showed up in the amines, with the 6-isomer **71** being essentially equipotent to the parent compound **53,** and the 7- and 8-isomers **63** and **61** were 4- and 7-fold, respectively, more potent than **53**. With an IC_{50} of 270 pM, amine **61** was the most potent inhibitor that we found in these classes of tricycles. Removal of the *m*-bromine led to 5- and 35-fold losses of activity, respectively, for the corresponding 8-nitro and 8-amino compounds, **54** and **58**. An 8-F substituent in combination with the 7-amino (compound **64**) was slightly deactivating. Simple alkylation of the amine at either the 7- or 8-position was moderately deactivating (**65**, **67,** and **68**).

Clearly these results do not parallel the quinazoline SAR and also do not open themselves up to a simple electron density explanation. However, low electron density in this ring does appear to be somewhat disfavored, with the 8-nitro compound being only 7-fold deactivated. The 7-nitro isomer **62** shows a deactivation expected by analogy with the quinazoline SAR. The 6-isomer **70** is more deactivating than expected, but steric effects could easily play a part at this position. However, replacing the nitro at this position with an aza substituent (pyridothienopyrimidine **75**) led to even greater deactivation although results with other heterocyclic nuclei suggest that factors other than simple electron density may come into play when the nucleus is altered. The 7- and 8-amino substituents suggest at best a weak activating effect of very electron-rich rings, with 6-amino being without significant effect. Even monoalkylation abolishes this effect, as is the case with the quinazolines. The methoxy substitutions examined are the most puzzling, with the 6- and 9-positions, as expected for steric reasons, being moderately deactivating, but the 7- and 8-position methoxy analogues examined were highly deactivated. It is difficult to attribute these results to steric factors, but they are strongly suggestive that simply increasing the electron density of the benzo ring is not useful for increasing affinity and that the modest activation seen with the 7- and 8-amino substituents may be due to some nonelectronic factor such as hydrogen bonding. The minor losses of affinity in the 6- and 9-positions relative to that seen with 5- and 8-substitution in the quinazolines are suggestive that further away from the pyrimidine ring the steric intolerances are less. One methoxy-substituted indolo[2,3-*d*]pyrimidine, **49**, was made, and in this case the substitution was highly favored with a 26-fold improvement in potency giving a 1.2 nM inhibitor, suggesting the possibility that the alternative ring fusion might lead to a more bicycle-like SAR in this ring.

Table 2. Inhibition of EGFr Autophosphorylation in A431 Cells

	IC_{50} (nM)[a]	
compd	enzyme	autophos
35	72	93
44	31	630
45	474	769
48	147	493
49	1.2	13
53	1.8	170
59	2.1	98
61	0.27	86
64	1.0	42
65	7.9	85
67	1.1	33

[a] Data values represent the average of two independent experiments with a variability of 25% or less.

A few aniline substituents, which have proved advantageous in other series, notably *m*-methyl and *m*-trifluoromethyl, were looked at with the 8-nitro and 8-amino compounds. Methyl proved to be as good as bromine in the nitro compound **55**, but 10-fold poorer in the amine **59**, whereas trifluoromethyl was 5−8-fold deactivating in the case of both **56** and **60**. These results have parallels elsewhere in the SAR.

As shown in Table 2 several of these compounds had good activity against cellular autophosphorylation. Compound **49** was the most potent, with only a 10-fold fall off in potency from that observed in the enzyme assay. This compound is as potent in cellular assays as the picomolar bicyclic compounds **5** and **6**, but this result was exceptional. In contrast, the most potent of these tricycles, compound **61**, had a 320-fold fall off in potency in the cellular assay and had a disappointing 86 nM IC_{50}, whereas compound **35** was nearly equipotent in enzyme and cellular assays. There does not appear to be a good correlation between enzymatic and cellular data in this subset, although we observed autophosphorylation IC_{50} values of <100 nM for inhibitors with IC_{50} values of <10 nM against the enzyme. The great insolubility of many of these tricycles presumably affects the cellular data more than the enzyme data and thus may render the results somewhat ambiguous.

Previous series in this SAR had shown excellent selectivity for EGFr inhibition over all of the non-erbB kinases examined, and these tricycles proved to be no exception, as demonstrated in Table 3. As the selectivity of this family appears to come from occupancy of a hydrophobic pocket behind the adenine binding site by the N-4 aromatic side chain, while the fused rings of the heteroaromatic nucleus point toward the solvent, these compounds should have similar poor affinity for most other kinases, as has been demonstrated for all of the other inhibitors containing the ar(alk)ylaminopyrimidine pharmacophore.

Compound **61** was examined in two in vivo models, using EGFr-transfected fibroblasts and MDA MB-468 human breast tumor cells, respectively, as ip xenografts

Table 3. Selectivity of Inhibitors Against Other Kinases

compd	IC_{50} (nM) EGFr	% inhibition @ 50 μM c-Src	FGFr	IR	PDGFr	PKC
49	1.2	8 @ 1.25 μM	30	0	2	ND
51	191	0	11	0	10	ND
52	538	0	20	13	0	7
53	1.8	0	21	0	0	18
59	2.1	47	24	14	17	44
60	47	13	17	0	12	51
61	0.27	ND	ND	0	ND	0
66	80	22	19	0	21	20

in nude mice. The low solubility of **61** (<30 μg/mL) proved to be very problematical, and even when dosed as an ip suspension (50 mg/kg in pH 4 lactate buffer containing 10% DMA) the compound proved completely inefficacious. The peak blood levels were measured in the low nanomolar range, considerably below that predicted to be required for efficacy from the cellular data. Since this was so far removed from our target of acceptable oral bioavailability, and since none of the other compounds examined in this SAR showed a significant enough amelioration of physicochemical properties to expect any better results, no further in vivo work was carried out in this series.

Conclusions

An expansion of the basic ATP-binding site competitive EGFr TK inhibitor pharmacophore of 4-anilinopyrimidine to 6,5,6-tricyclic pyrimidines has been achieved. Generally, the best compounds have potencies between those of linear and angular *X*,6,6-tricycles in the same SAR, as might be expected. The nature of the heteroatom introduced into the five-membered ring is surprisingly important. In the [3,2-*d*] series oxygen was strongly (27-fold) deactivating with respect to the corresponding bromoanilinoquinazoline, whereas nitrogen was mildly deactivating and sulfur was strongly activating (15-fold). In the pyrimidoindoles, the only series where different ring fusions were studied, the [2,3-*d*] fusion appears to be about twice as potent as the [3,2-*d*] ring fusion. The SAR of anilino substitution is similar to that of the quinazolines, and substitution at the 2- and 9-positions ([2,3-*d*] fusion), corresponding to the disfavored positions of the quinazoline (2 and 8), is detrimental, whereas substitution at C-6, corresponding to C-5 of the quinazolines, is mildly activating. This suggests that these tricyclic inhibitors can bind in the same or a very similar mode to the quinazolines to the EGFr ATP-binding site. No highly activating substituent was found for the tricyclic nuclei, and although the best compounds had sub-nanomolar IC_{50}s against EGFr, this activity was not sufficient to compensate for their very poor physical properties. Cellular activity was often much less than would have been predicted from the bicyclic series, and the one compound examined in vivo had plasma levels far below predicted efficacy levels even with ip dosing.

Experimental Section

General Methods. See Supporting Information.

4-Chloro-5-methyl-5*H*-pyrimido[5,4-*b*]indole (12). A mixture of 4-chloro-5*H*-pyrimido[5,4-b]indole hydrochloride[23] (**11**) (731 mg, 3.6 mmol), dimethyl sulfate (0.41 mL, 4.3 mmol), Cs_2CO_3 (3.5 g, 10.7 mmol), activated 3A molecular sieves (2.7 g), and acetone (10 mL) was heated at reflux for 6 h, then stirred at 25 °C for 36 h. The suspension was filtered over Celite and the filtrate was concentrated to a solid that was distributed between $CHCl_3$ and 1% aq NaOH. The organic layer was washed with H_2O (2×), dried, and concentrated to a solid that was purified by column chromatography eluting with 0% and then 1% EtOH in $CHCl_3$. The pooled product fractions were concentrated to a residue that was crystallized to give **12** (278 mg, 35%): mp 153−154 °C (lit.[23] mp 145−146 °C).

2-Amino-5-methoxy-1*H*-indole-3-carboxylic Acid Ethyl Ester (13b) and 2-Amino-1-hydroxy-5-methoxy-1*H*-indole-3-carboxylic Acid Ethyl Ester (20). To an ice-cold solution of ethyl cyanoacetate (10.9 mL, 102.4 mmol) in anhydrous THF (170 mL) under N_2 was added potassium *tert*-butoxide (12.07 g, 107.5 mmol). The formed white suspension was stirred for 15 min then treated with 3-fluoro-4-nitroanisole (**18**)[37] (8.86 g, 51.2 mmol). The suspension was heated at reflux for 1.5 h. The solution was poured into H_2O, and the aqueous mixture was acidified to pH 2 with concentrated HCl. The mixture was extracted with ether (3×) and then the combined organic phases were dried and concentrated to give an oil that was evacuated at 0.3 mm for 2 days. The oil was dissolved in CH_2Cl_2 and purified by column chromatography eluting with CH_2Cl_2. The product fractions were combined and concentrated to provide cyano(5-methoxy-2-nitrophenyl)acetic acid ethyl ester (**19**) (14.5 g) as a light yellow oil that was ∼93−95% pure: ^{1}H NMR ($CDCl_3$) δ 8.29 (1H, d, J = 9.2 Hz), 7.22 (1H, d, J = 2.7 Hz), 7.04 (1H, dd, J = 9.2, 2.7 Hz), 5.69 (1H, s), 4.31 (2H, q, J = 7.0 Hz), 1.34 (3H, t, J = 7.2 Hz). The material was used directly in the next step.

A solution of impure **19** (13.2 g, ∼46.3 mmol) in glacial AcOH (185 mL) was treated with a single charge of Zn dust (12.1 g, 185 mmol). The mixture was heated at 55 °C for 45 min, then treated with more Zn (4 g). After heating for another 105 min, the brown mixture was filtered through a pad of SiO_2. The pad was washed well with AcOH and the filtrate was concentrated to a residue that was distributed between CH_2Cl_2 and H_2O. The organic phase was washed with 5% aq $NaHCO_3$ and concentrated to a residue that showed a ∼1:1 mixture of products by TLC (3:1 CH_2Cl_2/EtOAc). The residue was purified by column chromatography eluting sequentially with 0%, 5%, and 10% EtOAc in CH_2Cl_2. The fractions containing the pure higher R_f product were combined and concentrated to a solid that was sonicated in *tert*-butyl methyl ether. The solid was collected to give pure **13b** (2.07 g, 19%) as an off-white solid: mp 149−151 °C. Further chromatography of the combined mother liquor and impure fractions afforded additional **13b** (120 mg, 1%): ^{1}H NMR δ 10.44 (1H, br s, exchanges with D_2O), 7.11 (1H, d, J = 2.2 Hz), 6.98 (1H, d, J = 8.4 Hz), 6.61 (2H, br s, exchanges with D_2O), 6.48 (1H, dd, J = 8.4, 2.7 Hz), 4.20 (2H, q, J = 7.0 Hz), 3.71 (3H, s), 1.32 (3H, t, J = 7.2 Hz); CIMS *m*/*z* (relative intensity) 235 (MH^+, 81), 234 (M^+, 100). Anal. ($C_{12}H_{14}N_2O_3$) C, H, N.

Processing of the pure cuts from the lower R_f fractions provided **20** (278 mg) following crystallization from EtOAc: mp 163−164 °C; ^{1}H NMR δ 11.11 (1H, br s, exchanges with D_2O), 7.18 (s, 1H), 7.00 (1H, d, J = 8.4 Hz), 6.75 (2H, br s, exchanges with D_2O), 6.57 (1H, dd, J = 8.4, 2.4 Hz), 4.21 (2H, q, J = 7.0 Hz), 3.73 (3H, s), 1.32 (3H, t, J = 7.1 Hz); CIMS *m*/*z* (relative intensity) 250 (M^+, 10). Anal. ($C_{12}H_{14}N_2O_4$) C, H, N.

2-[(Aminoiminomethyl)amino]-1*H*-indole-3-carboxylic Acid Ethyl Ester, Hydrochloride (14c). A suspension of 2-amino-1*H*-indole-3-carboxylic acid ethyl ester (**13a**)[25] (2.04 g, 10.0 mmol), cyanamide (534 mg, 12.7 mmol), and concentrated HCl (1 mL) in dioxane (91 mL) was heated at reflux for 48 h. After the reaction mixture had cooled to 25 °C, it was filtered and the solid was washed well with dry diethyl ether and then air-dried to give **14c** (1.08 g, 38%) as an off-white solid: mp >250 °C. The compound was used directly in the next step.

2-[(1-Iminoethyl)amino]-1*H*-indole-3-carboxylic Acid Ethyl Ester, Hydrochloride (14d). Dry HCl was bubbled into a suspension of 2-amino-1*H*-indole-3-carboxylic acid ethyl ester (**13a**)[25] (1.0 g, 4.9 mmol) and CH_3CN (65 mL) for 1.5 h. The mixture was heated at reflux for 2.5 h and then cooled to

25 °C. The precipitate was collected and dried to give **14d** (1.02 g, 74%): mp >300 °C; ^{1}H NMR δ 12.63 (1H, br s, exchanges with D_2O), 11.89 (1H, br s, exchanges with D_2O), 9.95 (1H, br s, exchanges with D_2O), 9.0 (1H, br s, exchanges with D_2O), 8.26 (1H, d, J = 7.2 Hz), 7.49 (1H, d, J = 7.7 Hz), 7.33−7.24 (2H, m), 4.29 (2H, q, J = 7.0 Hz), 2.40 (3H, br s), 1.34 (3H, t, J = 7.2 Hz). Anal. ($C_{13}H_{15}N_3O_2$·1.2HCl) C, H, N.

1,9-Dihydro-6-methoxy-4*H*-pyrimido[4,5-*b*]indol-4-one (15b). A solution of 2-amino-5-methoxy-1*H*-indole-3-carboxylic acid ethyl ester (**13b**) (2.15 g, 9.2 mmol), NaOMe (0.5 g, 9.3 mmol), and formamide (200 mL) was heated under N_2 at 220 °C for 1.5 h. The solution was cooled to 25 °C, stored for 2.5 days, and filtered. The solvent was evaporated by Kugelrohr distillation at 95 °C/0.8 mmHg to leave a residue that was triturated in H_2O and then heated in 35 mL of boiling DMF. The suspension was filtered hot over a pad of SiO_2, and the filtrate was concentrated to a solid that was sonicated in ~30 mL of MeOH. The solid was collected and dried to leave **15b** (1.71 g, 72%) that was ~83% pure: ^{1}H NMR δ 12.16 (1H, br s, exchanges with D_2O), 12.04 (1H, br s, exchanges with D_2O), 8.08 (1H, d, J = 3.4 Hz, exchanges to s with D_2O), 7.46 (1H, d, J = 1.9 Hz), 7.37 (1H, d, J = 8.7 Hz), 6.95 (1H, dd, J = 8.8, 2.5 Hz), 3.81 (s, 3 H); CIMS m/z (relative intensity) 216 (MH^+, 100), 215 (M^+, 52). The material was used directly in the next step.

2-Amino-1,9-dihydro-4*H*-pyrimido[4,5-*b*]indol-4-one (15c). A mixture 2-[(aminoiminomethyl)amino]-1*H*-indole-3-carboxylic acid ethyl ester hydrochloride (**14c**) (1.0 g, 3.5 mmol) and NaOH (1.5 g) in H_2O (50 mL) was heated at gentle reflux for 6 h. The mixture was acidified to pH 1 with 5% aq HCl, and filtered through Celite. The filtrate was extracted with EtOAc (3×) and then made basic with Na_2CO_3. The precipitate that slowly formed was collected and dried to leave **15c** (561 mg, 78%) as light tan crystals: mp >275 °C. The material was used directly in the next step.

1,9-Dihydro-2-methyl-4*H*-pyrimido[4,5-*b*]indol-4-one (15d). A solution of 2-[(1-iminoethyl)amino]-1*H*-indole-3-carboxylic acid ethyl ester hydrochloride (**14d**) (1.0 g, 3.6 mmol), EtOH (300 mL), and 10% aq NaOH (30 mL) was heated at reflux for 6 h. The cooled solution was concentrated to a residue that was diluted with 20 mL of H_2O. The solution was adjusted to pH 2 with aq HCl then stored at 25 °C for 30 min. The precipitated solid was collected and dried to leave **15d** (618 mg, 87%): mp >275 °C; ^{1}H NMR δ 12.11 (1H, br s, exchanges with D_2O), 11.97 (1H, br s, exchanges with D_2O), 7,93 (1H, d, J = 7.7 Hz), 7.43 (1H, d, J = 7.9 Hz), 7.28 (1H, t, J = 7.5 Hz), 7.20 (1H, t, J = 7.7 Hz), 2.41 (s, 3 H); CIMS m/z (relative intensity) 200 (MH^+, 100), 199 (M^+, 85). The material was used directly in the next step.

4-Chloro-1*H*-pyrimido[4,5-*b*]indole Hydrochloride (16a). A mixture of 9*H*-pyrimido[4,5-*b*]indol-4-ol[26] (**15a**) (5.3 g, 28.6 mmol), $POCl_3$ (50 mL), and *p*-dioxane (125 mL) was heated at reflux for 6 h, then stirred at 25 °C for 36 h. The mixture was filtered and the filtrate was concentrated to an oil that solidified. The solid was triturated in hot EtOH and collected to give **16a** (3.1 g, 45%) in two crops: mp 253−254 °C (lit.[26] mp 288−290 °C); ^{1}H NMR δ 12.84 (1H, br s, exchanges with D_2O), 8.79 (1H, s), 8.29 (1H, d, J = 8.0 Hz), 7.68−7.42 (3H, m). The material was used directly in the next step.

4-Chloro-6-methoxy-1*H*-pyrimido[4,5-*b*]indole (16b). A suspension of 1,9-dihydro-6-methoxy-4*H*-pyrimido[4,5-*b*]indol-4-one (**15b**) (800 mg, 3.1 mmol, ~83% pure) and $POCl_3$ (7 mL) was heated at 90 °C for 6 h. The suspension was concentrated to a solid that was evacuated at 1 mmHg for 1 h. The solid was cooled in a −78 °C bath then treated dropwise with cold H_2O. The bath was removed and the frozen solid was allowed to gradually melt. The solid was filtered, washed well with cold H_2O, and dried to leave **16b** (733 mg, 81%) that was ~80% pure: ^{1}H NMR δ 12.64 (1H, br s, exchanges with D_2O), 8.74 (1H, s), 7.74 (1H, d, J = 2.4 Hz), 7.57 (1H, d, J = 8.9 Hz), 7.28 (1H, dd, J = 8.9, 2.4 Hz), 3.88 (3H, s); CIMS m/z (relative intensity) 236 (^{37}Cl, MH^+, 29), 235 (^{37}Cl, M^+, 37), 234 (^{35}Cl, MH^+, 100), 233 (^{35}Cl, M^+, 87). The material was used directly in the next step.

4-Chloro-1*H*-pyrimido[4,5-*b*]indole-2-amine Hydrochloride (16c). A suspension of 2-amino-1,9-dihydro-4*H*-pyrimido[4,5-*b*]indol-4-one (**15c**) (490 mg, 2.5 mmol) and $POCl_3$ (7 mL, 75 mmol) in *p*-dioxane (13 mL) was heated at reflux for 4 h, then concentrated. The residue was triturated in EtOH and the solid was collected to give **16c** (170 mg, 27%): mp >250 °C. The material was used directly in the next step.

4-Chloro-9-methyl-9*H*-pyrimido[4,5-*b*]indole (17a). A mixture of 4-chloro-1*H*-pyrimido[4,5-*b*]indole (**16a**) (610 mg, 3 mmol), dimethyl sulfate (0.34 mL, 3.6 mmol), Cs_2CO_3 (2.93 g, 9 mmol), activated 3A molecular sieves (2.4 g), and acetone (9 mL) was heated at reflux for 1.5 h. The suspension was filtered through Celite, and the filtrate was concentrated to a solid that was dissolved in $CHCl_3$. The solution was washed with H_2O (2×), dried, and concentrated to a solid that was crystallized from 1:1 EtOAc:petroleum ether. The solid was collected to give **17a** (285 mg, 44%): mp 129−130 °C (lit.[28] mp 128−130 °C); ^{1}H NMR ($CDCl_3$) δ 8.80 (1H, s), 8.41 (1H, d, J = 8.0 Hz), 7.68−7.64 (1H, m) 7.54 (1H, d, J = 8.2 Hz), 7.48−7.44 (1H, m), 1.62 (3H, br s); CIMS m/z (relative intensity) 220 (^{37}Cl, MH^+, 34), 219 (^{37}Cl, M^+, 24), 218 (^{35}Cl, MH^+, 100), 217 (^{35}Cl, M^+, 41).

4-Chloro-*N*,*N*-diethyl-9*H*-pyrimido[4,5-*b*]indole-9-ethanamine (17b). A suspension of 4-chloro-1*H*-pyrimido[4,5-*b*]indole hydrochloride (**16a**) (407 mg, 2 mmol), 2-diethylaminoethyl chloride hydrochloride (413 mg, 2.4 mmol), Cs_2CO_3 (1.95 g, 6 mmol), and 4A molecular sieves (1.5 g) in acetone (6 mL) was heated at reflux under N_2 for 1.5 h. The mixture was filtered through Celite, and the filtrate was concentrated to a viscous oil that was dissolved in CH_2Cl_2. The solution was washed with H_2O, dried, and concentrated to a residue that was purified by column chromatography, eluting with 4% MeOH in $CHCl_3$, to give **17b** (495 mg, 82%) as a pale yellow oil: ^{1}H NMR δ 8.79 (1H, s), 8.41 (1H, d, J = 8.0 Hz), 7.66−7.58 (2H, m), 7.46−7.42 (1H, m), 4.57 (2H, t, J = 6.8 Hz), 2.90 (2H, t, J = 7.1 Hz), 2.63 (4H, d, J = 7.0 Hz), 0.99 (6H, t, J = 7.0 Hz); CIMS m/z (relative intensity) 305 (^{37}Cl, MH^+, 16), 304 (^{37}Cl, M^+, 9), 303 (^{35}Cl, MH^+, 47).

4-Chloro-*N*,*N*-diethyl-6-methoxy-9*H*-pyrimido[4,5-*b*]indole-9-ethanamine (17c). Reaction of a suspension of 4-chloro-6-methoxy-1*H*-pyrimido[4,5-*b*]indole (**16b**) (773 mg, 2.5 mmol, ~80% pure), 2-diethylaminoethyl chloride hydrochloride (582 mg, 3.4 mmol), Cs_2CO_3 (2.3 g, 7.1 mmol), 4A molecular sieves (2.1 g), and 2:1 acetone:DMF (12 mL) was refluxed for 16.5 h as described for **17b**. Similar workup followed by column chromatography, eluting with 0% and then 2% MeOH in CH_2Cl_2, gave **17c** (667 mg, 80%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 8.75 (1H, s), 7.87 (1H, d, J = 2.4 Hz), 7.47 (1H, d, J = 8.9 Hz), 7.25 (1H, dd, J = 8.9, 2.4 Hz), 4.50 (2H, t, J = 7.2 Hz), 3.96 (3H, s), 2.86 (2H, t, J = 7.1 Hz), 2.59 (4H, q, J = 7.1 Hz), 0.96 (6H, t, J = 7.1 Hz); CIMS m/z (relative intensity) 335 (^{37}Cl, MH^+, 7), 333 (^{35}Cl, MH^+, 22). Anal. ($C_{17}H_{21}N_4ClO$) C, H, N.

6-Nitrobenzo[*b*]thieno[3,2-*d*]pyrimid-4(3*H*)-one (21h). A mixture of methyl 3-amino-7-nitrobenzothiophene-2-carboxylate (242 mg, 0.96 mmol) and formamidine acetate (0.51 g, 4.9 mmol) was heated at 190 °C for 1 h. After cooling, the mixture was slurried in H_2O, and the formed precipitate was collected and dried to give **21h** (162 mg, 68%) as a brown solid: ^{1}H NMR δ 8.67 (d, 2H, J = 8.1 Hz, H-7,H-9), 8.39 (s, 1H, H-2), 7.85 (t, 1H, J = 7.8 Hz, H-8). The material was used directly in the next step.

Methyl 3-amino-7-nitrobenzothiophene-2-carboxylate was prepared as follows: Triethylamine (0.16 mL, 1.15 mmol) was added dropwise to a solution of 2-chloro-3-nitrobenzonitrile (191 mg, 1.05 mmol) and methyl thioglycolate (0.10 mL, 1.1 mmol) in DMSO, and the mixture was stirred under N_2 at 25 °C. After 30 min the mixture was poured onto H_2O, and the formed precipitate was collected and dried to give methyl 3-amino-7-nitrobenzothiophene-2-carboxylate (244 mg, 92%) as an orange-red solid: ^{1}H NMR δ 8.60 (dd, 1H, J = 1.0, 8.1 Hz, H-4), 8.53 (dd, 1H, J = 0.9, 7.9 Hz, H-6), 7.66 (t, 1H, J = 7.9 Hz, H-5), 7.31 (br s, 2H, NH_2), 3.77 (s, 3H, OMe).

2-Chloro-3-nitrobenzonitrile was prepared as follows: DMF

(5 drops) was added to a slurry of 2-chloro-3-nitrobenzoic acid (987 mg, 4.9 mmol) and oxalyl chloride (5.4 mmol) in CH_2Cl_2 (20 mL) at 25 °C. Following gas evolution, solution was gradually achieved. After 3 h, the mixture was concentrated to a yellow residue that was redissolved in CH_2Cl_2 (2 mL). The solution was treated rapidly with cold concentrated aq NH_3 (2 mL). After 10 min the formed precipitate was collected and dried. The solid was added to a solution of PPSA (prepared from P_2O_5 and hexamethyldisiloxane as described[38]) in 1,2-dichloroethane (30 mL), and the mixture was refluxed for 16 h. The reaction mixture was filtered through a pad of SiO_2, which was eluted with hexanes (250 mL) and then 5% MeOH in $CHCl_3$ (400 mL). The latter washes were concentrated to give a white solid that was dissolved in a minimum of $CHCl_3$ and eluted through a second pad of SiO_2, eluting with 1% MeOH in $CHCl_3$ (400 mL). Concentration of the eluate gave the desired nitrile (0.66 g, 74%) as a white solid: ^{1}H NMR δ 8.36 (dd, 1H, J = 1.6, 8.2 Hz, H-4), 8.27 (dd, 1H, J = 1.6, 8.0 Hz, H-6), 7.75 (t, 1H, J = 8.1 Hz, H-5).

4-Chlorobenzothieno[3,2-*d*]pyrimidine (22a). Vilsmeier's reagent was made by the dropwise addition of DMF (0.27 g, 3.5 mmol) to a solution of oxalyl chloride (0.44 g, 3.5 mmol) in 1,2-dichloroethane (10 mL) with stirring at 25 °C. After vigorous gas evolution had ceased, benzothieno[3,2-*d*]-3*H*-pyrimid-4-one (**21a**)[31] (337 mg, 1.53 mmol) was added and the mixture was heated to reflux. After 20 min, the mixture was cooled and then quenched with saturated aq $NaHCO_3$ (20 mL). The phases were separated, and the aqueous phase was extracted with $CHCl_3$ (3×). The combined organic phases were washed with H_2O (2×) and brine, dried, and concentrated to give **22a** (249 mg, 74%) as a light brown solid: ^{1}H NMR ($CDC1_3$) δ 9.09 (1H, s, H-2), 8.53 (1H, dd, J = 1.8, 7.6 Hz, H-6), 7.95 (1H, d, J = 7.8 Hz, H-9), 7.73 (1H, dt, J_d = 1.4 Hz, J_t = 7.7 Hz, H-7), 7.62 (1H, dt, J_d = 1.2 Hz, J_t = 7.5 Hz, H-8).

7-Amino-8-fluoro-4-thiomethylbenzothieno[3,2-*d*]pyrimidine (23d). A mixture of P_2S_5 (620 mg, 2.8 mmol) and 7-amino-8-fluoro-3*H*-[1]benzothieno[3,2-*d*]pyrimid-4-one (**21d**) (620 mg, 2.64 mmol) was stirred at 115 °C in diglyme (13 mL) under N_2 for 1 h and cooled to 25 °C. The mixture was treated with diisopropylamine (1.29 g, 10 mmol) and MeI (0.31 mL, 5 mmol) and stirred under N_2 for 10 min, then filtered. The filtrate was poured onto stirred ice H_2O and the formed precipitate was collected, triturated in $CHCl_3$, and dried to give **23d** (181 mg, 26%): ^{1}H NMR δ 8.96 (1H, s, H-2), 7.91 (1H, d, J = 11.2 Hz, H-9), 7.32 (1H, d, J = 7.6 Hz, H-6), 6.24 (2H, br s, NH_2), 2.74 (3H, s, SCH_3).

7-Ethylamino-8-fluoro-4-thiomethylbenzo[*b*]thieno-[3,2-*d*]pyrimidine (23e). A suspension of 7-ethylamino-8-fluoro-3*H*-[1]benzothieno[3,2-*d*]pyrimid-4-one (264 mg, 1.0 mmol) (**21e**) and Lawesson's reagent[32] (675 mg, 1.65 mmol) in diglyme (5 mL) was stirred under N_2 at 112 °C for 6.5 h. After cooling, the mixture was concentrated and the residue was dissolved in DMSO (3 mL). Diisopropylamine (263 mg, 2 mmol) was added, forming an orange-brown solution, followed by MeI (287 mg, 2 mmol). After 20 min, the mixture was diluted with H_2O. The formed precipitate was collected and dried to give **23e** (266 mg, 77%) as a dark brown solid: ^{1}H NMR δ 8.93 (1H, s, H-2), 7.87 (1H, d, J = 11.5 Hz, H-9), 7.32 (1H, d, J = 7.6 Hz, H-6), 6.48 (1H, br t, NH), 3.24 (2H, p, J = 6.6 Hz, NHCH_2), 2.72 (3H, s, SCH_3), 1.23 (3H, t, J = 7.1 Hz, CH_3).

Ethyl 3-Aminopyrido[3,2-*b*]thiophene-2-carboxylate (25). Ethyl thioglycolate (0.12 mL, 1.1 mmol) was added to a stirred mixture of NaH (0.036 g, 1.5 mmol) and DMSO (1 mL) under N_2 at 25 °C. After formation of H_2 had ceased, a solution of 2-chloro-3-cyanopyridine (**24**) (0.14 g, 1.0 mmol) in DMSO (2 mL) was added dropwise. After 3 h the mixture was poured onto stirred ice H_2O. The light yellow precipitate was collected and dried to give **25** (197 mg, 89%): ^{1}H NMR δ 8.68 (1H, dd, J = 4.6, 1.6 Hz, H-6), 8.54 (1H, dd, J = 8.2, 1.6 Hz, H-4), 7.46 (1H, dd, J = 8.2, 4.5 Hz, H-5), 7.31 (2H, br s, NH_2), 4.3 (2H, q, J = 7.1 Hz, OCH_2-), 1.29 (3H, t, J = 7.1 Hz, CH_3).

3*H*-Pyrido[3′,2′;4,5]thieno[3,2-*d*]pyrimid-4-one (26). A mixture of ethyl 3-aminopyrido[3,2-*b*]thiophene-2-carboxylate (**25**) (0.92 g, 4.14 mmol) and formamide (10 mL) was heated at 135 °C for 1 h and then at 190 °C for 4 h. The reaction mixture was cooled to 25 °C and the solid was collected and dried to give **26** (0.61 g, 73%) as yellow-brown needles: ^{1}H NMR δ 13.0 (1H, br s, NH), 8.86 (1H, dd, J = 4.6, 1.6 Hz, H-7), 8.63 (1H, dd, J = 8.0, 1.6 Hz, H-9), 8.4 (1H, s, H-2), 7.68 (1H, dd, J = 8.1, 4.6 Hz, H-8).

4-Chloropyrido[3′,2′;4,5]thieno[3,2-*d*]pyrimidine (27). Following reaction of Vilsmeier's reagent (15 mmol), made as described for **22a**, with 3*H*-pyrido[3′,2′;4,5]thieno[3,2-*d*]-pyrimid-4-one (**26**) (0.61 g, 3.0 mmol) in 1,2-dichloroethane (75 mL) at 85 °C for 2 h, the mixture was cooled and distributed between $CHCl_3$ and H_2O. The organic extracts were washed with H_2O and brine, dried, and concentrated to give **27** (0.64 g, 96%) as a yellow solid: ^{1}H NMR δ 9.3 (1H, br s, H-2), 9.0 (1H, dd, J = 4.7, 1.7 Hz, H-7), 8.9 (1H, dd, J = 7.3, 1.7 Hz, H-9), 7.8 (1H, dd, J = 7.3, 4.7 Hz, H-8).

Methyl 2-(2-Cyanophenoxy)ethanoate (30). Methyl bromoacetate (1.95 mL, 20 mmol) was added dropwise to a stirred solution of 2-cyanophenol (**29**) (2.38 g, 20 mmol) and K_2CO_3 (2.78 g, 20.1 mmol) in acetone (100 mL) at 25 °C. After 24 h, the mixture was filtered and the filtrate was concentrated to leave **30** (3.82 g, 100%) as a beige solid: ^{1}H NMR δ 7.76 (1H, dd, J = 7.6, 1.7 Hz, H-3), 7.64 (1H, dt, J_d = 1.6 Hz, J_t = 8.0 Hz, H-4), 7.20−7.10 (2H, m, H-5, H-6), 5.04 (2H, s, OCH_2-), 3.70 (3H, s, COOMe). The material was used directly in the next step.

Methyl 3-Aminobenzo[*b*]furan-2-carboxylate (31). A solution of methyl 2-(2-cyanophenoxy)ethanoate (**30**) (3.82 g, 20 mmol) in DMSO (40 mL) was added dropwise to a stirred suspension of NaH (0.504 g, 21 mmol) and DMSO (10 mL) under N_2 at 25 °C. After 10 min the mixture was poured onto ice H_2O and extracted with ether. The combined extracts were washed with H_2O and brine, dried, and concentrated to leave **31** (2.15 g, 56%) as a yellow solid: ^{1}H NMR δ 7.95 (1H, d, J = 7.7 Hz, H-4), 7.48 (2H, d, J = 3.4 Hz, H-6, H-8), 7.29−7.22 (1H, m, H-7), 6.40 (2H, br s, NH_2), 3.80 (3H, s, $COOCH_3$).

3*H*-Benzofurano[3,2-*d*]pyrimid-4-one (32). A solution of methyl 3-aminobenzo[*b*]furan-2-carboxylate (**31**) (0.28 g, 1.36 mmol) in formamide (5 mL) was heated at 135 °C for 4 h; then the temperature was raised to 170 °C. After 4 h the reaction was cooled to 25 °C and a dark purple solid precipitated. The solid was collected and air-dried to leave **32** (118 mg, 47%): ^{1}H NMR δ 13.0 (1H, br s, NH), 8.25 (1H, s, H-2), 8.05 (1H, d, J = 8.1 Hz, H-6), 7.84 (1H, d, J = 8.3 Hz, H-9), 7.68 (1H, t, J = 7.7 Hz, H-8), 7.51 (1H, t, J = 7.7 Hz, H-7).

4-Chlorobenzofurano[3,2-*d*]pyrimidine (33). Following reaction of Vilsmeier's reagent (3.1 mmol), made as described for **22a**, with 3*H*-benzofurano[3,2-*d*]pyrimid-4-one (**32**) (113,-mg, 0.61 mmol) in 1,2-dichloroethane (15 mL) at reflux for 1 h, the mixture was cooled and distributed between H_2O and $CHCl_3$. The combined extracts were washed with H_2O and then brine, dried, and concentrated to give **33** (116 mg, 93%) as a yellow solid: ^{1}H NMR δ 9.08 (1H, s, H-2), 8.30 (1H, d, J = 8.1 Hz, H-6), 8.02 (1H, d, J = 8.5 Hz, H-9), 7.90, (1H, dt, J_d = 1.3 Hz, J_t = 7.1 Hz, H-8), 7.64 (1H, dt, J_d = 1.0 Hz, J_t = 7.8 Hz, H-7).

General Procedure A. A stirred solution of the tricyclic 4-chloro- or 4-thiomethylpyrimidine and -aniline (2 molar excess) in a lower alcohol solvent (0.15−1.0 M solution) was heated at reflux until TLC showed complete consumption of the starting pyrimdine. Occasionally, reactions were run in DMA, ethylene glycol, or 2-methoxyethanol at the indicated temperatures. All reactions were catalyzed with anhydrous HCl. After cooling to 25 °C, the mixture was worked up to provide the desired product. The following compounds were made by this procedure.

***N*-Phenyl-5*H*-pyrimido[5,4-*b*]indole-4-amine Hydrochloride (34).** Reaction of 4-chloro-5*H*-pyrimido[5,4-*b*]indole hydrochloride (**11**)[23] (240 mg, 1.0 mmol), aniline (0.273 mL, 3 mmol), and EtOH (1 mL) was followed by dilution of the suspension with EtOH (4 mL). The solid was collected, washed with H_2O and EtOH, and then recrystallized from DMF/H_2O to give **34** (82 mg, 27%): mp >340 °C; ^{1}H NMR δ 12.79 (1H,

br s), 11.04 (1H, br s), 8.94 (1H, s), 8.27 (1H, d, J = 8.2 Hz), 7.96 (2H, d, J = 7.5 Hz), 7.85 (1H, d, J = 8.4 Hz), 7.71 (1H, t, J = 7.7 Hz), 7.49 (2H, t, J = 8.0 Hz), 7.41 (1H, t, J = 7.6 Hz), 7.24 (1H, t, J = 7.4 Hz); CIMS *m/z* (relative intensity) 261 (MH^+, 100), 260 (M^+, 57).

***N*-(3-Bromophenyl)-6-methoxy-1*H*-pyrimido[4,5-*b*]indole-4-amine (49)**. Reaction of 4-chloro-6-methoxy-1*H*-pyrimido[4,5-*b*]indole (**16b**) (107 mg, 0.37 mmol, 80% pure), 3-bromoaniline (0.15 mL, 1.4 mmol), DMA (1 mL), and 8.5 M HCl in 2-propanol (1 drop) at 120 °C for 5 h was followed by concentration to a residue that was triturated in 5% aq $NaHCO_3$ and collected. The solid was dissolved in a minimum volume of DMF and purified by preparative TLC (elution with 3:2 CH_2Cl_2/EtOAc). The product band was extracted from the SiO_2 by sonication in EtOAc and filtration. The filtrate was concentrated to a solid that was triturated in MeOH, collected, and dried to give **49** (39 mg, 28%): ^{1}H NMR δ 11.99 (1H, br s, exchanges with D_2O), 8.97 (1H, br s, exchanges with D_2O), 8.44 (1H, s), 8.02 (1H, s), 7.91 (1H, d, J = 2.4 Hz), 7.76 (1H, d, J = 8.0 Hz), 7.42 (1H, d, J = 8.7 Hz), 7.36−7.24 (2H, m), 7.08 (1H,-dd, J = 8.7, 2.2 Hz), 3.87 (3H, s); CIMS *m/z* (relative intensity) 371 (^{81}Br, MH^+, 74), 370 (^{81}Br, M^+, 71), 369 (^{79}Br, MH^+, 100), 368 (^{79}Br, M^+, 65).

4-(3-Bromoanilino)benzothieno[3,2-*d*]pyrimidine (53). Reaction of 4-chlorobenzothieno[3,2-*d*]pyrimidine (**22a**) (110.1 mg, 0.5 mmol), 3-bromoaniline (107.2 mg, 0.62 mmol), triethylamine (102.8 mg, 1.0 mmol), and ethoxyethanol (2 mL) at 110 °C for 18 h was followed by concentration to an oil that was purified by preparative TLC (elution with 2% MeOH in $CHCl_3$). Extraction of the product band gave a solid that was crystallized from EtOH to leave **53** (70 mg, 39%) as pale beige plates: mp 231−233 °C; ^{1}H NMR ($CDCl_3$) δ 8.88 (1H, s, H-2), 8.49 (1H, dd, J = 1.7, 7.1 Hz, H-9), 7.96 (1H, t, J = 1.9 Hz, H-2′), 7.89 (1H, dd, J = 1.6, 7.0 Hz, H-6), 7.65 (1H, dt, J_d = 1.5 Hz, J_t = 7 Hz, H-7), 7.60 (1H, dd, J = 1.5, 7.5 Hz, H-6′), 7.57 (1H, dt, J_d = 1.5 Hz, J_t = 7 Hz, H-8), 7.40 (1H, dt, J_d = 1.7 Hz, J_t = 8 Hz, H-4′), 7.28 (1H, t, J = 7.8 Hz, H-5′), 6.90 (1H, br s, NH); CIMS *m/z* (relative intensity) 358 (^{81}Br, MH^+, 68), 357 (^{81}Br, M^+, 48), 356 (^{79}Br , MH^+, 100), 355 (^{79}Br , M^+, 41).

7-Amino-4-(3-bromoanilino)-8-fluorobenzo[*b*]thieno-[3,2-*d*]pyrimidine (64). Reaction of 7-amino-8-fluoro-4-thiomethylbenzothieno[3,2-*d*]pyrimidine (**23d**) (53 mg, 0.2 mmol), 3-bromoaniline (69 mg, 0.4 mmol), 3-bromoaniline hydrochloride (125 mg, 0.6 mmol), and ethylene glycol (1 mL) at 140 °C for 38 h was followed by pouring the solution onto ice H_2O to precipitate a solid that was collected. Purification by preparative TLC (elution with 5% MeOH in $CHCl_3$) followed by extraction of the product band and crystallization from EtOH gave **64** (18 mg, 23%) as a mustard yellow solid: mp 244−246 °C; ^{1}H NMR δ 9.62 (1H, s, NH), 8.67 (1H, s, H-2), 8.16 (1H, t, J = 1.9 Hz, H-2′), 7.85 (1H, d, J = 11.1 Hz, H-9), 7.80 (1H, ddd, J = 1.0, 1.9, 8.2 Hz, H-6′), 7.32 (1H, t, J = 8.0 Hz, H-5′), 7.32 (1H, d, J = 7.7 Hz, H-6), 7.26 (1H, ddd, J = 1.0, 1.9, 8.0 Hz, H-4′), 6.04 (2H, br s, NH_2); CIMS *m/z* (relative intensity) 391 (^{81}Br, MH^+, 77), 390 (^{81}Br, M^+, 65), 389 (^{79}Br , MH^+, 100), 388 (^{79}Br , M^+, 50).

General Procedure B. A stirred mixture of the tricyclic 4-chloropyrimidine and the neat amine, usually with a catalytic amount of HCl, was heated at an elevated temperature until TLC showed complete consumption of starting pyrimidine. After cooling to 25 °C, excess amine was removed under vacuum and the residue was further processed to give the desired product. The following compounds were made by this procedure.

***N*-(Phenylmethyl)-5*H*-pyrimido[5,4-*b*]indole-4-amine (37).** Reaction of 4-chloro-5*H*-pyrimido[5,4-*b*]indole hydrochloride (**11**)[23] (240 mg, 1 mmol) and benzylamine (1 mL) at 150 °C for 6 h provided a soft solid that was dissolved in EtOAc. The solution was washed with saturated aq $NaHCO_3$, H_2O, and brine, dried, and concentrated to leave a residue that was triturated in CH_2Cl_2 to provide **37** (190 mg, 69%): mp 242−244 °C; ^{1}H NMR ($CDCl_3$) δ 10.58 (1H, br s), 8.60 (1H, s), 8.08 (1H, d, J = 8.0 Hz), 7.47−7.14 (8H, m), 4.82 (2H, d, J = 5.6 Hz), 2.41 (1H, br s); CIMS *m/z* (relative intensity) 275 (MH^+, 100), 274 (M^+, 17).

General Procedure C. A slurry of the 4-anilinonitrobenzo-[*b*]thieno[3,2-*d*]pyrimidine, Raney Ni, and an appropriate solvent was hydrogenated at ∼50 psi/25 °C until the theoretical amount of hydrogen had been taken up. The mixture was filtered over Celite and the filtrate was concentrated to give a residue that was further worked up to give the product. The following compounds were made by this procedure.

8-Amino-4-anilinobenzo[*b*]thieno[3,2-*d*]pyrimidine (58). Hydrogenation of 4-anilino-8-nitrobenzo[*b*]thieno[3,2-*d*]pyrimidine (**54**) (234 mg, 0.65 mmol), Raney Ni (0.2 g), and THF (40 mL)/MeOH (35 mL) for 17 h followed by crystallization from EtOH gave **58** (59 mg, 30%) as a khaki solid: mp 241−243 °C; ^{1}H NMR δ 9.54 (1H, br s, NH), 8.67 (1H, s, H-2), 7.78 (2H, dd, J = 0.9, 8.5 Hz, H-2′, H-6′), 7.75 (1H, d, J = 8.7 Hz, H-6), 7.49 (1H, d, J = 1.9 Hz, H-9), 7.38 (1H, t, J = 7.9 Hz, H-3′), 7.12 (1H, dt, J_d = 7.5, J_t = 1.1 Hz, H-4′), 7.10 (1H, dd, J = 2.3, 8.7 Hz), 5.45 (2H, br s, NH_2); CIMS *m/z* (relative intensity) 293 (MH^+, 100), 292 (M^+, 65).

Procedure D. 4-(3-Bromoanilino)-8-methylaminobenzo[*b*]thieno[3,2-*d*]pyrimidine (67) and 4-(3-Bromoanilino)-8-dimethylaminobenzo[*b*]thieno[3,2-*d*]pyrimidine (68). To a 0 °C mixture of 8-amino-4-(3-bromoanilino)benzo[*b*]-thieno[3,2-*d*]pyrimidine (**61**) (363 mg, 0.98 mmol), formic acid (0.13 g, 88%, 2.5 mmol), and H_2O (2 mL) was added dropwise formaldehyde (0.22 g, 2.6 mmol). The mixture was heated at 90 °C for 24 h then cooled and extracted with $CHCl_3$. The combined organic phases were washed with H_2O and brine, dried, and concentrated to a solid. Purification by preparative TLC (elution with 2% MeOH in $CHCl_3$) gave two products. The upper band was extracted to give **68** (29 mg, 7.4%) as a yellow solid: mp 190−192 °C; ^{1}H NMR δ 9.69 (1H, br s, NH), 8.69 (1H, s, H-2), 8.14 (1H, s, H-2′), 7.91 (1H, d, J = 9.0 Hz, H-6), 7.79 (1H, d, J = 7.8 Hz, H-6′), 7.48 (1H, *s*, H-9), 7.29 (1H, t, J = 7.6 Hz, H-5′), 7.24−7.20 (2H, m, H-7, H-4′), 2.98 (6H, s, 2 × Me); CIMS *m/z* (relative intensity) 401 (^{81}Br, MH^+, 100), 400 (^{81}Br, M^+, 95), 399 (^{79}Br, MH^+, 99), 398 (^{79}Br, M^+, 77).

The lower band was extracted to give **67** (12 mg, 3%) as a yellow solid: mp 193−194 °C; ^{1}H NMR δ 9.65 (1H, br s, NH), 8.68 (1H, s, H-2), 8.14 (1H, d, J = 1.9 Hz, H-2′), 7.79−7.76 (2H, m, H-6, H-6′), 7.29−7.23 (3H, m, H-5′, H-4′, H-9), 6.99 (1H, dd, J = 2.4, 8.8 Hz, H-7), 6.03 (1H, q, J = 5.1 Hz, N*H*CH_3), 2.73 (3H, d, J = 4.9 Hz, NHC*H*$_3$); CIMS *m/z* (relative intensity) 387 (^{81}Br, MH^+, 92), 386 (^{81}Br, M^+, 97), 385 (^{79}Br, MH^+, 100), 384 (^{79}Br, M^+, 77).

Enzyme Assay. EGFr was prepared from human A431 carcinoma cell shed membrane vesicles by immunoaffinity chromatography as previously described,[39] and the assays were carried out as reported previously.[18] The substrate used was based on a portion of phospholipase C-γ1, having the sequence Lys-His-Lys-Lys-Leu-Ala-Glu-Gly-Ser-Ala-Tyr^{472}-Glu-Glu-Val. The reaction was allowed to proceed for 10 min at room temperature, then was stopped by the addition of 2 mL of 75 mM phosphoric acid. The solution was then passed through a 2.5-cm phosphocellulose disk which bound the peptide. This filter was washed with 75 mM phosphoric acid (5×), and incorporated label was assessed by scintillation counting in an aqueous fluor. Control activity (no drug) gave a count of approximately 100 000 cpm. At least two independent dose−response curves were done and the IC_{50} values computed. The reported values are averages; variation was generally ±15%.

EGFr Autophosphorylation in A431 Human Epidermoid Carcinoma Cells. Cells were grown to confluence in 6-well plates (35-mm diameter) and exposed to serum-free medium for 18 h. The cells were treated with compound for 2 h and then with 100 ng/mL EGF for 5 min. The monolayers were lysed in 0.2 mL of boiling Laemlli buffer (2% sodium dodecyl sulfate, 5% β-mercaptoethanol, 10% glycerol, and 50 mM Tris, pH 6.8), and the lysates were heated to 100 °C for 5 min. Proteins in the lysate were separated by polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose. The membrane was washed once in a mixture of 10 mM Tris, pH 7.2, 150 mM NaCl, and 0.01% azide (TNA),

and blocked overnight in TNA containing 5% bovime serum albumin and 1% ovalbumin. The membrane was blotted for 2 h with antiphosphotyrosine antibody (UBI, 1 μg/mL in blocking buffer) and then washed twice in TNA, once in TNA containing 0.05% Tween-20 and 0.05% nonidet P-40, and twice in TNA. The membranes were then incubated for 2 h in blocking buffer containing 0.1 μCi/mL [^{125}I]protein A and then washed again as above. After the blots were dry they were loaded into a film cassette and exposed to X-AR X-ray film for 1−7 days. Band intensities were determined with a Molecular Dynamics laser densitometer.

Supporting Information Available: Experimental procedures and spectral data for compounds **22b**−**j**, **35**, **36**, **38**−**48**, **50**−**52**, **54**−**57**, **59**−**63**, **65**, **66**, **69**, and **70**−**77**. This material is available free of charge via the Internet at http://pubs.acs.org.

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