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Novel C-5 aminomethyl pyrrolotriazine dual inhibitors of EGFR and HER2 protein tyrosine kinases

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Abstract—Novel C-5 aminomethyl pyrrolotriazines were prepared and optimized for dual EGFR and HER2 protein tyrosine kinase inhibition. The homopiperazine, **1p**, emerged as a key lead and it showed promising oral efficacy in EGFR and dual EGFR/HER2 driven human tumor xenograft models. It is hypothesized that the C-5 homopiperazine side chain binds in the ribose–phosphate portion of the ATP binding pocket.

The epidermal growth factor receptor (EGFR, ErbB1 or HER1) and the human epidermal growth factor receptor 2 (HER2, ErbB2) are members of the ErbB family of receptor tyrosine kinases that have been clinically validated as targets for cancer therapy.[1] Their frequent co-expression in a variety of tumor types and their capacity to form heterodimers with other members of the ErbB family provide a strong rationale for simultaneously targeting both of these receptors.[2] We have reported pyrrolotriazine dual EGFR and HER2 kinase inhibitors with solubilizing groups that are linked to C-5 via a methylene ether, i.e. **1a** (Fig. 1).[3a] Morpholine analog **1b**, which showed potent kinase inhibition and good oral efficacy in EGFR and HER2 driven tumor models, emerged as the lead compound from that study. It was hypothesized that its C-5 solubilizing group extends into the ribose-phosphate portion of the ATP binding pocket where it participates in multiple hydrogen bonding interactions. We examined other solubilizing groups and linkers at C-5 to further probe this binding site and here report our initial results for analogs with C-5 aminomethyl groups, that is, **1c**.

To make C-5 aminomethyl analogs **1c**, we examined the reaction of 4-chloro-5-(bromomethyl)-pyrrolotriazine **2**[3a] with primary or secondary amines. This gave the desired C-5 methylamines but together with significant amounts of C-4 amine side products. The 5-methyl

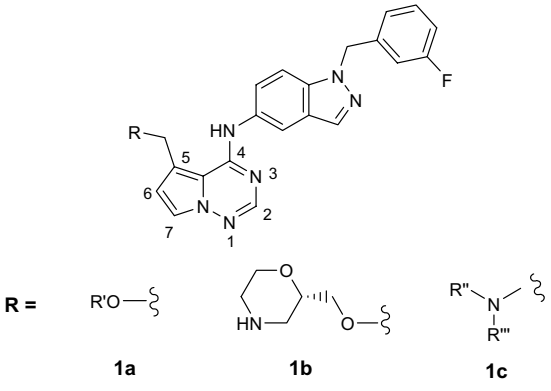

Figure 1. C-5 Substituted pyrrolotriazine dual EGFR and HER2 kinase inhibitors.

Keywords: Pyrrolotriazine; EGFR; HER2; Kinase inhibitor.
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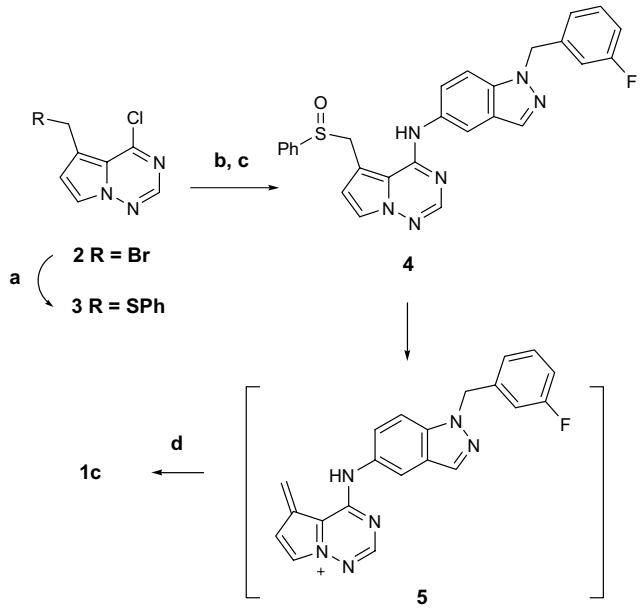
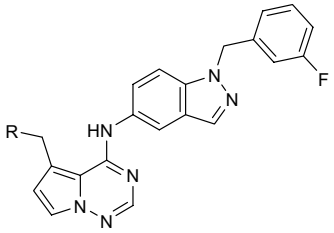
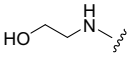
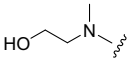
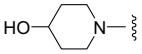
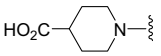
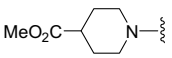


Scheme 1. Reagents and conditions: (a) PhSH (1.0 equiv), $(i\text{-Pr})_2$EtN (1.0 equiv), CH_2Cl_2, −20 °C, 3 h; (b) 1-(3-fluorobenzyl)-1*H*-indazol-5-amine (0.9 equiv), 1,2-dichloroethane/ *n*-BuOH (1:1), 85 °C, 2.5 h; (c) *m*-chloroperbenzoic acid (1 equiv), $CHCl_3$, 0 °C, 1 h , 55% overall; (d) amine (about 30 equivalents), 135 °C, sealed tube, overnight or amine (about 5 equiv), dimethyl sulfoxide, 140 °C, sealed tube, overnight.

sulfide, **3**, was found to form selectively on reaction of **2** with thiophenol and so it was examined as an intermediate to make aminomethyl analogs **1c** (Scheme 1).[4] Reaction of **3** with 1-(3-fluorobenzyl)-1*H*-indazol-5-amine followed by oxidation gave the stable sulfoxide, **4**. On being heated neat with an excess of a primary or a secondary amine, it gave the C-5 aminomethyl product, **1c**, presumably via cationic intermediate **5**. If necessary, this coupling was followed by protecting group removal and the overall procedure was used to make most of the analogs listed in Table 1. 5-Methyl-pyrrolotriazines with acetate or chloride as leaving groups were also used to prepare C-5 aminomethyl analogs (see Schemes 2 and 3). For coupling reactions where the amine was difficult to come by, smaller amounts of the amine were employed and dimethyl sulfoxide was used as the solvent. The piperidine ester and acid, **1j** and **1i**, were obtained by heating **4** with methyl piperidine-4-carboxylate. This gave a mixture of the ester and the acid which were separated by preparative HPLC. Sulfonamide **1r** was prepared by reacting **1p** with methansulfonyl chloride in the presence of base. The 3-hydroxyl-homopiperazines, **1t** and **1u**, were made by heating **4** with excess 3-hydroxy-homopiperazine[5] and separating the enantiomers by preparative chiral HPLC. Ketone **1v** was prepared by perruthenate oxidation of a mixture of alcohols **1t** and **1u** in acetonitrile.[6]

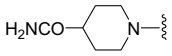
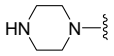
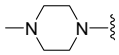
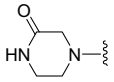
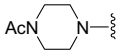
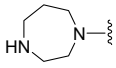
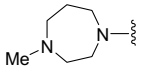
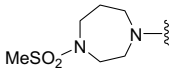
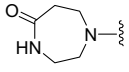
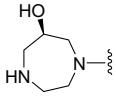
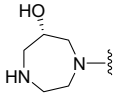
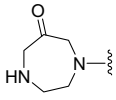
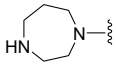
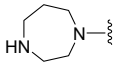
Table 1. Structure–activity relationship for the C-5 aminomethyl analogs



Compound	R	IC_{50}[a] (μM)				Metabolic stability[e]	4-h Plasma level[f] (nM)
		HER2	EGFR	N87[b]	HT29[c] or A2780[d]		
1d	H	0.088	0.081	0.38	>10[c]	0.14	4100
1e		0.047	0.045	1.95	nd	nd	nd
1f		0.11	0.11	1.2	nd	nd	nd
1g		0.36	0.50	nd	nd	nd	nd
1h		0.063	0.068	0.14	nd	0.29	250
1i		0.11	0.22	>5	>10[c]	0.23	600
1j		>1	>1	>5	>10[c]	nd	27

(*continued on next page*)

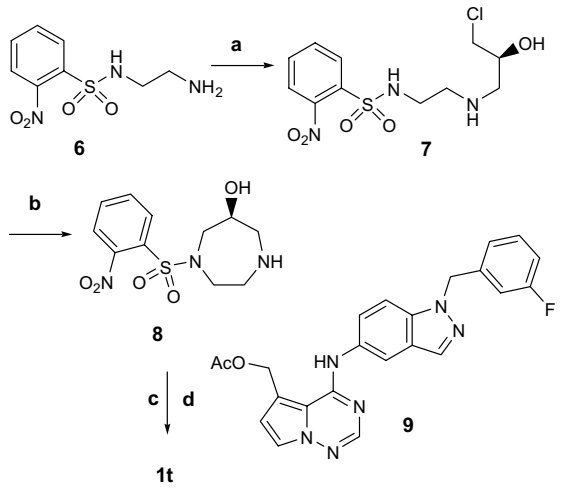
Table 1 (*continued*)

Compound	R	IC_{50}[a] (μM)				Metabolic stability[e]	4-h Plasma level[f] (nM)
		HER2	EGFR	N87[b]	HT29[c] or A2780[d]		
1k		0.31	0.14	0.40	nd	nd	nd
1l		0.081	0.090	0.73	nd	0.00	6300
1m		0.12	0.17	0.49	nd	nd	480
1n		0.20	0.35	0.52	>10[c]	nd	nd
1o		0.61	0.71	nd	nd	nd	nd
1p		0.027	0.033	0.11	3.6[c]	0.05	1300
1q		0.12	0.13	0.62	7.9[c]	0.21	93
1r		0.12	0.21	0.33	>10[c]	nd	0
1s		0.075	0.24	0.20	>10[c]	0.27	44
1t		0.016	0.026	0.16	>10[c]	0.12	1506
1u		0.076	0.20	0.95	>10[c]	0.12	880
1v		0.039	0.078	0.65	>10[c]	nd	nd
11[g]		0.17	0.19	3.7	>5[d]	nd	nd
14[h]		0.074	0.13	0.68	>2.1[d]	0.00	nd

[a] IC_{50} values are reported as means of at least three determinations. Variability around the mean value was <15% for the enzymatic assays and <25% for cell proliferation assays.
[b] Cell line N87 is a human gastric carcinoma that overexpresses both EGFR and HER2.
[c,d] Cell lines HT29 (human colon carcinoma) and A2780 (human ovarian carcinoma) do not express EGFR or HER2.
[e] Rate of metabolism (mmol min^{-1} mg $protein^{-1}$) by mouse liver microsomes after a 10-min incubation at 3 μM in the presence of NADPH.
[f] Averaged 4 h plasma levels in three male Balb/C mice for compounds administered orally at 50 mpk in Tween 80/PEG400/water = 10:40:50.
[g] The 6-methoxy analog of **1p**.
[h] The 6-methyl analog of **1p**.

Enantiomer **1t** was independently made from *N*-(2-aminoethyl)-2-nitrobenzene-sulfonamide **6** as outlined in Scheme 2.[4] Reaction of **6** with (*S*)-epichlorohydrin in the presence of $MgSO_4$[7] gave chlorohydrin **7** which cyclized to homopiperazine **8** on heating with cesium carbonate.[8] The monoprotected homopiperazine was linked to the pyrrolotriazine by heating it with acetate **9**. Deprotection with thiophenol[9] gave material that was identical with **1t**.

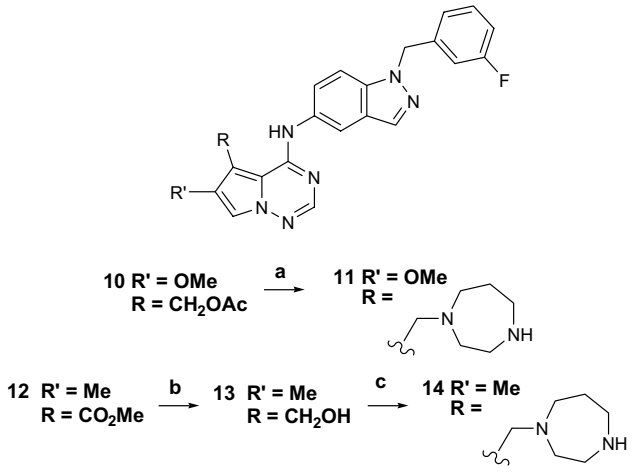
The 6-methoxy analog of homopiperazine **1p**, **11**, was prepared by heating acetate **10**[3a] with excess homopiperazine (Scheme 3). To make the 6-methyl analog, **14**, ester **12**[10] was first reduced to alcohol **13**. This was treated



Scheme 2. Reagents and conditions: (a) *S*-(+)-epichlorhydrin (0.5 equiv), $MgSO_4$ (0.5 equiv), MeOH, rt, 8 h , 41%; (b) Cs_2CO_3 (3 equiv), acetonitrile, 65 °C, 6.5 h, 31%; (c) **8** (1 equiv), Et(*i*-Pr)$_2$N (1.5 equiv), CH_3CN, pressure vessel, 102 °C, 17 h, 85%; (d) PhSH (2 equiv), K_2CO_3 (5 equiv), DMF, rt, 50 min, 92%.

with thionyl chloride to give a chloromethyl intermediate that was reacted directly with homopiperazine to give **14**.

Aminomethyl analogs that showed promising EGFR and HER2 kinase inhibition were screened for their antiproliferative activity against the N87 cell line. This is a human gastric carcinoma that overexpresses both EGFR and HER2.[11,12] Analogs were also screened in HT29 (human colon carcinoma) or A2780 (human ovarian carcinoma) cell proliferation assays. These are cell lines that do not depend on EGFR or HER2 signaling and provided a check for off-target antiproliferative effects. The SAR data are summarized in Table 1. All of the analogs carried the C-4 *m*-fluorobenzylindazolylamino side chain since it was previously found to provide optimal dual EGFR and HER2 kinase inhibition.[3a,b]



Scheme 3. Reagents and conditions: (a) homopiperazine (10 equiv), CH_3CN, microwave, 170 °C, 30 min, 61%; (b) $LiAlH_4$ (3 equiv), THF, 6 h, 46%; (c) $SOCl_2$ (1.2 equiv), CH_2Cl_2, 1 h; then homopiperazine (2 equiv), *N*-methylmorpholine (2 equiv), CH_2Cl_2, rt, 20 h, 21%.

Compound **1e** showed a profile that was typical of secondary amine analogs: kinase inhibition that was comparable to that of the 5-methyl parent compound, **1d**, but weaker antiproliferative activity. Tertiary amine analogs, for example **1f** and **1g**, were generally less potent but the hydroxypiperidine, **1h**, was remarkable in that it showed good potency in both the kinase and cell assays. The piperidine acid, ester, and amide analogs, **1i**, **1j**, and **1k**, were less potent while the piperazine, **1l**, showed good kinase inhibition but not antiproliferative activity. The latter did however demonstrate better metabolic stability in the presence of mouse liver microsomes and mouse oral exposure than the hydroxypiperidine **1h**. This was in line with previous observations[3a] that pyrrolotriazines with basic primary or secondary amine substituents showed better metabolic stability. *N*-Methyl piperazine **1m** and amide analogs, **1n** and **1o**, were less potent, indicating that the basic secondary amino group on the piperazine side chain was required for potent kinase inhibition. Homopiperazine **1p** showed both potent kinase inhibition and antiproliferative activity but did not significantly inhibit proliferation of the HT29 cell line. Its *N*-methyl derivative, **1q**, sulfonamide, **1r**, and amide analog, **1s**, showed reduced potency and oral exposure. The (*R*)-3-hydroxyhomopiperazine, **1t**, showed a profile similar to that of **1p**, while its enantiomer, **1u**, was less potent. Ketone **1v** and the 6-methoxy and 6-methyl analogs, **11** and **14**, were also less potent. The latter results indicate that additional substitution at C-6 is less tolerated.

Figure 2 shows a potential binding mode for piperazine **1p** in the ATP binding site that was modeled using the published X-ray structure of the complex between lapatinib and EGFR kinase.[13] The docked poses were energy-minimized in Maestro[14] using the OPLS-AA force field[15] and the GBSA continuum model[16], an implicit solvation model. The pyrrolotriazine core is oriented

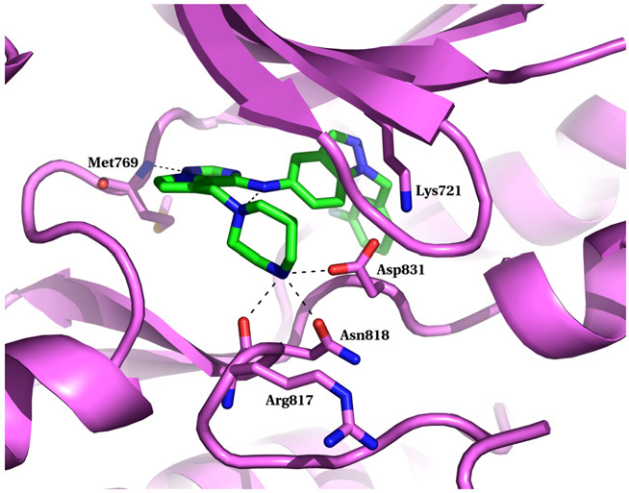


Figure 2. Predicted binding mode of compound **1p** modeled in the X-ray structure of the lapatinib/EGFR kinase complex, see Ref. 13. The C-5 side chain extends into the ribose phosphate binding region where the protonated azepine NH may form hydrogen bonds with Asp831, Asn818 and/or Arg817. Image created with Pymol from DeLano Scientific LLC, San Carlos, CA, USA. http://www.pymol.org

Table 2. Pharmacokinetic parameters[a] for **1p** in mice

C_{max} (nM) po	T_{max} (h) po	AUC $_{0-8\ h}$ (nM h) po	$t_{1/2}$ (h) iv	MRT (h) iv	Cl (mL/min/kg) iv	V_{ss} (L/kg) iv	F_{po} (%)
7951	3	48913	3.7	4.0	39	9.5	77

[a] p*K* parameters were obtained from composite plasma concentration–time profiles with an average of three male Balb/C mice at each time point. Mice were dosed at 90 (po) and 10 (iv) mg kg^{-1} using Tween 80/PEG400/water (10:40:50, v/v) as the vehicle.

in the ATP binding site such that there is a hydrogen bond between *N*-1 and the hinge region Met769 NH, and the C-4 benzyl indazole group extends back into a deep hydrophobic pocket formed partially by the alpha-C-helix. The C-5 substituent extends out into the ribose phosphate pocket where the protonated homopiperazine NH can hydrogen bond with the side chains of Asp831, Asn818 and/or the backbone carbonyl oxygen of Arg817. In this model, there is also an intramolecular hydrogen bond between the C-4 aniline NH and the hompiperazine tertiary nitrogen atom. This binding model is similar to that previously suggested[3a] for morpholine analog **1b** and has been hypothesized[17] for quinazoline EGFR and HER2 kinase inhibitors with solubilizing side chains at C-5. It also offers an explanation for why (*R*)-3-hydroxy-homopiperazine **1t** is more potent than its *S*-enantiomer, **1u**. The hydroxy group of the former homopiperazine is better situated to hydrogen bond with Asp831 and/or the conserved Lys745 that are normally involved in binding the phosphate group of ATP.

The PK parameters for **1p** in mice are summarized in Table 2. Absorption of the compound after oral administration was excellent (77% bioavailable) and this was consistent with its high permeability in Caco-2 cells. After intravenous administration, **1p** showed a moderate plasma clearance of 39 mg/min/kg, with a half-life ($t_{1/2}$) and mean residency time (MRT) of 3.7 and 4 h, respectively. A relatively high steady-state volume of distribution (V_{ss}) was observed in mice. The compound was 98.4% bound to mouse serum proteins, yielding a free fraction of 1.6% in that species. The aqueous solubility of **1p** was pH-dependent, ranging from 9.7 mg/mL with a solution pH of 3.9 for the HCl salt to 0.012 mg/mL in a pH 7.5 phosphate buffer. The relatively low aqueous solubility at neutral pH is similar to that of other ATP competitive EGFR kinase inhibitors.[18]

Table 3. In vivo antitumor activity of orally administered **1p** against established N87 and GEO xenografts implanted subcutaneously in athymic mice[a]

Tumor model	Dose[a], mg kg^{-1}	Schedule	% TGI[b]	*P*[c]
N87	90	BID × 14	97	0.0037
	135		111	0.0001
	180		121	0.0018
GEO	90	BID × 14	60	0.0001
	135		78	0.0018

[a] Vehicle: Tween 80/PEG400/water, 10:40:50.

[b] Percent tumor growth inhibition during treatment. Activity is defined as %TGI $\geqslant$50%.

[c] Probability for median tumor weight at the end of drug treatment, compared with controls.

Compound **1p** was evaluated in EGFR/HER2 driven N87 human gastric and EGFR driven GEO human colon carcinoma xenograft models in athymic mice. In both studies it was administered orally, twice a day (BID), for 14 consecutive days. It was robustly active in the N87 model yielding tumor growth inhibition of 97 to 121% over the dose-range of 90–180 mg/kg (Table 3). A clear maximum-tolerated dose (MTD) was reached at the 180 mg/kg dose which was associated with approximately 12% drug related mortality. In the GEO model, **1p** was tested at 90 and 135 mg/kg and was active at both dose levels. The 4-hydroxy-homopiperazine, **1t**, was also evaluated in vivo but it did not demonstrate an advantage over **1p**.

In summary, pyrrolotriazines with diamino solubilizing groups that are tethered to C-5 showed potent inhibition of both EGFR and HER2 kinases. Modeling studies suggested that the solubilizing group can extend into the ribose–phosphate binding region of the ATP binding pocket where it can participate in multiple hydrogen bonding interactions. The homopiperazine analog, **1p**, emerged as a key lead and it exhibited potent kinase inhibition, antiproliferative activity, and oral efficacy in tumor xenograft models. This encouraged a broader examination of other C-5 amino and diamino solubilizing groups and the results will be described in future publications.

References and notes

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