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Development of multitargeted inhibitors of both the insulin-like growth factor receptor (IGF-IR) and members of the epidermal growth factor family of receptor tyrosine kinases

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ABSTRACT

Emerging clinical and pre-clinical data indicate that both insulin-like growth factor receptor (IGF-IR) and members of the epidermal growth factor (EGF) family of receptor tyrosine kinases (RTKs) exhibit significant cross-talk in human cancers. Therefore, a small molecule that successfully inhibits the signaling of both classes of oncogenic kinases might provide an attractive agent for chemotherapeutic use. Herein, we disclose the structure activity relationships that led to the synthesis and biological characterization of **14**, a novel small molecule inhibitor of both IGF-IR and members of the epidermal growth factor family of RTKs.

The development and maintenance of cancer requires dysregulation of normal cellular signaling. Receptor tyrosine kinases serve to propagate external cellular stimuli into intracellular responses that range from proliferation, to induction of cell death. In a variety of cancers, RTKs are either mutated or overexpressed leading to enhanced proliferation, resistance to chemotherapy or decreased apoptotic potential. Therefore, several small molecule inhibitors have been developed that inhibit RTKs, such as Bcr-Abl (Gleevec®), and KDR (Nexavar®).[1] For the agents mentioned above, these drugs inhibit multiple kinase targets, potentially enabling broader clinical utility versus a more selective agent.[2] A key issue in developing the next generation of RTK inhibitors is to determine which kinases should be targeted by a single small molecule; one potential combination is IGF-IR and members of the EGF family of RTKs.

IGF-IR is a member of a complex system of growth factors and receptors that includes: insulin receptor (IR), insulin-like growth factor I/II (IGF-I/IGF-II), and six insulin-like growth factor binding proteins.[3] In normal tissues, IGF-IR is essential for growth, development, and the suppression of apoptosis. This is a consequence of the ability of IGF-IR to simultaneously activate the anti-apoptotic phosphoinositide-3-kinase/Akt pathway, and the mitogenic extracellular signal regulated kinase (ERK)/mitogen-activated protein kinase pathway (MAPK).[4] In malignant cells, the ability of IGF-IR to simultaneously control both the pro-survival and the proliferative aspects of the cellular machinery allow these cells to avoid apoptosis, induce the production of key angiogenic factors, and promote tumor cell invasion.[5]

The epidermal growth factor family of RTKs consists of four family members: the epidermal growth factor receptor (EGFR/ErbB-1), ErbB-2, ErbB-3 and ErbB-4. ErbB-2 is an orphan receptor and ErbB-3 lacks kinase activity. Normally, the ErbB-family members bind growth factor then proceed to either homodimerize or heterodimerize leading to phosphorylation and activation of the receptor pair.[6] However, receptor overexpression of ErbB-2, via gene amplification, can drive ligand independent activation. For EGFR, aberrant activation can be achieved either via autocrine overexpression of its ligands,[7] or by possessing activating mutations in and around the kinase domain of the receptor.[8] Analogous to IGF-IR, the ErbB-family of RTKs signal through both MAPK and Akt pathways, thereby enabling the receptors to control various cellular processes including proliferation, migration and evasion of apoptosis.

Recent literature has suggested that simultaneous inhibition of IGF-IR and EGFR/ErbB-2 with combinations of either small molecules or antibodies affords enhanced inhibition of cellular

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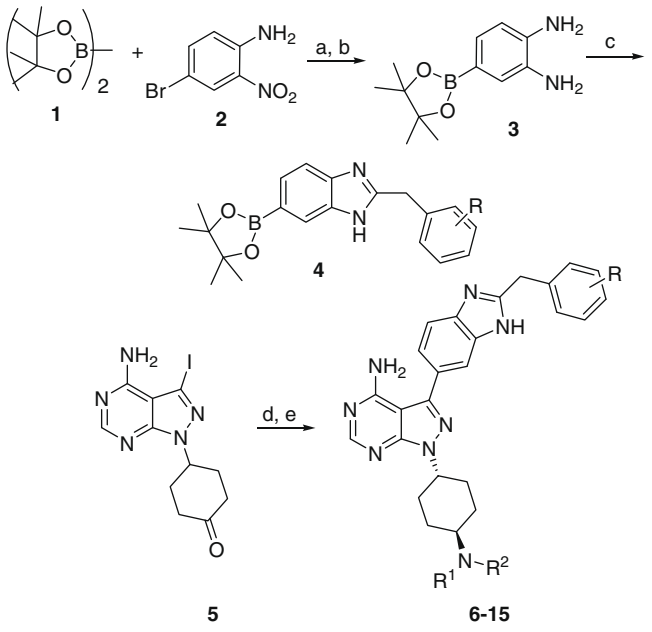


Scheme 1. Reagents and conditions: (a) $PdCl_2$·dppf, KOAc, DMF, 100 °C, 85–99%; (b) 10% Pd/C, 60 psi H_2, EtOAc, 91%; (c) 1—CDI, THF, R-CO_2H; 2—**3**, AcOH, 90 °C, 60–70%; (d) amine (R_1R_2NH), HCO_2H, NMP, 100 °C, 25% yield of *trans*-diastereomer; (e) **4**, $(Ph_3P)_2PdCl_2$, 2 M aq Na_2CO_3, DME/H_2O 2/1 (v/v), 80 °C, 40–87%.

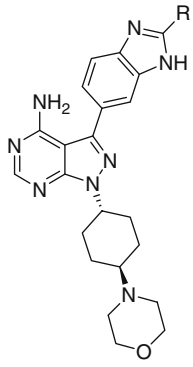
proliferation versus the single agents. In particular, the combination of IGF-IR and EGFR antibodies showed improved activity versus an A549 mouse tumor xenograft.[9] Combinations of gefitinib and NVP-ADW742 (selective EGFR and IGF-IR inhibitors, respectively) caused synergistic decreases in cellular proliferation across a diverse set of cancer cell lines.[10] The impressive combination data provides rationale for a small molecule discovery program targeting simultaneous inhibition of IGF-IR and EGFR/ErbB-2. We have previously disclosed that a subset of pyrazolo[3,4-*d*]pyrimidines possesses potent IGF-IR inhibitory properties,[11] however, these analogs displayed poor cellular activity versus EGFR/ErbB-2. Therefore, the synthetic efforts were focused on the preparation of a scaffold that provided balanced enzyme and cellular activities versus the three targets of interest: IGF-IR, EGFR, and ErbB-2.

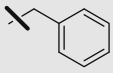
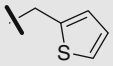
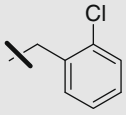
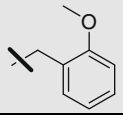
The synthesis of the pyrazolopyrimidine analogs begins with boronation of the commercially available bromide, **2**, and subsequent reduction of the nitro-arene intermediate affords **3** in 77–80% yield over the two steps (Scheme 1). Acylation of the desired phenylacetic-acid derivative with 1,1′-carbonyldiimidazole (CDI) yields the crude acyl-imidazole, which was treated with **3** in the presence of acetic acid[12] to afford the benzimidazole **4** in 60–70% yield. Reductive amination of the known ketone **5**,[13] followed by Suzuki coupling with **4** affords the fully elaborated set of analogs (**6**–**15**).

Previous SAR indicated that a *trans*-substituted morpholino-pyrazolopyrimidine afforded potent IGF-IR inhibition.[14] With the amine held constant, we assessed the effect of benzimidazole substitutions on EGFR/ErbB-2 enzyme potency (Table 1).[15] The simple phenyl (**6**) and the isosteric thiophene (**7**) analogs yielded potent enzyme inhibition for IGF-IR, but only moderate activity versus the ErbB-family members. The 2-chloro substituent, in **8**, which was previously reported to provide IGF-IR activity,[11] demonstrated increased potency versus EGFR, but not against ErbB-2. Changing from 2-Cl to 2-OMe yielded **9**, a compound that possessed balanced enzyme inhibition across the desired targets.

With the preferred benzimidazole substituent (2-OMe) identified, we focused on varying the amine moiety. The results from the amine-group SAR are shown in Table 2. In addition to

Table 1
Enzyme activities of substituted benzimidazoles



Analog	R=	Enzyme (IC_{50}, nM)		
		IGF-IR	EGFR[a]	ErbB-2
6		64	270	186
7		25	361	250
8		36	65	664
9		81	58	53

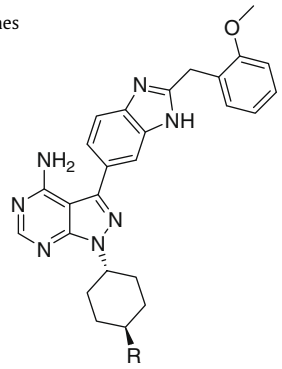
[a] EGFR kinase construct possessed the L858R activating mutation.

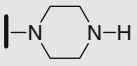
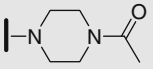
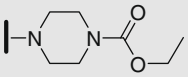
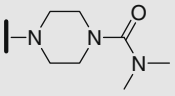
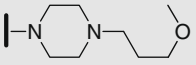
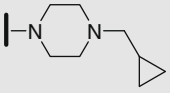
examining the kinase activity of the compounds, analogs were assessed for their ability to inhibit cellular phosphorylation in relevant cancer cell lines: MiaPaCa-2, which expresses both IGF-IR and EGFR and N87, which expresses ErbB-2.[16] The simple piperazine **10**, afforded balanced enzyme activity, however, suffered from poor cellular activity. A survey of nitrogen-based functional groups (amide, carbonate, and urea) was prepared to tune the basicity of the piperazinyl-nitrogen yielding analogs (**11**, **12**, and **13**). Collectively, this set of analogs demonstrated poor cellular activity. However, an alkyl-substituted piperazine **14** displayed the desired balance of enzyme and cellular activity required for further evaluation. Although the enzyme and cellular activity for **15** were comparable to **14**, phamacokinetic evaluation of **15** in mice resulted in much lower oral exposure (data not shown).

The ADME and pharmacokinetic (PK) properties of **14** are summarized in Figure 1. The in vitro ADME characterization of **14** reveals that the compound possesses relatively low plasma protein binding and very high aqueous solubility. In addition, analog **14** displays a fairly benign cytochrome P450 inhibition profile. The murine PK of **14** shows low clearance coupled with moderate oral bioavailability, which increases via IP administration (12% vs >100%).

The balanced kinase and cellular activity of **14** coupled with the moderate murine PK indicated that this compound warranted further investigation in our in vivo phamacodynamic (PD) model. The experimental design of the PD model is such that the compound is dosed either IP or PO and the mice ($N = 3$) are challenged 4 h later with growth factor stimulation, via an IV dose of IGF-I and EGF. The level of inhibition is determined by examining the phosphorylation status of the receptor in either lung (IGF-IR) or liver (EGFR). As shown in Figure 2, analog **14** dosed via either IP or PO (50 mg/

Table 2
Enzyme and cellular activity of 2-OMe substituted pyrazolopyrimidines



Analog	R=	Enzyme (IC_{50}, nM)			Cellular phosphorylation (EC_{50}, nM)		
		IGF-IR	EGFR[a]	ErbB-2	IGF-IR (MiaPaCa-2)	EGFR (MiaPaCa-2)	ErbB-2 (N87)
10		26	30	44	711	335	210
11		27	130	59	143	742	660
12		309	30	270	247	520	1600
13		69	25	17	208	808	1580
14		81	58	54	115	85	94
15		40	3	13	190	91	147

[a] EGFR kinase construct possessed the L858R mutation.

In vitro ADME		
P450 inhibition Profile	CYP2C9	IC_{50} > 20 μM
	CYP3A4	IC_{50} > 20 μM
	CYP2D6	IC_{50} = 1.6 μM
Aqueous Solubility	pH = 7.2	> 30 μM
Human Protein Binding		96.7%

Mouse PK		**Units**
PO DNAUC[a]	0.42	μmol·hr/L/mg/kg
IV DNAUC	3.50	μmol·hr/L/mg/kg
IP DNAUC	2.71	μmol·hr/L/mg/kg
F (PO)	12	%
F (IP)	> 100	%
Cl	0.47	L/hr/kg
$t_{1/2}$	3.1	hr

[a] DNAUC = dose normalized AUC

Figure 1. ADME and murine PK characterization of **14**.

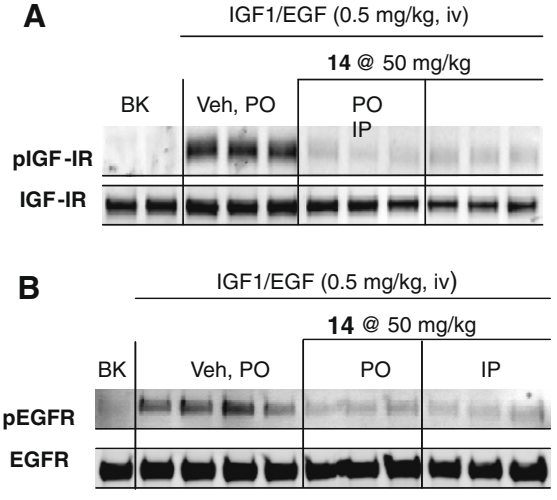


Figure 2. In vivo activity of **14** in a murine PD model of efficacy. (A) Represents the inhibition of lung IGF-IR phosphorylation after an IP or PO dose of **14** (BK = background). (B) Represents the inhibition of liver EGFR phosphorylation after an IP or PO dose of **14** (BK = background).

kg) resulted in near complete inhibition of IGF-IR and EGFR kinase activity (receptor phosphorylation).

In conclusion, we have disclosed that appropriate functionalization of a novel pyrazolopyrimidine scaffold yields a series of multitargeted kinase inhibitors. In particular, analog **14** displayed a balanced enzyme and cellular inhibitory profile versus IGF-IR and members of the epidermal growth factor RTK family (EGFR and ErbB-2). The in vitro activity parlayed into in vivo activity as determined in a murine PD model, in which **14** completely inhibited

receptor phosphorylation of both IGF-IR and EGFR. Additional in vivo characterization of **14** will be disclosed in due course.

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14. This class of compounds possesses equivalent enzyme and cellular potency for both IGF-IR and IR.
15. The kinase inhibitory activity of the disclosed compounds were assayed by a homogeneous time-resolved fluorescence assay as previously described, see Ref. 11 The IC_{50}s for IGF-IR (0.25 nM, Cell Signaling Technology) and EGFR–L858R (4 nM, Upstate) were obtained using 50 μM and 100 μM ATP, respectively.
16. MiaPaCa-2 and N87 cells were treated with serial dilutions (0.0003–30 μM) of compounds for 6 h in growth media containing 10% fetal bovine serum. MiaPaCa-2 cells were then stimulated with IGF-I and EGF for 10 min. Cells were lysed and phosphorylation of IGF-IR, EGFR, and ErbB-2 was determined using the human p-IGF-IR, human p-EGFR, and human p-ErbB-2 DuoSet IC ELISA kits from R&D Systems (Minneapolis, MN).