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Pyrazolo[3,4-*d*]pyrimidines as potent inhibitors of the insulin-like growth factor receptor (IGF-IR)

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Abstract—A high throughput screen of Abbott's compound repository revealed that the pyrazolo[3,4-*d*]pyrimidine class of kinase inhibitors possessed moderate potency for IGF-IR, a promising target for cancer chemotherapy. The synthesis and subsequent optimization of this class of compounds led to the discovery of **14**, a compound that possesses in vivo IGF-IR inhibitory activity.

Receptor tyrosine kinases (RTKs) have recently become validated targets for cancer chemotherapy. The clinical successes of agents that inhibit RTKs, such as Bcr-Abl (Gleevec®), EGFR (Tarceva®), and EGFR/ErbB-2 (Tykerb®), have revealed that a variety of RTKs can be successfully exploited for cancer treatment.[1] Therefore, the search for additional RTKs has led to the proposal that small-molecule inhibitors of the insulin-like growth factor receptor (IGF-IR) might yield effective anti-cancer agents.[2] IGF-IR is a member of a complex system of growth factors and receptors that includes the following: insulin receptor (IR), insulin-like growth factor I/II (IGF-I/IGF-II), and six insulin-like growth factor binding proteins.[3] The complexity of the signaling family is further increased due to the presence of hybrid receptors, which are composed of both IGF-IR and IR.[4] In normal tissue, 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.[5] In malignant cells, the ability of IGF-IR to simultaneously control both the pro-survival and the proliferative aspects of the cellular machinery allows these cells to avoid apoptosis, induce the production of key angiogenic factors, and promote tumor cell invasion.[6]

A variety of approaches have been or are currently being employed to target IGF-IR, which include antibodies to the extracellular domain of the receptor, dominant-negative receptor proteins, antisense RNA, siRNA, and small-molecule inhibitors.[7] The latter agents can be divided into roughly two categories, substrate inhibitors of IGF-IR[8] and ATP-competitive inhibitors of IGF-IR.[9] One of the first examples of an ATP-competitive inhibitor of IGF-IR in both enzymatic and cellular assays was recently disclosed (**1**, NVP-ADW742, Fig. 1).[10] Prior to the completion of our SAR efforts, compound **2** was reported to also possess IGF-IR enzymatic and cellular activity.[11] During a high throughput screen of our compound repository, we noted that the pyrazolopyrimidine class of kinase inhibitors, such as **3** possessed moderate potency for IGF-IR.[12] The remainder of this communication will focus on the synthesis and IGF-IR inhibitory properties of this promising class of compounds.

The synthesis of the pyrazolopyrimidines begins with boronation of the appropriate bromide **5** with diborane reagent **4** to afford **6** in 85–99% yield (Scheme 1). The known ketone **7** was treated with morpholine and formic acid, which after recrystallization from DMF/IPA afforded the desired *trans*-diastereomer,[13] which was reacted with the aforementioned boronates **6** under Suzuki

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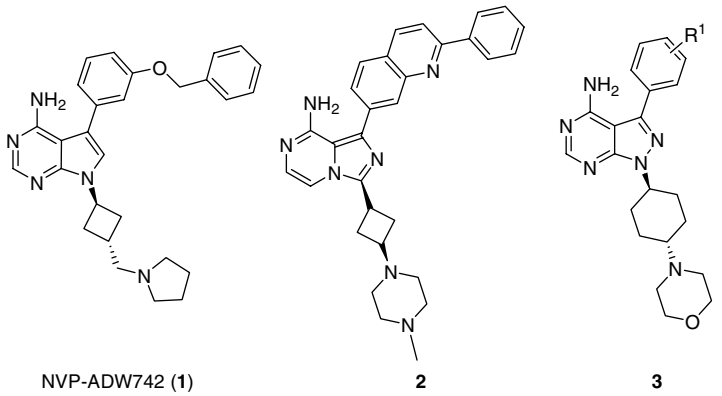
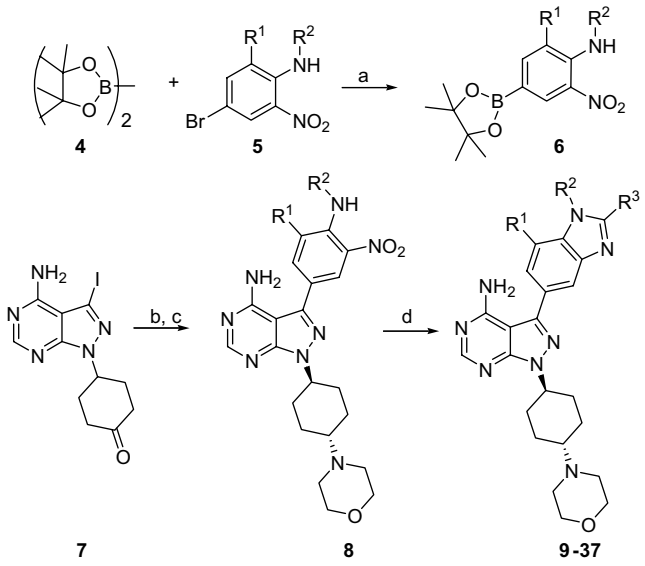


Figure 1. Structures of reported IGF-IR kinase inhibitors (**1**, **2**) and generic structure for the pyrazolo[3,4-*d*]pyrimidines **3**.



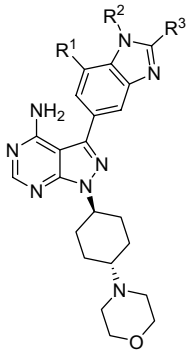
Scheme 1. Reagents and conditions: (a) $PdCl_2 \cdot dppf$, KOAc, DMF, 100 °C, 85–99%; (b) morpholine, HCO_2H, NMP, 100 °C, 25% yield of *trans*-diastereomer; (c) **6**, $(Ph_3P)_2PdCl_2$, 2 M aq Na_2CO_3, DME/H_2O 2/1 (v/v), 80 °C, 40–87%; (d) R_3-CHO, 1 M $Na_2S_2O_4$, EtOH, 130 °C, 20 min, microwave heating, 35–55%.

reaction conditions to afford **8**, in 40–87% yield. The nitro-aniline was treated with the appropriate aldehyde in the presence of 1 M $Na_2S_2O_4$[14] in a microwave reactor at 130 °C for 20 min to afford analogs **9**–**37**, in 35–55% yield.

The analogs, described herein, were synthesized to probe the IGF-IR inhibitory properties of differentially substituted benzimidazoles, in the presence of the fixed morpholino-cyclohexane moiety.[15] Therefore, analogs **9**–**37**, in addition to **1**, and **2**, were tested for intrinsic potency versus IGF-IR enzyme,[16] and their ability to inhibit intracellular phosphorylation (p-IGF-IR) of the receptor in MiaPaCa-2 cells, a human pancreatic tumor cell line.[17] The initial set of analogs tested revealed that the C_2-phenyl substituted benzimidazole provided a potent inhibitor of the enzyme (**9**, Table 1). Insertion of a methylene unit affording **10** further increased enzyme activity, while providing equipotent cell activity. Methylation of N_1 of the benzimidazole did not affect the enzyme or cell activity; however, the C_7-methyl-substituted benzimidazole was completely void of enzyme activity (**13** vs **12**).

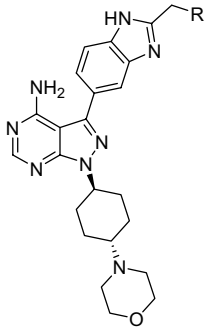
The data provided in Table 1 indicated that the benzyl-substituted benzimidazole analog **10** provided an acceptable balance of cell and enzyme activity. Therefore, we focused our attention toward further defining the optimal substituent(s) on the aryl ring. As shown in Table 2, a variety of substituents were tolerated at the *ortho*-position, such as, –F, Cl, –Me, –OMe, and even –Br, yielding analogs **14**, **15**, **16**, **17**, and **18**, respectively. All the aforementioned analogs provided potent enzyme inhibitors, however, the cellular activity suffered, with **14** providing the only analog with improved cell and enzyme activity relative to **10**.

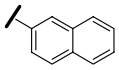
Table 1. Enzyme and cellular activities of substituted benzimidazole pyrazolopyrimidines



Analog	Benzimidazole			IGF-IR enzyme (IC_{50}, nM)	Cellular phosphorylation (p-IGF-IR, IC_{50}, nM)
	R^1	R^2	R^3		
1	—	—	—	78	323
2	—	—	—	37	58
9	–H	–H	–Ph	176	787
10	–H	–H	$–CH_2Ph$	64	94
11	–H	–H	$–CH_2CH_2Ph$	328	487
12	–Me	–H	$–CH_2Ph$	4334	2794
13	–H	–Me	$–CH_2Ph$	35	132

Table 2. Enzyme and cellular activities of substituted benzyl benzimidazole pyrazolopyrimidines



Analog	R	IGF-IR enzyme (IC_{50}, nM)	Cellular phosphorylation (p-IGF-IR, IC_{50}, nM)
10	–Ph	64	94
14	–2-Cl–Ph	37	90
15	–2-Me–Ph	38	117
16	–2-F–Ph	51	168
17	–2-OMe–Ph	81	142
18	–2-Br–Ph	71	65
19	3-Cl–Ph	123	372
20	–3-Me–Ph	186	267
21	–3-F–Ph	58	140
22	–4-Cl–Ph	642	637
23	4-Me–Ph	70	257
24	4-F–Ph	924	1250
25	2,6-di-F–Ph	79	104
26	2-F, 6-Cl–Ph	90	113
27	3,5-di-F–Ph	152	183
28	2,3-di-F–Ph	173	197
29	3,4-di-F–Ph	674	819
30	2,6-di-Cl–Ph	125	252
31	3,4-di-Cl–Ph	488	429
32	2,3-di-Cl–Ph	2322	ND[a]
33		23	56
34		32	84
35		2337	2646
36		1309	2335
37		3310	1542

[a] ND, not determined.

The SAR indicated that for the simple mono-substituted analogs, the preferred position was the *ortho*-position, while the substituents at the *meta*- and *para*-position were tolerated, the enzymatic and cellular activity was usually decreased. A series of disubstituted analogs were prepared affording compounds **25**–**32**. The preferred analogs possessed 2,6-disubstitution patterns, for example, the di-fluoro analog **25** possessed enzyme and cellular activity comparable to the 2-F, 6-Cl-analog **26**, which was analogous to the di-chloro-analog **30**. A survey of putative heterocyclic replacements for the aryl ring in **10** indicated that the thiophene-derived analogs (**33**, or **34**) provided improved cellular activity, with comparable enzymatic activity. However, other rings systems like pyridyl-analog **35**, the thiazole-analog **37**, and the larger naphthyl-ring-analog **36** were all found to lack relevant enzymatic and cellular activity.

From the data in Table 2, we decided to evaluate the murine pharmacokinetic (PK) properties of three of the more promising analogs (**10**, **14**, **33**, Table 3). Comparison of key PK parameters dose normalized AUC (DNAUC), clearance (CLp), and half-lives ($t_{1/2}$) indicated that the compounds were characterized as possessing short half-lives and moderate to high clearance. Although the isosteric replacement of a phenyl with a thiophene (**10** → **33**) was tolerated at the enzyme and cellular level, the aforementioned substitution yielded much lower oral and intravenous (IV) murine exposure. Fortunately, the 2-chloro-substituent yielded an analog (**14**) that exhibited increased oral and IV exposure, as well as decreased clearance. As a result of the promising enzyme and cellular profile of **14**, in combination with improved pharmacokinetic properties, this compound was chosen for further evaluation in a pharmacodynamic model for in vivo IGF-IR activity (Fig. 2). Compound **14** given orally at 12.5 mg/kg produced drug plasma concentrations of 2.4–3.7 μM at a 4 h time point after dosing, and resulted in nearly complete inhibition of IGF-IR kinase activity (receptor autophosphorylation) in mouse lung tissue (92–99%).[18] This confirms that the in vitro IGF-IR inhibitory activity observed for this class of compounds does indeed translate to in vivo activity.

Table 3. Comparison of the murine oral and intravenous pharmacokinetic properties of **10**, **14**, and **33**

Compound	PO DNAUC[a]	IV DNAUC[a]	CLp (L/h/kg)	$t_{1/2}$ (h)	F (%)
10	0.16	1.1	2.3	1	15
14	0.43	1.5	1.2	1.2	28
33	0.039	0.42	4.6	0.60	9

[a] Units of DNAUC: μmol h/L/mg/kg.

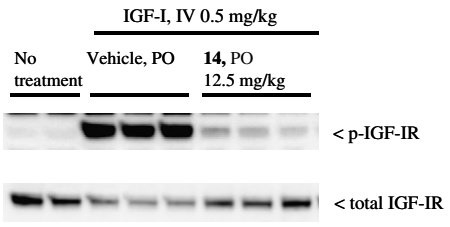


Figure 2. Inhibition of IGF-IR in vivo by **14**.

In conclusion, we have disclosed a novel scaffold for inhibiting the insulin-like growth factor receptor, the pyrazolo[3,4-*d*]pyrimidine. Appropriate substitution of the scaffold afforded **14**, which possessed potent enzyme and cellular activity (<100 nM), while simultaneously displaying promising murine pharmacokinetics and activity versus an in vivo pharmacodynamic model. Further in vitro and in vivo characterization of **14**, as well as further SAR exploration of the pyrazolo[3,4-*d*]pyrimidines, will be disclosed in due course.

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15. The SAR of changing the morpholine ring in related pyrazolo[3,4-*d*]pyrimidine-based analogs to other ring systems will be the focus of future publications.
16. IGF-IR kinase activity was assayed by a homogeneous time-resolved fluorescence in vitro kinase assay. Specifically, 10 μL of C-terminal GST-tagged, recombinant, human IGF-IR, amino acids 954–1367 expressed by baculovirus in Sf21 cells (Cell Signaling Technology) was mixed with 10 μL of an inhibitor (various concentrations, 2% final DMSO) and 10 μL of ATP (50 μM final concentration) in reaction buffer (50 mM HEPES, pH 7.5, 10 mM $MgCl_2$, 2 mM $MnCl_2$, 0.1% BSA, and 1 mM DTT, 40 μL final volume).
17. MiaPaCa-2 cells were treated with serial dilutions (0.0003–30 μM) of compounds for 6 h in growth media containing 10% fetal bovine serum before stimulation with IGF-I for 10 min. Cells were then lysed and phosphorylation of IGF-IR was determined using the human p-IGF-IR DuoSet IC ELISA kit from R&D Systems (Minneapolis, MN).
18. A pharmacodynamic model to determine the activity of IGF-IR kinase inhibitor in mice was adapted from García-Echeverría, C. Pearson, M. A.; Marti, A.; Meyer, T.; Mestan, J.; Zimmermann, J.; Gao, J.; Brueggen, J.; Capraro, H.-G.; Cozens, R.; Evans, D. B.; Hofman, D. *Cancer Cell* **2004**, *5*, 231. Compound **14** or vehicle was administered orally at 12.5 mg/kg in C57BL6 mice; 4 h later IGF-I at 0.5 mg/kg was injected intravenously, and the lung tissue was collected 10 min thereafter. p-IGF-IR and total IGF-IR in lung tissue lysates were determined by Western Blot using specific antibodies (Biosource, Camarillo, CA and Santa Cruz Biotechnology, Inc., Santa Cruz, CA).