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Discovery of an inhibitor of insulin-like growth factor 1 receptor activation: Implications for cellular potency and selectivity over insulin receptor

Edgar R. Wood [a,*], Lisa Shewchuk [b], Anne Hassel [b], Jim Nichols [a], Anne T. Truesdale [a], Danielle Smith [a], H. Luke Carter [a], Kurt Weaver [a], George Barrett [a], Tony Leesnitzer [c], Emilio Alvarez [c], Ana Isabel Bardera [c], Amelia Alamillo [c], Juan Cantizani [c], Julio Martin [c], Gary K. Smith [c], David E. Jensen [a], Hongbo Xie [a], Robert Mook [d], Rakesh Kumar [e], Kevin Kuntz [d]

[a] Department of Biological Reagents and Assay Development, Research Triangle Park, GlaxoSmithKline, Inc., 5 Moore Drive, NC 27709, United States
[b] Department of Computational and Structural Chemistry, Research Triangle Park, GlaxoSmithKline, Inc., 5 Moore Drive, NC 27709, United States
[c] Department of Screening and Compound Profiling, Research Triangle Park, GlaxoSmithKline, Inc., 5 Moore Drive, NC 27709, United States
[d] Department of Oncology Chemistry, GlaxoSmithKline, Inc., 5 Moore Drive, Research Triangle Park, NC 27709, United States
[e] Department of Oncology Biology, 1250 South Collegeville Road, Collegeville, PA 19426, United States

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ABSTRACT

Insulin-like growth factor 1 receptor (IGF-1R) is an attractive target for anti-cancer therapy due to its anti-apoptotic effect on tumor cells, but inhibition of insulin receptor (IR) may have undesired metabolic consequences. The primary sequences of the ATP substrate-binding sites of these receptors are identical and the crystal structures of the activated kinase domains are correspondingly similar. Thus, most small-molecule inhibitors described to date are equally potent against the activated kinase domains of IGF-1R and IR. In contrast, the non-phosphorylated kinase domains of these receptors have several structural features that may accommodate differences in binding affinity for kinase inhibitors. We used a cell-based assay measuring IGF-1R autophosphorylation as an inhibitor screen, and identified a potent purine derivative that is selective compared to IR. Surprisingly, the compound is a weak inhibitor of the activated IGF-1R tyrosine kinase domain. Biochemical and structural studies are presented that indicate the compound preferentially binds to the ATP site of non-phosphorylated IGF-1R compared to phosphorylated IGF-1R. The potential selectivity and potency advantages of this binding mode are discussed.

1. Introduction

The insulin-like growth factor 1 receptor (IGF-1R[1]) is a transmembrane receptor tyrosine kinase. The mature receptor has a heterotetrameric structure of two extracellular ligand-binding α-chains linked by disulfide bonds to two β-chains that span the membrane and contain the tyrosine kinase catalytic activity [1]. The unliganded receptor exists in a low activity, non-phosphorylated form. Binding of the ligands, insulin-like growth factors 1 or 2 (IGF-1 or IGF-2), elicits a conformational change that allows trans-phosphorylation of the β-chains. Three tyrosine residues in the kinase domain activation loop are phosphorylated resulting in structural rearrangement and a significant increase in kinase activity [2]. Activation of the kinase results in phosphorylation of intracellular substrates including insulin receptor substrates 1–4 (IRS1–4) and different Shc isoforms. These phosphorylation events trigger two key signal transduction cascades leading to AKT and extracellular-signal-regulated kinase (ERK) activation, which promote growth and survival of cells [3–5].

IGF-1R signaling plays a significant role in cancer. IGF-1R overexpression results in cellular transformation to a malignant phenotype and cells lacking IGF-1R are resistant to transformation by a variety of other oncogenes [3,5,6]. Furthermore, many human tumors overexpress IGF-1R and activation increases metastatic propensity [7]. For these and other reasons, IGF-1R has become an attractive target for anti-cancer therapy [8,9]. Inhibitors of kinase activity are one approach for targeting receptor tyrosine kinases [10,11]. For example, the epidermal growth factor receptor (EGFR)

* Corresponding author at: P.O. Box 13398, Research Triangle Park, NC 27709-13398, United States. Tel.: +1 919 483 3910; fax: +1 919 315 6720.
E-mail address: erw39216@gsk.com (E.R. Wood).

[1] The abbreviations used are: IGF-1R, insulin-like growth factor 1 receptor; IR, insulin receptor; IRS, insulin receptor substrate; ERK, extracellular-signal-regulated kinase; EGFR, epidermal growth factor receptor; DMEM, Dulbecco's modified Eagles medium; ECL, electrochemiluminescence; GST, glutathione *S*-transferase; BSA, bovine serum albumin; FBS, fetal bovine serum; DTT, dithiothreitol; DMSO, dimethylsulfoxide; DELFIA, dissociation-enhanced lanthanide fluorescent immunoassay.

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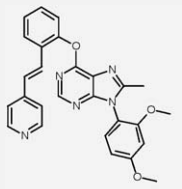
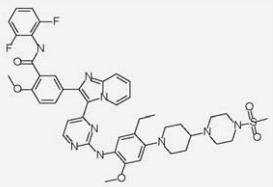
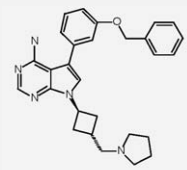
Table 1
Properties of IGF-1R inhibitors.

Compound	Cellular PY		Activated enzyme substrate PY	
	IGF-1R	IR	GST-IGF-1R$_{cat}$	GST-IR$_{cat}$
	IC_{50} (nM)		IC_{50} (nM)	
 Compound 1	110 (50)	2994 (618)	3300 (2300)	4000 (700)
 GSK4529	22	19	1.6	1.3
 NVP-AEW541	90	2300	150	140

For compound 1, inhibition of ligand-stimulated receptor autophosphorylation was evaluated in NIH3T3 cells stably overexpressing either IGF-1R or IR (Cellular PY). Peptide substrate phosphorylation reactions were conducted using purified, fully activated GST-IGF-1R$_{cat}$ or GST-IR$_{cat}$. Data represents the average of at least three independent experiments. The standard deviation of the mean is shown in parentheses. Results for GSK4529 and NVP-AEW541 have been described previously [15,19].

and ErbB-2 inhibitor, lapatinib, has been approved for advanced breast cancer and selective EGFR inhibitors, gefitinib and erlotinib, are approved for the treatment of non-small cell lung cancer [12,13]. Several inhibitors of IGF-1R tyrosine kinase activity have been described that have activity in pre-clinical cancer models [14–17].

A challenge for the development of IGF-1R-targeted kinase inhibitors may be the catalytic domain similarity to the highly related insulin receptor (IR). Inhibition of IR would be expected to have significant effects on glucose homeostasis and chronic treatment may result in symptoms of diabetes [3]. The majority of kinase inhibitors described to date bind to the ATP site of the catalytic domain. For IGF-1R and IR, the amino acid sequences of the catalytic domains are 84% identical, and the ATP sites are 100% identical [18]. GSK4529 is a typical ATP-competitive IGF-1R inhibitor [19]. The compound is an equipotent inhibitor of purified IGF-1R and IR kinase domains. In cells, GSK4529 inhibits IGF-1R and IR autophosphorylation with similar potency that is reduced relative to the K_i for inhibition of the purified enzyme. This is expected for an ATP-competitive compound since the cellular ATP concentration is high (1–2 mM). BMS-554417 is a potent inhibitor of IGF-1R [16]. The compound inhibits the purified enzyme (IC_{50} = 68 nM), IGF-stimulated receptor autophosphorylation in cell culture and tumor growth in mice. The compound is also an equipotent inhibitor of purified IR (IC_{50} = 51 nM). Treatment with BMS-554417 was found to result in transient increases in insulin and glucose levels in mice. The dual inhibition of IGF-1R and IR and the physiological effects on glucose homeostasis reported for these compounds are consistent with the structural similarity and biological functions of the receptors. Another compound, NVP-AEW451, appears to have a different mode of inhibition [15]. This compound inhibits purified IGF-1R and IR with similar potency (IC_{50} = 150 and 140 nM respectively). However, in intact cells the compound is selective for IGF-1-stimulated IGF-1R autophosphorylation (IC_{50} = 90 nM) compared to insulin-stimulated IR autophosphorylation (IC_{50} = 2300 nM). Moreover, treatment with NVP-AEW451 at doses that blocked tumor growth did not result in an increase in insulin or glucose levels in mice. With NVP-AEW451, selectivity for IGF-1R over IR is observed in intact cells but not in assays measuring substrate phosphorylation catalyzed by the purified, activated catalytic domains.

We conducted a high-throughput screen of the GlaxoSmithKline compound collection for inhibitors of IGF-1R. Typically, receptor tyrosine kinase inhibitor screens are conducted using recombinantly expressed, purified, catalytic domains [20,21]. However, because of the challenges of IGF-1R selectivity, we developed a high-throughput assay that measures IGF-1-stimulated receptor autophosphorylation in intact cells. We identified compound 1 that potently inhibited receptor autophosphorylation (Table 1). Interestingly, compound 1 is a poor inhibitor of the purified, activated catalytic domain. Moreover, we also observe selectivity over insulin-stimulated IR autophosphorylation.

2. Materials and methods

2.1. Materials

Dimethylsulfoxide (DMSO), bovine serum albumin (BSA), Triton X-100, IGEPAL CA630, sodium deoxycholate, sodium orthovanadate, sodium fluoride, insulin, HEPES, $MgCl_2$, ATP, dithiothreitol (DTT), CHAPS, EDTA, MOPS, and sodium citrate were from Sigma–Aldrich (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM)/F-12, fetal bovine serum (FBS), and