The growth factor receptor tyrosine kinases EGFR (ErbB-1) and ErbB-2 (ErbB-2) play a major role in controlling cell growth and differentiation. These two receptor tyrosine kinases are often overexpressed and/or abnormally active in a wide variety of tumor types. Based on EGFR and ErbB-2’s critical roles in cell proliferation, differentiation and survival, inhibition of these kinases is an attractive and promising therapeutic approach for the treatment of tumors. In a series of experiments, we show that ARRY-334543 is an orally active dual inhibitor of EGFR and ErbB-2. The compound behaves as a reversible ATP-competitive inhibitor with nanomolar potency both in vitro and in cell-based proliferation assays using A431 and BT-474 cells. Selectivity against a panel of kinases has been demonstrated in vitro. In mouse xenograft models utilizing the EGFR over expressing tumor line A431, ARRY-334543 demonstrated significant dose related tumor growth inhibition when administered orally, BID, for 21 days. Based on potency, selectivity and efficacy data, the compound is currently in development as an anti-cancer agent.

The EGF family of receptor tyrosine kinases consists of ErbB-1 (EGFR), ErbB-2 (ErbB-2), ErbB-3 and ErbB-4. In response to the binding of various ligands, these kinases undergo heterodimerization and homodimerization, resulting in activation of numerous downstream targets. Several new cancer therapies have been developed that target the EGF family of kinases. Currently approved therapies include ErbB-1 specific small molecule inhibitors (BIBX1382) and monoclonal antibodies (Herceptin). Despite the clinical success of these drugs, there still remains a need to develop more efficacious and broadly applicable compared to current therapies. Based on potency, selectivity and efficacy data, the compound is currently in development as an anti-cancer agent.

Methods & Results

ARRY-334543 is an ATP-Competitive Inhibitor of ErbB-2,  Ki = 1 nM

**References:**


**Cancer – EGFR Signaling Pathway**

**Figure 1**

**Figure 2a**  Purified full-length ErbB-1 (Biolmol) or histidine-tagged ErbB-2 kinase domain (residues 691-1255) were assayed with 1 µM [3H]ATP.  Each reaction was terminated at 15 min by addition of 10% ice cold Triton-X 100 and plated onto nitrocellulose.  Quantitation of enzyme activity was completed by addition of TMB substrate and spectrophotometric measurement at 450 nm in a microtiter plate Reader.

**Figure 2b**  Western Blot showing that ARRY-334543 inhibits ErbB-2 phosphorylation on Tyr1068.  Immunoblotting of 60 µg of protein from each of the following cell lines were probed with anti-phospho Tyr1068 antibody: A431, BT-474, and MDA-MB-453.  The effect of ARRY-334543 was assessed in the presence of 50 nM ATP.

**Figure 3**

**Figure 3a**  Western Blot showing that ARRY-334543 inhibits ErbB-2 phosphorylation on Tyr1068.  Immunoblotting of 60 µg of protein from each of the following cell lines were probed with anti-phospho Tyr1068 antibody: A431, BT-474, and MDA-MB-453.  The effect of ARRY-334543 was assessed in the presence of 50 nM ATP.

**Figure 3b**  Western Blot showing that ARRY-334543 inhibits ErbB-2 phosphorylation on Tyr1068.  Immunoblotting of 60 µg of protein from each of the following cell lines were probed with anti-phospho Tyr1068 antibody: A431, BT-474, and MDA-MB-453.  The effect of ARRY-334543 was assessed in the presence of 50 nM ATP.

**Figure 4**

**Figure 4a**  Western Blot showing that ARRY-334543 inhibits ErbB-2 phosphorylation on Tyr1068.  Immunoblotting of 60 µg of protein from each of the following cell lines were probed with anti-phospho Tyr1068 antibody: A431, BT-474, and MDA-MB-453.  The effect of ARRY-334543 was assessed in the presence of 50 nM ATP.

**Figure 4b**  Western Blot showing that ARRY-334543 inhibits ErbB-2 phosphorylation on Tyr1068.  Immunoblotting of 60 µg of protein from each of the following cell lines were probed with anti-phospho Tyr1068 antibody: A431, BT-474, and MDA-MB-453.  The effect of ARRY-334543 was assessed in the presence of 50 nM ATP.

**Figure 5**

**Figure 5a**  Western Blot showing that ARRY-334543 inhibits ErbB-2 phosphorylation on Tyr1068.  Immunoblotting of 60 µg of protein from each of the following cell lines were probed with anti-phospho Tyr1068 antibody: A431, BT-474, and MDA-MB-453.  The effect of ARRY-334543 was assessed in the presence of 50 nM ATP.

**Figure 5b**  Western Blot showing that ARRY-334543 inhibits ErbB-2 phosphorylation on Tyr1068.  Immunoblotting of 60 µg of protein from each of the following cell lines were probed with anti-phospho Tyr1068 antibody: A431, BT-474, and MDA-MB-453.  The effect of ARRY-334543 was assessed in the presence of 50 nM ATP.

**Figure 6**

**Figure 6a**  Western Blot showing that ARRY-334543 inhibits ErbB-2 phosphorylation on Tyr1068.  Immunoblotting of 60 µg of protein from each of the following cell lines were probed with anti-phospho Tyr1068 antibody: A431, BT-474, and MDA-MB-453.  The effect of ARRY-334543 was assessed in the presence of 50 nM ATP.

**Figure 6b**  Western Blot showing that ARRY-334543 inhibits ErbB-2 phosphorylation on Tyr1068.  Immunoblotting of 60 µg of protein from each of the following cell lines were probed with anti-phospho Tyr1068 antibody: A431, BT-474, and MDA-MB-453.  The effect of ARRY-334543 was assessed in the presence of 50 nM ATP.

**Summary**

**Based on data provided, these experiments demonstrate:**

ARRY-334543 is a selective kinase inhibitor of ErbB-1 and ErbB-2 that is competitive with ATP.

ARRY-334543 inhibits phosphorylation of ErbB-1 and ErbB-2 in cells that contain these activated receptors.

ARRY-334543 is a potent inhibitor of the AKT pathway in cells that contain active ErbB-2 receptors.

When dosed orally, ARRY-334543 inhibits growth of human tumor xenografts that overexpress ErbB-1 (A431) or ErbB-2 (MDA-MD-453) in a dose-dependent manner. This activity is superior to that seen with a benchmark compound. 

**References:**