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Discovery of 4,6-bis-anilino-1*H*-pyrrolo[2,3-*d*]pyrimidines: Potent inhibitors of the IGF-1R receptor tyrosine kinase

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ABSTRACT

The evaluation of a series of 4,6-bis-anilino-1*H*-pyrrolo[2,3-*d*]pyrimidines as inhibitors of the IGF-1R (IGF-IR) receptor tyrosine kinase is reported. Examples demonstrate nanomolar potencies in in vitro enzyme and mechanistic cellular assays as well as promising in vivo pharmacokinetics in rat.

The IGF-1R signaling pathway is activated in many human cancers including prostate,[1] colon,[2] breast,[3] and pancreas[4] by overexpression of IGF-1R or its ligands IGF-1 and 2 and/or by decreased levels of IGF binding proteins. Inhibition of IGF-1R signaling using a variety of approaches (e.g., anti-IGF-1R antibodies, antisense, IGF binding proteins, siRNA) has resulted in decreased proliferation and survival of tumor cells in vitro and in vivo.[5] Further, IGF-1R activation has been associated with resistance to targeted agents such as trastuzumab[6] in breast cancer. Therefore, inhibition of IGF-1R signaling may reverse resistance to targeted agents under certain conditions. These observations have led to the development of a number of potent small-molecule inhibitors of IGF-1R in diverse chemical space.[7] We report herein the investigation of a series of 4,6-bis-anilino-1*H*-pyrrolo[2,3-*d*]pyrimidines, whose syntheses have been described elsewhere,[8] as potent inhibitors of the IGF-1R receptor tyrosine kinase.

Pyrrolopyrimidine **1** was identified from a focused library consisting of small molecules with known kinase inhibitory motifs. A remarkable kinase selectivity profile was observed for **1**, wherein 48 of 52 kinases screened in the initial panel showed IC_{50} values of >100 nM (50-fold selectivity relative to IGF-1R). However, in addition to IGF-1R, pyrrolopyrimidine **1** also potently inhibited JNK1, ALK, and IR, with IC_{50}s of 12, 1.0, and 6.3 nM, respectively.

Figure 1. Model of IGF-1R (carbon atoms in grey) in complex with **1** (carbon atoms in green). Intermolecular H-bond interactions are highlighted with yellow lines.

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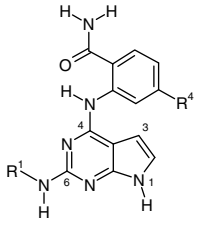
To provide a structure-based rationale for medicinal chemistry efforts, a docking model of the IGF-1R kinase domain in complex with **1** was constructed using available crystallographic data (Fig. 1).[9] Lead **1** forms three H-bond interactions at the hinge region, one with the backbone carbonyl of Glu1050 and the second with the backbone amino group of Met1052. A third H-bond is also highlighted between the C6 amino group and the backbone carbonyl of Met1052. The 2-aminobenzamide occupies the inner hydrophobic region of the ATP-binding site with the phenyl making van der Waals contact with Leu975, Gly976, Gln977 and Val983. The carboxamide is directed toward Lys1003 (catalytic lysine) and Asp1123 (of the DFG motif), but does not form an H-bond interaction with either residue. Instead, the carboxamide forms an intramolecular H-bond with the C4 amino group forming a pseudo-six membered ring. The C6 aniline occupies the outer hydrophobic region of the pocket where it makes van der Waals contact with Leu975, Gly1055 and Met1112. The C2′ methyl is directed toward the hinge region where it interacts with Leu975, Leu1051 and Thr1053. Lastly, the 1-propyl-tetrahydro-3-pyridinyl lies outside the pocket and is thus solvent exposed.

Initial structural optimizations were focused on the C6 aniline, wherein it was anticipated substitution at C2′ in particular would be crucial for efficient binding to IGF-1R because of both spatial proximity to Leu1051 and influence on the C6 aniline N–H pK_a. As illustrated in Table 1, a C2′ methoxy substituent proved optimal (see **5** and **6**). Bulkier alkoxy substituents (**7**) and strong electron withdrawing substituents (**4**) were less potent in screening at both the enzyme[10] and cellular[11] level (Table 1). Smaller substituents (**2** and **3**) gave reasonable enzyme potency but suffered from a reduced kinase selectivity profile.[12] Simple fluorination at R^4 (i.e., **5** vs **6**) had only minimal influence on inhibitor potency, but proved important in reducing in vivo metabolism (vida infra).

Consistent with the model, tolerability to a wide variety of substituents was anticipated at C4′ because of its orientation toward solvent (Fig. 1), and removal of the unsaturated piperidine was desirable from a safety perspective.[13] Indeed, both saturated 3- and 4-substituted piperidines (Table 1, **8** and **9**) and 1,4-disubstituted piperazines (Table 1, **10** and **11**) possess excellent potency against IGF-1R. Additionally, the distal latent amine was not required, as morpholine-substituted **12** proved to be an exceptionally potent inhibitor of IGF-1R. Initially, synthetic tractability and promising pharmacokinetic properties (vida infra) led us to explore the piperazine subseries exemplified by **10** and **11** more carefully.

Crystallographic confirmation of the binding mode for the pyrrolopyrimidine series of IGF-1R inhibitors was achieved through the use of an IR double mutant (C981S, D1132N). Given that the sequence identity between IGF-1R and IR is roughly 85% over their kinase binding domains and identical within the ATP-binding cleft, IR was a reasonable surrogate for IGF-1R. A co-crystal structure of our IR mutant in complex with **13** was produced and is illustrated in Figure 2. The overall binding mode for **13** was similar to that modeled for **1** in complex with IGF-1R. Key intermolecular interactions included the H-bonding of **13** to IR at the hinge region via the inhibitor's pyrrolopyrimidine. Also, as with our docking model, the H-bond interaction between the C6 amino group and the backbone carbonyl of Met1079 was at a non-ideal angle and distance. Interestingly, the carboxamide did interact indirectly with Asp1150 through a water-mediated interaction. Finally, the C2′ methoxy was directed towards the hinge region in similar fashion to the C2′ methyl of **1**, while

Table 1
IGF-1R enzyme IC_{50} and phospho IGF-1R cellular IC_{50} results for **1–12**. (values represent an average of ⩾2 individual measurements)



Compound	R^4	R^1	X	IGF-1R Enzyme IC_{50} (nM)	Phospho IGF-1R Cellular IC_{50} (nM)
1	H		–Me	5.0	—
2	H		–H	1.6	—
3	H		–F	1.6	317
4	H		$-CF_3$	20	>10,000
5	H		–OMe	0.6	104
6	F		–OMe	0.8	82
7	F		–O*i*Pr	3.2	2240
±8	F		–Pr	0.4	60
9	F		–Pr	0.3	47
10	F		–Pr	1.6	122
11	F		–*i*Pr	0.8	130
12	F		—	1.6	68