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Imidazole moiety replacements in the 3-(1*H*-benzo[d]imidazol-2-yl)pyridin-2(1*H*)-one inhibitors of insulin-like growth factor receptor-1 (IGF-1R) to improve cytochrome P450 profile

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Abstract—A series of 3-(1*H*-benzo[d]imidazol-2-yl)pyridin-2(1*H*)-one inhibitors of insulin-like growth factor receptor-1 (IGF-1R) were examined in which the pendant imidazole moiety was replaced to improve selectivity for IGF-1R inhibition over cytochrome P450 (CYP). Synthesis and SAR of these compounds is presented.

Insulin-like growth factor receptor-1 (IGF-1R) plays an important role in normal cell physiology, and is also implicated in the establishment and maintenance of the malignant phenotype.[1] IGF-1R is a tyrosine kinase receptor that is activated by the binding of its ligands, IGF-I or IGF-II, to the extracellular domain. Ligand binding causes a conformational change resulting in autophosphorylation of tyrosine residues in the intracellular kinase domain resulting in activation of both the mitogenic (Ras/Raf/MAPK) and survival pathways (PI3K/Akt/mTor).[1,2] In addition, recent studies have shown that IGF-1R regulates cell adhesion and motility.[3] Overexpression of IGF-1R and IGF-I has been demonstrated in a variety of tumors,[4] including glioma, lung, ovary, breast, carcinomas, sarcomas, and melanoma. Therefore, IGF-1R seems to be a very promising target for cancer therapy.[5] As a consequence, the identification of potent inhibitors of IGF-1R has recently attracted considerable attention and several classes of inhibitors have been described.[6]

We have recently reported the discovery and biological activity associated with the benzimidazole-pyridone derivatives stemming from the identification of **1** as a prototype of a new structural class of IGF-1R kinase inhibitors.[7] Further optimization of **1** by installing basic amine side chains at the C-4-position of pyridone led to the identification of **2**, **3**, and **4** with IC_{50}'s of 390, 530, and 180 nM, respectively.[8] Additionally, compound **4** reduced autophosphorylation of IGF-1R in CD8-IGF1R mouse xenografts when dosed in vivo. However, compounds **1**–**4** exhibited potent cytochrome P450 (CYP) inhibition profiles across 3A4, 2C9, 2C19, 2D6, and 1A2 isozymes (Table 1). Such potent inhibition of drug-metabolizing CYP enzymes may cause unfavorable drug–drug interactions in the clinic.[9] Many lead candidate molecules in pharmaceutical development fail due to potent inhibition of one or more CYP enzyme isoforms. Consequently, we sought to improve the CYP inhibition of this chemotype while maintaining or improving the IGF-1R enzyme potency. By examining the structural elements within the chemotype **1**, it was reasoned that the pendant imidazole moiety could be responsible for potent CYP inhibition, as some

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Table 1. IGF1R and CYP activities[11] for compounds **2**, **3**, and **4**

IC_{50} (μM)	2	3	4
IGF-1R	0.39	0.53	0.18
CYP1A2	<0.03	<0.04	<0.04
CYP2C9	<0.03	<0.04	<0.04
CYP2C19	0.1	<0.04	<0.04
CYP2D6	5.7	0.56	ND
CYP3A4-BFC	0.08	0.06	<0.046

imidazole derivatives such as ketoconazole and clotrimazole are known to show high affinity for the heme iron of major drug-metabolizing CYP enzymes Figure 1.[9b,10]

The crystal structure of **1** bound to a truncated IGF-1R revealed that the pendant imidazole ring is solvent exposed, and is not involved in any favorable hydrogen bonding interactions with the receptor.[7a] Therefore, we explored replacing of the imidazole ring with other groups in an effort to improve selectivity for IGF-1R inhibition over cytochrome P450 inhibition.

At the outset, we elected to replace the imidazole moiety with both the bromo and cyano groups as these groups are considered versatile chemical handles for analog synthesis. Accordingly, the syntheses of bromo derivative **10** and cyano derivative **24** were accomplished as outlined in Schemes 1 and 2. Commercially available 4-bromo-2-methyl-6-nitro aniline (**5**) was reduced to furnish diamine, which was immediately treated with 2-methoxy-4-iodo-pyridine-3-carboxaldehyde[12] (**7**) to afford benzimidazole **8**. The methoxy group in **8** was cleaved, and the resultant iodopyridone was heated with (*S*)-phenyl alaninol to provide **10**. Suzuki coupling with aryl boronic acids was rather sluggish presumably for solubility reasons, and therefore the primary hydroxyl was protected as its trityl ether **11** which underwent Suzuki couplings smoothly with several aryl boronic acids. Finally, the trityl group was deprotected with trifluoroacetic acid to afford biaryls (**12**, **13**, and **14**).

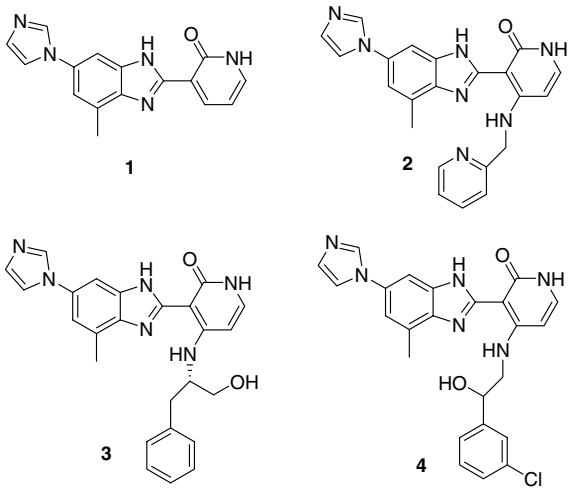
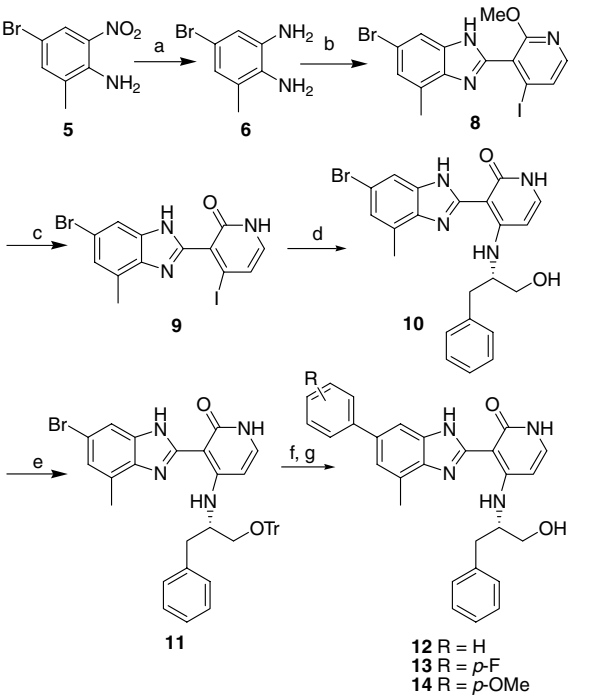


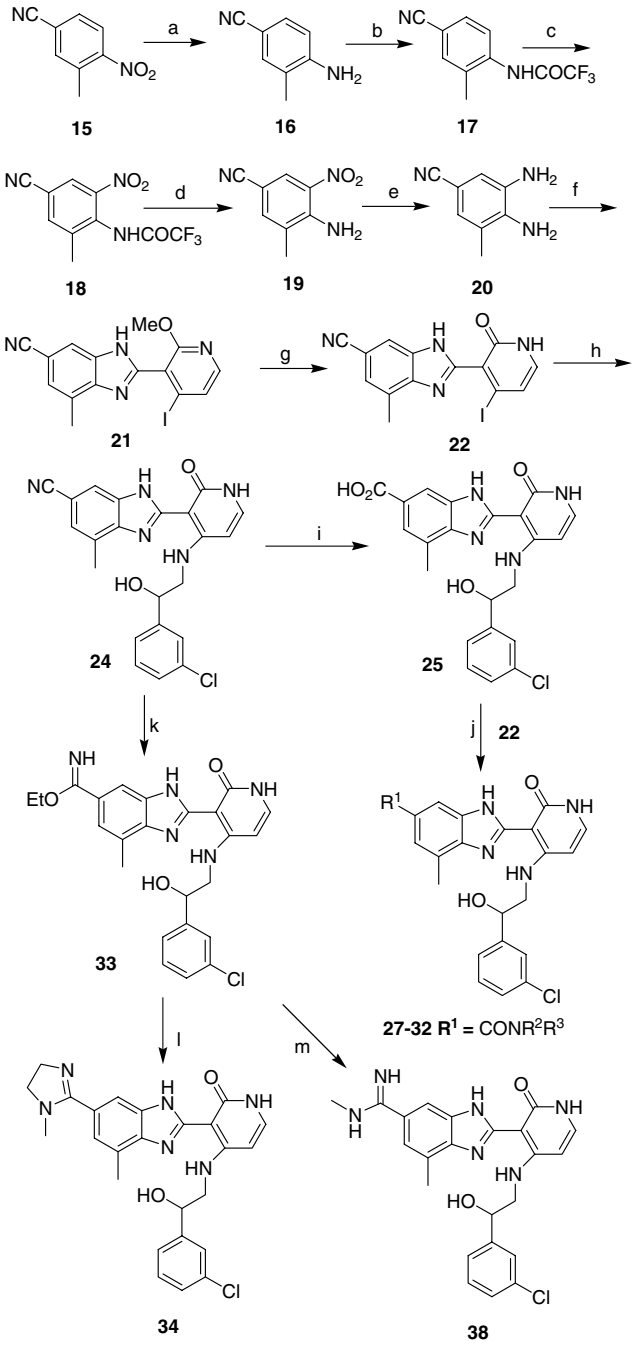
Figure 1. Representative benzimidazole IGF-1R inhibitors.



Scheme 1. Reagents and conditions: (a) $SnCl_2{\cdot}2H_2O$ EtOH, reflux, 59%; (b) 2-methoxy-4-iodo pyridine-3-carboxaldehyde (**7**), MeOH, rt, 50%; (c) 4 N HCl in dioxane, H_2O, rt, 100%; (d) phenyl alaninol, Et_3N, DMF, 90 °C, 89%; (e) triphenylmethyl chloride, Et_3N, DMAP, CH_2Cl_2, 72%; (f) aryl boronic acids, $Pd(PPh_3)_4$, K_3PO_4, THF, reflux, 60–67%; (g) TFA, CH_2Cl_2, 100%.

Similarly, the cyano derivative **24**, which is useful to synthesize other heterocycles, was synthesized starting from 3-methyl-4-nitro-benzonitrile (**15**). The nitro group was reduced and the resultant aniline **16** was trifluoroacetylated. The amide **17** was subjected to nitration by treating with potassium nitrate in sulfuric acid, and then the trifluoroacetyl group was cleaved. The nitroaniline **19** was reduced and the resultant phenylene diamine **20** was immediately treated with the 2-methoxy-4-iodo-pyridine-3-carboxaldehyde[12] (**7**) to furnish the benzimidazole **21**. The methoxy group was cleaved to give the iodo pyridone **22**, which was heated with *meta*-chloro phenethylamine **23** to afford **24**. The nitrile group was hydrolyzed to the corresponding acid **25**, which was used to make amides **27**–**32**. Additionally, the cyano compound **24** was also converted to imidate ester **33** by exposure to anhydrous HCl gas in ethanol. Heating **33** with several 1,3 or 1,4 diamines furnished imidazolines (**34**, **35**, and **36**) or tetrahydropyrimidine **37**, respectively. The carbon attached imidazole **39** was built from the imidate ester **33** using a two-step procedure.[13]

As outlined in Table 2, biaryls **12**–**14** represented the first examples with a reduced cytochrome P450 inhibition profile in this series of IGF-1R inhibitors. These results suggest that the pendant imidazole was primarily responsible for potent CYP inhibition as anticipated. Interestingly, the bromo compound **10** displayed reduced CYP3A4 inhibition, while still retaining significant affinity for other CYP isozymes.



Scheme 2. Reagents and conditions: (a) Fe, HOAc, 74%; (b) trifluoroacetic anhydride, Et_3N, CH_2Cl_2, 100%; (c) KNO_3, concd sulfuric acid, 52%; (d) 2 M NH_3 in MeOH, 95%; (e) $SnCl_2{\cdot}2H_2O$, EtOH reflux, 90%; (f) 2-methoxy-4-iodo-pyridine-3-carboxaldehyde (7), I_2, MeOH, 46%; (g) 4 N HCl in dioxane, H_2O, 100%; (h) 2-amino-1-(3-chlorophenyl)-ethanol (**23**), DMF, Et_3N, 80 °C, 94%; (i) 6 N HCl, 80 °C, 54%; (j) DPPA, Et_3N, DMF, amines, 40–60%; (k) anhydrous HCl, EtOH, 90%; (l) *N*-methyl 1,3-propane diamine, EtOH, reflux, 100%; (m) NH_2Me, heat, 100%.

However, the biaryls (**12**–**14**) were found to be less potent against IGF-1R relative to the imidazole containing compounds (**2**, **3**, and **4**). With these promising initial results in improving the CYP profile our efforts shifted to cyano compound **24** and derivatives thereof Table 3.

Table 2. IGF1R and CYP activities[11] for imidazole replacements

IC_{50} (μM)	**10**	**12**	**13**	**14**
IGF-1R[a]	1.4	3.1	5.1	4.0
CYP1A2	0.052	0.58	3.2	12.0
CYP2C9	0.72	2.3	4.0	11.0
CYP2C19	0.32	0.53	1.0	2.2
CYP2D6[b]	ND	ND	ND	ND
CYP3A4	3.1	3.6	6..3	6.8

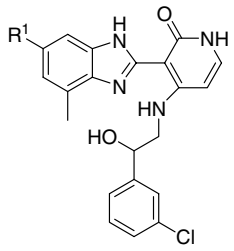
[a] IC_{50} values represent the average of two determinations and standard deviations ranged from 0.16 to 0.84.
[b] Overlap with reference, IC_{50} could not be determined.

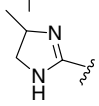
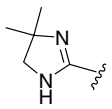
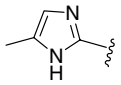
The compound that lacks imidazole, **26**, displayed a fourfold reduction in IGF-1R enzyme potency with an improved CYP profile. Examination of various functional groups at the imidazole location led to the identification of amides that retained IGF-1R enzyme potency while improving the CYP profile. The primary and secondary amides (**27** and **28**) displayed equipotency of 180 nM while the tertiary amide (**29**) was less active (550 nM). It was found that increasing bulk on the secondary amide led to a decrease in enzyme potency as represented by compounds **28** and **30** through **32**. The enhanced IGF-1R potency and acceptable CYP profile (3.7 μM for 3A4) of secondary amide **28** prompted us to make the corresponding methyl amidine **38**, which displayed similar IGF-1R potencies of 170 nM with an improved CYP profile across all isozymes. In order to fine-tune the potency of amidine containing compounds, cyclic amidines such as imidazolines were synthesized. We were delighted to observe a significant improvement in IGF-1R activity with these imidazolines (**34**, **35**, and **36**) and tetrahydropyrimidine **37** with a reduction in affinity for CYP enzymes. For instance, *N*-methyl imidazoline **34** displayed an impressive 93 nM IGF-1R activity and even further reduction in cytochrome P450 inhibition (>17 μM against all isozymes). Alpha methyl substitutions on *N*-methyl imidazoline as represented by **35** and **36** further enhanced selectivity of IGF1R versus CYP inhibition with 46 nM for compound **36** while its CYP3A4 affinity was 13 μM.

The corresponding six-membered tetrahydro pyrimidine **37** also maintains excellent IGF-1R inhibition (68 nM) with less affinity for CYP enzymes (>8.5 μM). Interestingly, the unsaturated version of imidazoline, the carbon attached imidazole **39**, regained some of its CYP inhibition. Taken together, these findings demonstrate that saturated heteroaryls such as imidazolines were optimal for greater separation of IGF-1R activity versus CYP inhibition. The reduced CYP inhibition of these imidazolines could be in part due to enhanced basicity. Further investigation of these imidazolines for selectivity over other tyrosine kinases, cell-based activities, and optimization for desirable ADME properties are ongoing.

In summary, the results described herein have demonstrated that the pendant imidazole was primarily responsible for potent cytochrome P450 inhibition. By replacing this moiety with other groups such as imidazolines, greater separation of IGF-1R activity versus CYP inhibition was observed. Future studies will expand on

Table 3. IGF1R and CYP activities[11] for imidazole replacements



Compound	R_1	IC_{50}[a]-IGF1R (μM)	CYP1A2	CYP2C9	CYP2C19	CYP3A4
4	Imidazole	0.18	<0.046	<0.04	<0.04	<0.046
26	H	0.77	0.38	1.5	0.26	0.71
27	$CONH_2$	0.18	ND	ND	ND	ND
28	CONHMe	0.18	21.0	2.9	2.1	3.7
29	$CONMe_2$	0.55	15	2.8	1.7	2.2
30	CONH-cyclopropyl	0.27	93	8.1	3.5	10
31	CONH-cyclopentyl	1.3	9.8	3.5	0.48	1.6
32	CONH-cyclohexyl	2.5	59	6.6	1.3	11
34		0.093	53	91	17	35
35		0.054	10	67	4.7	4.3
36		0.046	11	47	5.1	13
37		0.068	20	100	8.5	16
38		0.17	25	76	3.9	15
39		0.14	5.8	7.8	2.0	0.36

[a] IC_{50} values represent the average of two determinations. Standard deviations ranged from 0.03 to 0.26.

these results to identify compounds with superior ADME properties for further in vivo evaluation.

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11. (a) *IGF-1R In Vitro Assay*. The primary screen is an in vitro kinase assay that utilized the fusion of glutathione-*S*-transferase (GST) to the IGF-1R receptor cytoplasmic domain that contains the catalytic activity. The cytoplasmic sequence of the human IGF-1R was expressed as a recombinant protein using baculovirus insect cells and was purified by affinity chromatography on glutathione-Sepharose. The IGF-1 receptor tyrosine kinase was assayed using the synthetic polymer poly(Glu/Tyr) (Sigma Chemicals) as a phosphoacceptor substrate. Each reaction mixture was in a total volume of 50 μl and contained 125 ng of enzyme, poly(Glu/Tyr) at 50 μg/ml (2.5 ng/well final), and 1–25 μM ATP, and 0.1 μCi [γ-^{33}P]ATP. The mixtures contained also 20 mM MOPS, pH 7.0, 5 mM $MnCl_2$, 0.5 mM dithiothreitol, and bovine serum albumin at 0.1 mg/ml. The reaction mixtures were incubated at 27 °C for 1 h and kinase activity was determined by quantitation of the amount of radioactive phosphate transferred to the poly(Glu/Tyr) substrate. Incorporation was measured by acid precipitation of the proteins and scintillation counting. Compounds were dissolved in dimethylsulfoxide to a concentration of 10 mM and were added to the kinase assays such that the final concentration of dimethylsulfoxide was no more than 1%, which has been shown to have no effect on kinase activity;
(b) *CYP Enzyme Assay*. The ability of test compounds to inhibit CYP microsomes (supersomes) was measured using 3-cyano-7-ethoxycoumarin (CYP1A2 and CYP2C19), 7-benzyloxy-4-trifluoromethyl coumarin (CYP3A4), 7-methoxy-4-trifluoromethyl coumarin (CYP2C9), and 3-[2-(*N*,*N*-diethyl-*N*-thylamino)ethyl]-7-methoxy-4-methyl coumarin (CYP2D6) as substrates. Assays were performed in 384-well microplates in a total volume of 30 μl. Incubations were performed with microsomes derived from baculovirus-infected cells, which were obtained from BD Gentest (Woburn, MA).
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