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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 2317–2321

Discovery and initial SAR of 3-(1*H*-benzo[*d*]imidazol-2-yl)pyridin-2(1*H*)-ones as inhibitors of insulin-like growth factor 1-receptor (IGF-1R)

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Received 19 September 2006; revised 12 January 2007; accepted 18 January 2007
Available online 4 February 2007

Abstract—The discovery and synthesis of 3-(1*H*-benzo[*d*]imidazol-2-yl)pyridin-2(1*H*)-one inhibitors of insulin-like growth factor 1-receptor (IGF-1R) are presented. Installing amine containing side chains at the 4-position of pyridone ring significantly improved the enzyme potency. SAR and biological activity of these compounds is presented.

Insulin-like growth factor-1 receptor (IGF-1R) is a member of the insulin receptor family of tyrosine kinases that is widely expressed in human tissues.[1] Signaling through the IGF-1R activates several downstream pathways which include Ras/Raf/MAPK and PI-3K/Akt pathways.[1,2] Growing evidence implicates that (IGF-1R) mediated signaling plays a crucial role in the development and progression of cancer.[2] For example, cells lacking IGF-1R kinase activity grow very slowly in culture and cannot be transformed by either viral or cellular oncogenes. Transfection of these cells with wild-type IGF-1R restores the ability of these cells to be transformed by viral and cellular oncogenes.[3] Reduction of receptor number or enzymatic activity by several strategies that include antisense and antibody approaches, as well as dominant negative mutants (lacking enzyme activity), reverses the transformed phenotype in tumor cells.[4] From a clinical perspective, overexpression of IGF-1R and IGF-1 has been demonstrated in a variety of tumors, including glioma, lung, ovary, breast, carcinomas, sarcomas, and melanoma.[5] These findings underline the desirability of identifying small-molecule IGF-1R inhibitors and evaluating their potential value for the treatment of human cancers.

Several IGF-1R inhibitors have been recently reported. Of these, CP-751871 is a fully human immunoglobulin G2 antibody that is in Phase I clinical trials.[2d] Small-molecule antagonists such as pyrrolopyrimidines,[6] pyrrole-5-carboxaldehyde,[7] picropodophyllin,[8] and tyrphostin[9] analogs have also shown to have IGF-1R inhibitory activity. We recently reported the identification of a small molecule inhibitor for IGF-1R from

Keywords: Insulin-like growth factor-1 receptor; Tyrosine kinase; Benzimidazole; Pyridone.
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doi:10.1016/j.bmcl.2007.01.102

the benzimidazole class that has shown robust in vivo efficacy.[10] This report describes early SAR leading to the identification of this molecule. The X-ray crystal structure of our initial hit **1** (IC_{50} = 3.5 μM) bound to the active site of truncated IGF-1R revealed that benzimidazole NH and pyridone carbonyl form hydrogen bonds with Met 123, whereas NH of pyridone forms hydrogen bond with Glu 121 in the ATP binding site[10a] (Fig. 1). The crystal structure suggests that the potency could be enhanced via analog design by exploiting the unfilled pocket that could be accessed via the methyl group on the benzimidazole or 4-position of the pyridone ring. Herein, we report the initial SAR at these positions that led to improvements in enzyme[11] and cellular potencies.[12]

Analogs off of the methyl group were accessed via the chemistry illustrated in Scheme 1. Commercially available 2-nitro-5-chloro benzaldehyde (**2**) was protected as ketal **3** and imidazole was then incorporated through an SN_{Ar} reaction. The nitro group was hydrogenated and the resultant aniline **4** was coupled with 2-chloronicotinyl chloride to furnish the amide **5**. Nitration under acidic conditions followed by aqueous work-up cleaved the ketal. However, non-aqueous work-up[13] facilitated the isolation of nitro ketal **6**. Reduction of nitro group with concomitant cyclization to benzimdazole **7** was accomplished by treating with iron in acetic acid. Finally, 2-pyridone **8** was obtained by heating chloropyridine **7** in acetic acid. The benzaldehyde **8** was subjected to reductive amination with several amines to furnish benzylamines (**9**–**16**).

Substitutions at the 4-position of pyridone ring of **1** were explored via chemistry illustrated in Scheme 2. Acylation of **17** followed by nitration of the resultant acetamide furnished nitro derivative **18**. The acetyl group was cleaved and the resultant nitro aniline **19** was hydrogenated to furnish phenylene diamine **20**. The diamine **20** was immediately treated with 4-iodo-2-methoxy-3-pyridine carboxaldehyde[14] (**21**) to afford the benzimidazole **22**. The methoxy group in **22** was cleaved to the iodo pyridone **23**[15] by exposure to 4 N HCl in dioxane. The iodo pyridone **23** served as a useful intermediate for the introduction of wide variety of amines. The biological data of selected compounds are summarized in Figures 2, 3 and Tables 1, 2.

As evident from the results in Table 1, analogs that are derived from reductive amination of aldehyde **8** (Scheme 1) with various alkyl amines, piperazine, benzylamines, and *N*-alkyl anilines did not result in improved potency over **1**. We then explored substitutions at the 4th position of the pyridone. Several diverse amines were incorporated on the iodopyridone **23** using parallel synthesis.

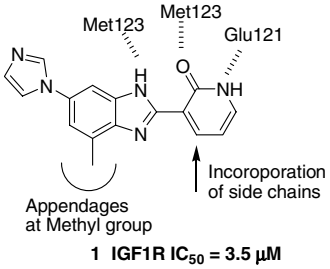
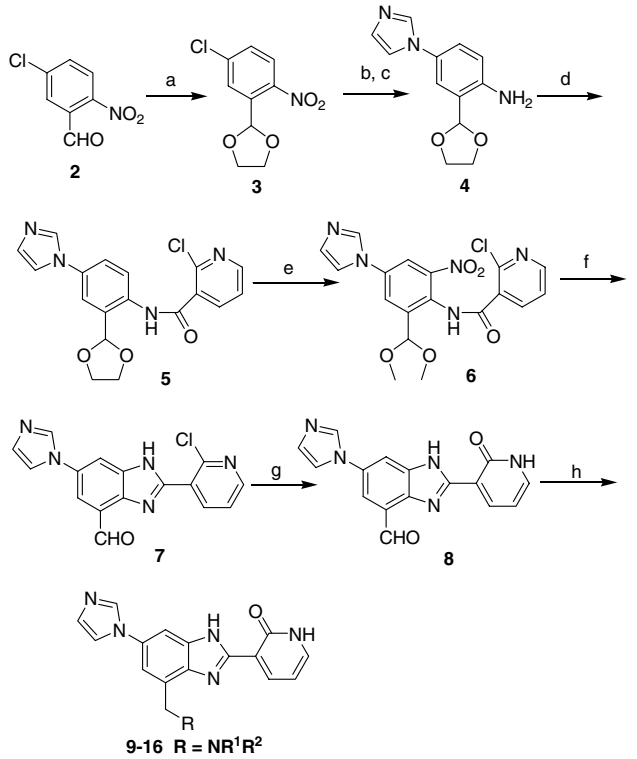
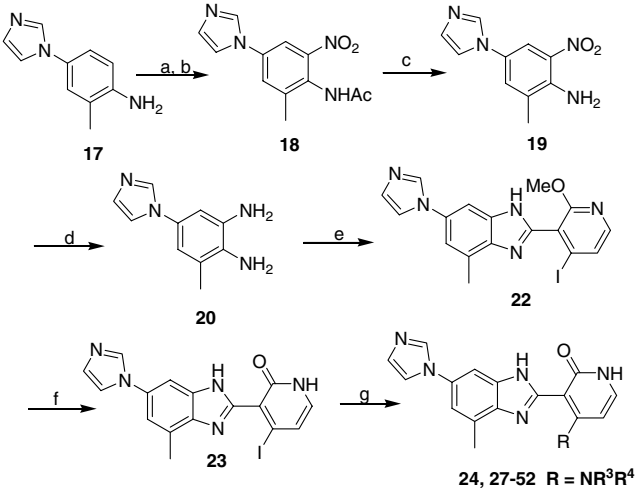


Figure 1. The benzimidazole hit from the screening.



Scheme 1. Reagents and conditions: (a) ethylene glycol, PTSA, toluene, reflux, 96%; (b) imidazole, K_2CO_3, DMSO, 100 °C, 3 h, 82%; (c) 10% Pd(C), H_2, MeOH, rt, 95%; (d) 2-chloronicotinyl chloride, DMAP, CH_2Cl_2; (e) KNO_3, concd H_2SO_4; worked up with 2 M NH_3 in MeOH at −60°C, 76%; (f) Fe, AcOH, 100 °C, 43%; (g) AcOH, 130 °C, 56%; (h) R^1R^2NH, $NaCNBH_3$, MeOH.



Scheme 2. Reagents and conditions: (a) Ac_2O, Py, DMAP, CH_2Cl_2, 95%; (b) KNO_3, concd, H_2SO_4, 68%; (c) K_2CO_3, MeOH, 84%; (d) 10% Pd(C), H_2, MeOH; (e) 2-methoxy-4-iodo pyridine-3-carboxaldehyde (**21**), MeOH, rt, 61%; (f) 4 N HCl in dioxane, H_2O, rt, 72%; (g) R^3R^4NH, DMF, Et_3N, heat.

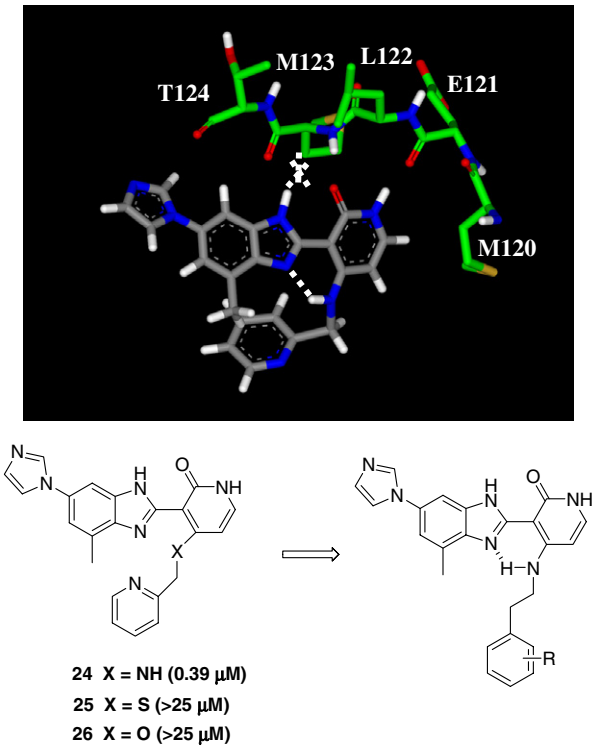


Figure 2. The X-ray crystal structure of **24** bound to IGF-1R and intramolecular hydrogen bonding.

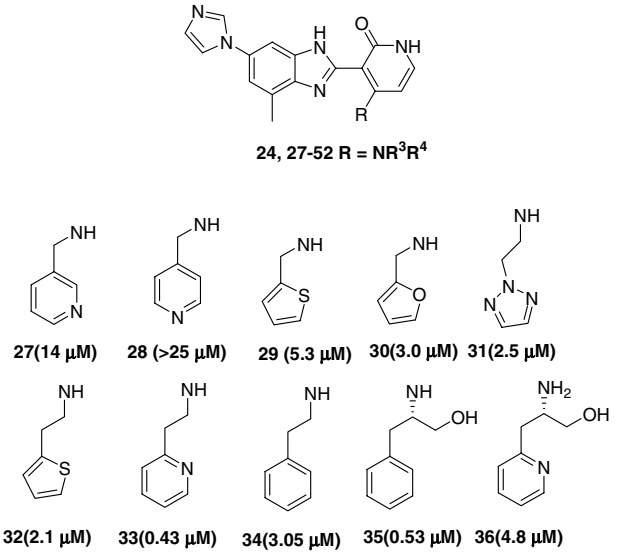
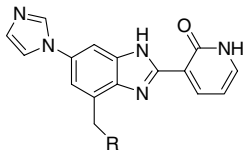


Figure 3. IGF-1R activities[10] of the side chain appended at the 4 position of pyridine. IC_{50} values represent the average of two determinations. Standard deviations ranged from 0.07 to 0.44.

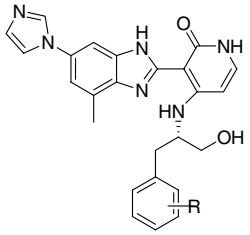
The addition of secondary amines and anilines provided compounds devoid of IGF-1R inhibitory activity. However, the addition of primary amines provided compounds with encouraging IGF-1R inhibitory activity as shown in Figures 2, 3 and Table 2. This effort led to the identification of pyridyl methylamine analog **24**, which displayed 390 nM IGF-1R inhibitory activity. We further investigated role of secondary amine nitrogen on the side chain by making sulfur linked (**25**) and

Table 1. IGF-1R activities[11] for substituents at bottom methyl group



Compound	R	IGF-1R IC_{50} (μM)
1	H	3.5
9	NH_2	>25
10	NHMe	>25
11	Piperazyl	>25
12	2-Imidazolyl ethylamine	13.0
13	1-Furyl amine	19.1
14	Benzylamine	>25
15	3-Pyridylmethylamine	>25
16	*N*-Methylaniline	>25

Table 2. IGF-1R activities[11] of the side chain appended at the 4 position of pyridone



Compound	Substitution	IGF-1R IC_{50}[a] (μM)
35	H	0.53
37	4-Chloro	5.4
38	4-Fluoro	1.4
39	4-Methoxy	3.1
40	4-*tert*-Butoxy	1.7
41	3-Trifluoromethyl	3.5
42	3-Nitro	3.3
43	3-Methoxy	0.66
44	3-Bromo	0.59
45	3-Chloro	0.73
46	3-Fluoro	0.59
47	2-Cyano	2.2
48	2-Trifluoromethoxy	1.3
49	2-Chloro	0.86
50	2-Methoxy	0.57
51	2-Methyl	0.53
52	2-Bromo	0.29

[a] IC_{50} values represent the average of two determinations. Standard deviations ranged from 0.01 to 0.19

oxygen linked (**26**) analogs. As delineated in Figure 2, the compounds **25** and **26** were totally devoid of any IGF-1R inhibitory activity suggesting that the secondary NH at C-4 pyridone as in **24** has an important role to play in facilitating binding to the ATP site. The X-ray crystal structure[16] of **24** bound to the IGF-1R revealed that the –NH at the 4-position of pyridone forms an intramolecular hydrogen bond[17] to the benzimidazole nitrogen thus stabilizing the benzimidazole tautomer and thereby allowing top –NH on the benzimidazole ring to form hydrogen bonding with Met 123. The

intramolecular hydrogen bond between the side chain NH and nitrogen atom of the benzimidazole further locks the conformation of benzimidazole and pyridone across the biaryl bond as depicted in Figure 2.

This result led us to examine other secondary amine side chain analogs at C-4 pyridone to further enhance the potency via a focused library approach. The submicromolar activity of 2-pyridyl methylamine **24** prompted us prepare the regio isomeric 3-pyridyl **27** and 4-pyridyl **28** methylamines. Both analogs were inactive, suggesting that a heteroatom at the 2-position on the side chain is desirable. In the X-ray crystal structure (Fig. 2), the nitrogen of pyridyl points to the sulfur Met183. Further exploration of other heterocycles that contain a heteroatom in the 2-position (**29**–**32**) displayed weak activity. The homolog of **24** with two carbon spacer (**33**) retained the potency, whereas the corresponding phenethylamine **34** displayed reduced potency. Interestingly, introduction of hydroxymethyl group as in **35** restored the potency.[18] However, the combination analog **36** of **33** and **35** resulted in reduced potency.

Attempting to optimize the ring substitution of **24** with both electron withdrawing and donating groups led to no improvement in potency (data not shown), whereas ring substitution on phenyl ring **35** yielded modest improvements in IGF-1R inhibition (Table 2).

Substitution at the *para* position with both electron donating (**39** and **40**) and halogenated (**37** and **38**) groups proved to be detrimental to the activity. According to the crystal structure, the receptor appears to be rather tight in that region. Electron withdrawing groups on both *meta* (**42**) and *ortho* positions (**47**) appeared to have detrimental effect as well. Electron donating groups and halogens at *meta* (**43**–**46**) and *ortho* (**49**–**52**) positions appear to maintain IGF-1R inhibitory activity. Importantly, halogen substituents especially in the *ortho*-position improved the enzyme potency as exemplified by 2-bromo analog **52** that displayed 290 nM (IC_{50}) potency against IGF1-R. The fact that bromine is preferred at *ortho* position is consistent with the productive interaction of aryl bromide with Met197. Further modifications such as bis-substitution on the phenyl ring did not improve enzyme potency suggesting mono substitution was optimal on the phenyl ring. At this stage, selected compounds were evaluated in HT29 whole-cell assay to demonstrate cell-based activity.[12] As shown in Table 3, compound **52** demonstrated sub-micromolar cell-based potency in a MTT proliferation assay.

Table 3. Cell-based activities[12] for selected compounds in HT29 cells

Compound	IGF-1R IC_{50} (μM)	HT29 (μM)
24	0.39	7.6 ± 1.3
35	0.53	0.78 ± 0.3
43	0.66	1.6
44	0.59	1.3
45	0.98	1.1
46	0.59	2.1 ± 1.2
50	0.57	1.2
52	0.29	0.83 ± 0.33

The results described herein represent our initial efforts toward the development of 3-(1*H*-benzo[*d*]imidazol-2-yl)pyridin-2(1*H*)-one inhibitors of insulin-like growth factor-1 receptor. This preliminary exploration unraveled some important features such as the importance of stabilization of the tautomeric form of benzimidazole for productive binding to Met 123 that is critical for IGF-1R inhibitor activity. Future efforts will focus on expanding upon current findings reported herein to identify analogs with desired ADME properties and in vivo efficacy.

Acknowledgments

We thank Dr. Stella Huang and Dr. Dan Schroeder for analytical support.

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11. 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 to 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.
12. Tumor cell lines maintained in RPMI medium (GIBCO) containing 10% heat inactivated fetal bovine serum (GIBCO). The in vitro cytotoxicity is assessed in tumor cells by MTT proliferation assay. Colorimetric assay resulting in the conversion of the MTT tetrazolium into a formazan product.
13. After the reaction with KNO_3 and concentrated sulfuric acid, the acid was very slowly neutralized with 2 M ammonia in methanol at −60 °C, and the resultant ammonium sulfate was filtered, washed with methanol. Evaporation of the solvent furnished the transketalized product **6**.
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15. Varied amounts of chloropyridone and iodopyridones were formed depending on the concentration of HCl and reaction time, and formation of the mixture is inconsequential as both pyridones participate equally well in nucleophilic substitution with amines.
16. Coordinates were submitted to RCSB Protein Data Bank and the pdb ID code is '2OJ9'.
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18. The corresponding *R*-enantiomer was found to be less active (data not shown).