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Insulin-like growth factor-1 receptor (IGF-1R) kinase inhibitors: SAR of a series of 3-[6-(4-substituted-piperazin-1-yl)-4-methyl-1*H*-benzimidazol-2-yl]-1*H*-pyridine-2-one

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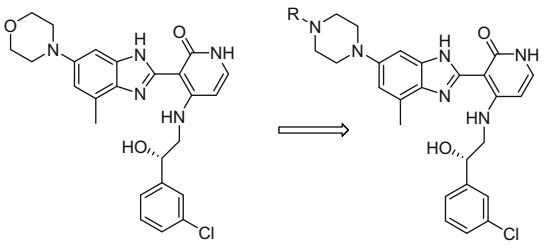
ABSTRACT

A series of 3-[6-(4-substitued-piperazin-1-yl)-4-methyl-1*H*-benzimidazol-2-yl]-1*H*-pyridine-2-one were synthesized to modulate CYP3A4 inhibition and improve aqueous solubility of our prototypical compound BMS-536924 (**1**), while maintaining potent IGF-1R inhibitory activity. Structure–activity and structure–solubility studies led to the identification of BMS-577098 (**27**), which demonstrates oral in vivo efficacy in animal models. The improvement was achieved by replacing morpholine with more polar bio-isoster piperazine and modulating the basicity of distal nitrogen with appropriate substitutions.

The Insulin-like growth factor-1 receptor (IGF-1R) is a transmembrane receptor tyrosine kinase that plays a critical role in mitogenesis and survival in a variety of human tumor cells.[1] Stimulation of IGF-1R through binding of either IGF-1 or IGF-2 ligands leads to autophosphorylation and activation, which in turn, recruits and phosphorylates downstream intracellular substrates such as IRS-1 and Shc.[2] This phosphorylation results in further activation of two major downstream pathways, the Ras/Raf/MAPK kinase pathway primarily responsible for mitogenesis and the anti-apoptotic PI3 K/Akt/*m*-TOR pathway.[3] There is considerable evidence linking IGF signaling with cellular transformation and the onset and progression of tumors.[3] Epidemiological studies have highlighted the importance of IGF-1R in key tumor types by correlating elevated IGF-1 levels with increased risk of developing colon, breast, prostate, and lung tumors.[4] Inhibition of IGF-1R by various approaches, including anti-sense, antibodies, dominant negative mutants, and small molecule inhibitors, has been shown to reduce tumor growth in human tumor xenograft models.[5]

The emerging importance of this target is the driving force behind the search for antagonists of IGF-1R. More than two dozen antibodies and small molecules have been developed and clinical trials are underway for at least 12 of these.[6] Among several small molecule IGF-1R inhibitors from various structural classes,



1 (BMS-536924)

Figure 1. Piperazine replacements of morpholine in BMS-536924.

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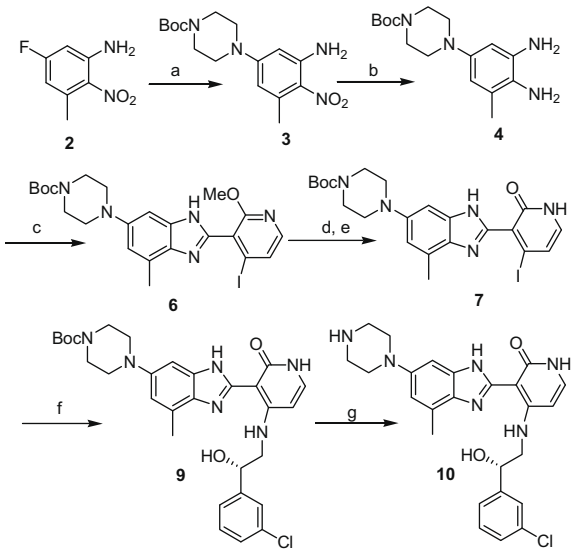


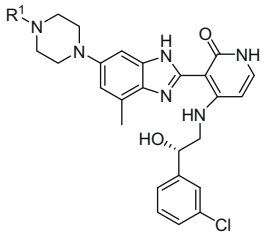
Scheme 1. Reagents and conditions: (a) *N*-Boc piperazine, Et(^{i}Pr)$_2$N, NMP, 80 °C, 95%; (b) H_2, methanol, 10% Pd/C; (c) 2-methoxy-4-iodo-pyridine-3-carboxaldehyde (**5**), MeOH, rt, air; (d) 6 N HCl, dioxane, 80 °C, 16 h; (e) di-*t*-butyl dicarbonate, TEA, CH_2Cl_2, 90%; (f) (*S*)-2-amino-1-(3-chlorophenyl)ethanol (**8**), Et(^{i}Pr)$_2$N, DMSO, 70%; (g) TFA, CH_2Cl_2.

OSI-906, XL-228, AXL1717, and BMS-754807 have been progressed to clinical development.[6,7]

As a consequence of our group's efforts[8] toward the discovery of novel IGF-1R antagonists, we identified BMS-536924 (**1**), a small molecule inhibitor from the benzimidazole class that shows robust in vivo efficacy in IGF-Sal tumor model.[8a] Liabilities of **1** include its potential for drug–drug interactions due to potent CYP3A4 inhibition (~0.5 μM) and poor aqueous solubility (<1 μg/mL). We reported approaches to improve the overall profile of **1** by modifying the morpholine portion[8f,g] of the molecule as well as the phenethylamine side chain.[8e,g] We describe herein an effort to mitigate CYP3A4 inhibition, improve aqueous solubility and IGF-1R enzyme potency by exploring bio-isosteric replacements of morpholine with more polar N-substituted piperazines (Fig. 1). We reasoned that modulating the basicity of the piperazine distal nitrogen atom could result in improving CYP3A4 inhibition, aqueous solubility, enzyme and cellular potencies. Appropriate changes in piperazine basicity may also impart desirable PK parameters such as oral exposure and plasma protein binding.

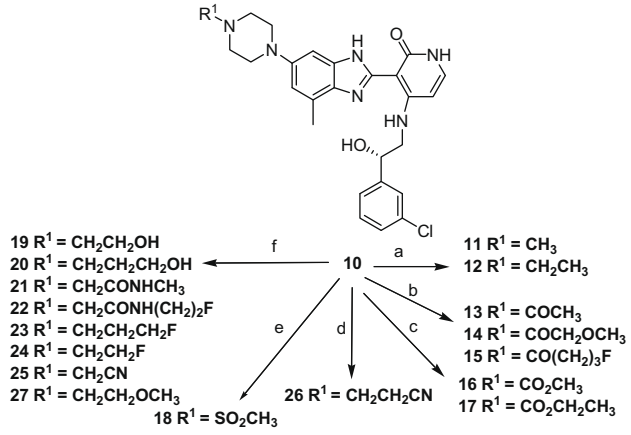
The synthesis of piperazine **10** is accomplished in seven steps as outlined in Scheme 1: S_NAr reaction of 5-fluoro-3-methyl-2-nitroaniline (**2**) with mono Boc-protected piperazine delivers nitroaniline **3**. Hydrogenolysis yields the labile diamine **4** which is immediately condensed with 2-methoxy-4-iodo-pyridine-3-carboxaldehyde (**5**) to furnish the benzimidazole **6**. Treatment of **6** with 6 N HCl cleaves both the methoxy and Boc groups, thus the piperazine is re-protected to give iodo-pyridone **7**. Incorporation of the requisite side chain using (*S*)-2-amino-1-(3-chlorophenyl)ethanol (**8**) followed by Boc-deprotection provides the desired piperazine **10**. As depicted in Scheme 1, alkyl substitutions of **10** are achieved either by reductive amination (for compounds **11** and **12**) or by direct alkylation with appropriate alkyl halides (for compounds **19** through **25** and **27**). Amides **13–15** and carbamates **16–17** are synthesized from the corresponding acyl halides and carbamoyl chlorides. Cyanoethyl compound **26** is fashioned by Michael reaction of **10** with acrylonitrile.

Table 1
SAR of substituted piperazines



Compound	R^1	IC_{50}-IGF-1R[a] (μM)	IGF-Sal[b] (μM)	CYP3A4[c] (μM)	AUC[d] (μM h)	Solubility[e] (μg/mL)
1	—	0.100	0.110	0.5	50.0	<1
10	H	0.032	0.120	3.0	26.5	63.0
11	CH_3	0.018	0.030	3.5	3.40	67.0
12	CH_2CH_3	0.017	0.060	2.2	1.0	89.0
13	$COCH_3$	0.100	0.230	1.1	3.1	—
14	$COCH_2OCH_3$	0.028	0.080	0.8	0.0	4.0
15	$CO(CH_2)_3F$	0.062	0.090	0.8	4.5	2.0
9	CO_2-*t*-Bu	0.210	0.140	1.9	8.3	1.0
16	CO_2CH_3	0.175	0.210	0.7	57.1	2.0
17	$CO_2CH_2CH_3$	0.270	0.200	1.0	25.3	<1.0
18	SO_2CH_3	0.027	0.080	0.6	7.2	—
19	CH_2CH_2OH	0.026	0.230	3.3	0.0	76.0
20	$CH_2CH_2CH_2OH$	0.016	0.180	1.9	0.0	80.0
21	$CH_2CONHCH_3$	0.038	0.168	2.6	1.6	86.0
22	$CH_2CONHCH_2CH_2F$	0.041	0.111	2.4	1.4	41.0
23	$CH_2CH_2CH_2F$	0.030	0.070	0.45	11.4	—
24	CH_2CH_2F	0.100	0.100	2.2	17.7	—
25	CH_2CN	0.038	0.070	1.4	10.3	9.0
26	CH_2CH_2CN	0.061	0.080	1.1	22.1	
27	CH_2CH_2OMe	0.016	0.065	2.9	11.1	95.0

[a] IC_{50} values represent the average of two determinations (variability in IC_{50} is roughly twofold).
[b] This cell line is derived from salivary tumors that develop in transgenic mice that overexpress IGF-1R construct under the MMTV promoter.[9]
[c] IC_{50} values are determined for inhibition of dealkylation of BFC(7-benzyloxy-4-trifluoromethylcoumarin).
[d] Compounds are evaluated in (0–4 h) exposure studies in mice at 20 mpk and formulated as solutions in 80:20 PEG400 and water.
[e] Solubility studies are conducted at pH 6.5 in the presence of phosphate buffer.

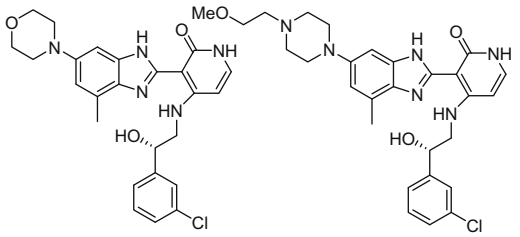


Scheme 2. Reagents and conditions: (a) RCHO, $NaCNBH_3$, MeOH; (b) R′COCl, Et_3N, CH_2Cl_2; (c) R″OCOCl, Et_3N, CH_2Cl_2; (d) acrylonitrile MeOH, rt; (e) $MeSO_2Cl$, Et_3N, CH_2Cl_2; (f) R_1-Br, $Et(^iPr)_2N$, DMSO.

Table 2
Pharmacokinetic parameters of **27** in mouse and rat

	Mouse	Rat
IV/PO dose (mg/kg)	5/75	5/75
F_{po} (%)	26%	26%
Cl_{tot} (mL/min/kg)	25.2	27.8
V_{ss} (L/kg)	4.9	2.9
$T_{1/2}$ (h)	2.9	1.9

Table 3
Comparison of activities for compounds **1** and **27**



	BMS-536924 (**1**)	BMS-577098 (**27**)
IGF-1R[a] (μM)	0.100	0.016
IGF-Sal[a] (μM)	0.110	0.065
CYP3A4[a] (μM)	0.5	2.9
AUC[b] (μM h)	50.0	11.1
Solubility[c] (μg/mL)	<1.0	95.0
Plasma protein binding[d] (%, mouse)	99.9	98
%TGI[e] (Colo205)[f]	72	74
%TGI[e] (RD1)[g]	53	73

[a] IC_{50} values represent the average of two determinations (Variability in IC_{50} is roughly twofold).
[b] Compounds are evaluated in (0–4 h) exposure studies in mice at 20 mpk and formulated as solutions of 80:20 PEG400 and water.
[c] Solubility studies are conducted at pH 6.5 in the presence of phosphate buffer.
[d] Plasma protein binding was determined at 10 μM using equilibrium dialysis.
[e] Percent tumor growth inhibition at the end of treatment or averaged over at least one tumor volume doubling time during a prolonged dosing regimen (value >50% is considered an active result).
[f] Dosed at 200 mpk qdX21.
[g] Dosed at 150 mpk bidX14for **1** and 270 mpk qdX14 for **27**.

The structure–activity relationship data is delineated in Table 1. The unsubstituted piperazine **10** shows threefold improvement in enzymatic potency while cell-based activity is maintained relative to morpholine **1**. The CYP3A4 profile is improved sixfold and the aqueous solubility is significantly increased, perhaps due to the introduction of basic piperazine nitrogen atom. Installation of small alkyl groups on the distal nitrogen of the piperazine (**11** and **12**) results in improved enzyme potency, cell potency, and solubility with modest improvement in CYP inhibition profile relative to **1**. However, systemic exposure (0–4 h AUC) upon oral dosing of **11** and **12** at 20 mpk in mice is reduced. In an effort to further modulate the electronic properties of the piperazine nitrogen atom and thereby improve oral exposure, electron withdrawing groups such as amides, carbamates, and sulfonamides are incorporated. Amides **13–15** maintain enzyme and cell potencies, while the oral exposure, solubility, and CYP3A4 inhibition properties all trend in the wrong direction. Significant reduction of basicity of the piperazine nitrogen atom in **13–15** presumably led to considerable reduction of solubility vis-à-vis **10–12**. While carbamates (**9**, **16**, and **17**) display diminished enzyme potency, their oral exposure is generally increased relative to alkyl piperazines. These carbamates also show potent CYP3A4 inhibition and poor solubility. The sulfonamide **18** exhibits improved enzyme and cell potencies, but it still has 0.6 nM CYP3A4 inhibition. Alkyl piperazines **19** and **20** with the polar hydroxyl group on the alkyl chain show improved solubility, and somewhat improved CYP3A4 profile. The alkylated piperazine amides **21**–**22** lower the pK_a of the piperazine and, while these analogs display enhanced solubility, they also lack oral exposure and improvement of CYP3A4 profile. Electron withdrawing groups (**23**, **24**, **25**, and **26**) on the alkyl chain attached to the piperazine nitrogen provide compounds with modest oral exposure. The best overall balance is accomplished with a methoxyethyl sidechain on the piperazine (compound **27**). This analog benefits from the well known effect of a β-oxygen atom has on lowering of the adjacent nitrogen atom's pK_a (*N*-methylmorpholine pK_a = 7.24 vs *N*-methylpiperazine pK_a = 10.5). Compound **27** demonstrates sixfold improvement in enzyme potency and moderation in CYP3A4 inhibition (sixfold) relative to **1**. In addition, compound **27** shows enhanced aqueous solubility with acceptable oral exposure (see Scheme 2).

Encouraged by its oral exposure and solubility data, compound **27** was evaluated in a full PK study in mouse and rat, and the data is summarized in Table 2. Compound **27** has 26% oral bioavailability in both species, moderate clearance (25.2 mL/min/kg for mouse and 27.8 mL/min/kg for rat) and high volume of distribution (4.9 L/kg for mouse and 2.9 L/kg for rat).

The compound **27** is confirmed to be an ATP-competitive inhibitor of IGF-1R with K_i of 28 ± 4 nM for unphosphorylated rIGF-1R. Safety profiling of compound **27** showed it to be negative in Ames mutagenicity and SOS chromotest. As shown in Table 3 when **27** is evaluated in vivo by oral administration once a day for 21 days in the Colo205 (human colon carcinoma) and RD1 (human rhabdomyosarcoma) xenografts upon oral administration once a day for 14 days, it is found to be as efficacious as BMS-536924 (**1**). This similar in vivo efficacy could be attributed to the differences in plasma protein binding[10] (0.01% free fraction for **1** vs 2% free fraction for **27**) despite five times lower AUC for compound **27** relative to **1**.

In summary, we describe a novel series of IGF-1R inhibitors wherein morpholine was replaced with substituted piperazines. By modulating the basicity of distal nitrogen on the piperazine, we show that **27** has the best balance of CYP3A4 inhibition, oral exposure and aqueous solubility. Additionally, this compound **27** demonstrates oral efficacy in Colo205 and RD1 xenograft models.

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