Identification of clinical candidate OSI-906 as a potent, selective and orally bioavailable IGF-1R inhibitor

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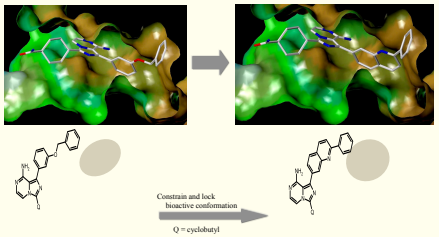
ABSTRACT

The insulin-like growth factor receptor (IGF-1R) is a transmembrane tyrosine kinase which has been implicated as a key driver in certain forms of cancers. Receptor over-expression and/or over-stimulation through either of its cognate ligands, IGF-I or IGF-II, leads to signal transduction processes which synergize to promote cellular proliferation, inhibit apoptosis, and increase cell survival. The validated role of IGF-1R in tumors such as colorectal, NSCLC and ovarian, has made it an attractive candidate for molecular targeted therapy. Here we present our efforts which led to the identification of the clinical candidate, *cis*-3-[8-amino-1-(2-phenyl-quinolin-7-yl)-imidazo[1,5-*a*]pyrazin-3-yl]-1-methyl-cyclobutanol (OSI-906) as a potent, selective and orally bioavailable IGF-1R inhibitor. Initial efforts focused on optimizing substituents at the C1 position of the imidazo[1,5-*a*]pyrazine core, establishing fundamental SAR around a benzyloxyphenyl moiety. Through structure based design efforts utilizing IGF-1R and IR co-crystal structures, the benzyloxyphenyl substituent was replaced with a bioactive, conformationally constrained 2-phenylquinolinyl moiety which resulted in a 13x boost in cellular potency. The progression towards the clinical candidate continued with optimization of the partially solvent exposed C3 imidazopyrazine substituent, optimizing for favorable DMPK properties (i.e. identifying and blocking key sites of metabolism and incorporating functionality to improve solubility). Upon optimization of both the C1 and C3 imidazopyrazine substituents for IGF-1R potency and ideal DMPK and physiochemical properties, efforts shifted to exploring a novel imidazo[5,1-*f*][1,2,4]triazine core, as a potential bioisostere to the imidazo[1,5-*a*]pyrazine series. In summary, the general SAR around the benzyloxyphenyl substituent, progression to the quinolinyl series, optimization of the C3 substituent, and comparison of the two cores will be presented.

RESULTS

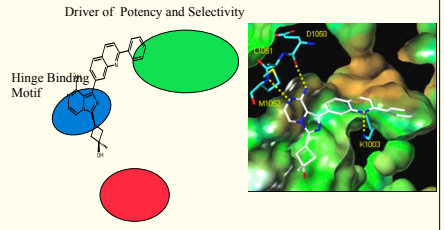
Novel IR co-crystal and docked IGF-1R structures with imidazopyrazine derivatives provided critical structural insights



Identification of key pharmacophoric interactions as well as structural observations based upon ligand-based SAR and X-ray co-crystal structures of insulin receptor (IR) and IGF-1R with an earlier 3-benzyloxyphenyl-derived imidazopyrazine series (**1**, left panel) led to efforts focused on replacing the benzyloxyphenyl moiety with a more constrained quinolinyl moiety (**2**, right panel).* This modification maintained key pharmacophores, including the proximal and terminal phenyl rings, filled an unoccupied hydrophobic pocket adjacent to the *para*-position of the proximal phenyl ring, replaced a potentially metabolically labile benzyl ether moiety, and ultimately locked the benzyloxyphenyl moiety in a preferred bioactive conformation. The result was a 13-fold increase in IGF-1R potency.

* Mark J. Mulvihill, Qun-Sheng Ji, Heather R. Coate, Andrew Cooke, Hanqing Dong, Lixin Feng, Maryland Rosenfeld-Franklin, Ayako Honda, Gilda Mak, Kristen M. Mulvihill, Anthony I. Nigro, Matthew O'Connor, Caroline Pirrit, Arno G. Steinig, Kam Siu, Kathryn M. Stolz, Yingchuan Sun, Paula A. R. Tavares, Yan Yao, and Neil W. Gibson. *Bioorganic Medicinal Chemistry* **2008**, *16*, 1359-1375.

Structure of OSI-906 and key pharmacophoric elements

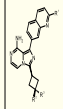


SAR of benzyloxy-imidazopyrazine series

R	Biochemical IC_{50} (μM)	Cellular (pY-IGF1R) IC_{50} (μM)
H	>10.0	ND
Me	>10.0	ND
CH_2-Ph	0.606	1.16
Cyclopentyl	3.5	ND
Cyclohexyl	1.05	ND
CH_2-Cyclohexyl	1.11	ND
CH_2-CH_2-OMe	6.28	ND
CH_2-2-F-Ph	0.224	2.06
CH_2-3-F-Ph	0.51	3.29
CH_2-4-F-Ph	1.23	ND
CH_2-2,6-di-F-Ph	0.215	1.59
CH_2-2,5-di-F-Ph	0.329	9.76
CH_2-2-Cl-6-F-Ph	0.248	2.67
CH_2-2-CN-Ph	>10.0	ND
CH_2-2-Cl-Ph	0.343	ND

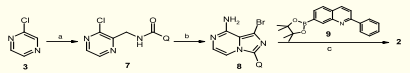
• For a more comprehensive description of the SAR around the benzyloxyphenylimidazopyrazine series, see: Mark J. Mulvihill, Qun-Sheng Ji, Doug Werner, Patricia Beck, Cara Cesario, Matthew Cox, Andrew Crew, Hanqing Dong, K. W. Foreman, Gilda Mak, Anthony Nigro, Matthew O'Connor, Lydia Sarogliou, Kathryn M. Stolz, Izabela Sujka, Brian Volk, Qinghua Weng and Robin Wilkes. *Bioorganic & Medicinal Chemistry Letters* **2007**, *17(4)*, 1091. ND = Not Determined

SAR of imidazopyrazine-quinoline series



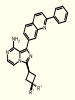
Compound	R^1	R^2	R^3	Cellular IC_{50} (μM) (pY-IGF1R)
2a	Ph	H	H	0.09
2b	Ph	OH	Me	0.02
2c	Me	OH	Me	>0.50
2d	Et	OH	Me	>0.50
2e	Cyclohexyl	OH	Me	0.02
2f	*t*-butyl	OH	Me	0.20
2g	O-Ph	OH	Me	0.15
2h	2-F-Ph	OH	Me	0.01
2i	2-Me-Ph	OH	Me	0.01
2j	2-OMe-Ph	OH	Me	0.19
2k	2-pyridyl	OH	Me	0.10

Scheme 1: General synthesis to imidazopyrazines



Reagents and conditions: (a) (i) 2M *n*BuLi in hexanes, tetramethylpiperidine, -78 °C, then DMF, MeOH and $NaBH_4$; (ii) phthalimide, DIAD, PPh_3, THF; (iii) NH_2NH_2, methanol, reflux 1.5 h then HCl gas, toluene, (80%, 2-step yield); (iv) QCO_2H, EDC, HOBT, DIEA, CH_2Cl_2, rt; (b) (i) $POCl_3$, DMF, EtOAc, 30 °C; (ii) NBS, DMF, acetone -10°C, 1 h; (iii) NH_3, *i*PrOH, 110 °C, 24 h; (c) $Pd(PPh_3)_4$, DMF/H_2O, Cs_2CO_3, 85 °C. Boronates **9** and **15**, from Schemes 1 and 2 respectively, were synthesized via the following procedures: 7-chloro-2-phenylquinoline, $Pd(OAc)_2$, 1,3-*bis*-(2,6-diisopropylphenyl)-imidazolium chloride, KOAc, and *bis*(pinacolato)diboron in THF at 75 °C for 12 h.

Cyclobutyl substituent effects on microsomal stability



Compound	R^2	R^3	ER (H/M)	Cellular IC_{50} (μM) (pY-IGF1R)
2a	H	H	0.85 / 0.88	0.086
2i	OH	H	0.74 / 0.69	0.089
2b (OSI-906)	OH	Me	0.58 / 0.53	0.021
2m	OH	Et	0.74 / 0.50	0.028
2n	OH	Ph	0.53 / 0.42	0.225
2o	OH	CH_2-Ph	0.39 / 0.46	>0.500

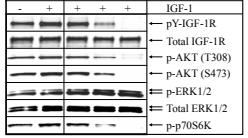
- Substitution at R^2 and R^3 blocked metabolism and improved potency
- Generally, larger substituents had an adverse affect on potency

Head to head comparison of imidazotriazine and imidazopyrazine cores: PSA, cellular IC_{50}, and microsomal stability

Compound	PSA	Cellular IC_{50}	ER (H/M)	Compound	PSA	Cellular IC_{50}	ER (H/M)
	78.3	2.929	0.95 / 0.96		65.4	1.161	0.93 / 0.97
	81.9	0.335	0.91/ 0.89		69.1	0.086	0.85 / 0.88
	102.2	0.140	0.71 / 0.62		89.3	0.021	0.58 / 0.53
	102.2	0.279	0.54 / 0.51		89.3	0.028	0.74 / 0.50

- Like-for-like comparisons indicate that the imidazotriazine series showed the same trends in SAR, but did not achieve similar potency
- OSI-906 emerged as the best-in-series
- OSI-906 demonstrated biochemical selectivity of >10 μM vs 45 kinases (100 μM ATP)

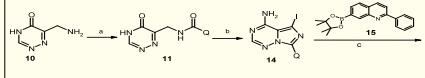
Inhibition of p-IGF-1R and downstream signaling by OSI-906 in GEO cells and effects on proliferation and apoptosis



In vitro Assay	IC_{50} (μM)	Cell Line
p-AKT (Ser473)	0.110	GEO
Proliferation	0.021	GEO
Apoptosis (ED_{50}) PARP cleavage	4.0	GEO

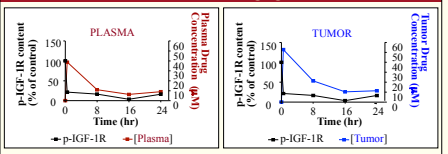
For a more comprehensive description of the GEO cell line and the IGF-II-mediated autocrine loop and biology thereof, see: Qun-sheng Ji, Mark Mulvihill, Maryland Franklin, Andrew Cooke, Lixin Feng, Gilda Mak, Matthew O'Connor, Yan Yao, Caroline Pirritt, and Darla Landfair. *Molecular Cancer Therapeutics* **2007**, *6(8)*, 2158-2167.

Scheme 2: General synthesis to imidazotriazines



Reagents and conditions: (a) (i) QCO_2H, EDC, HOBT, DIEA, CH_2Cl_2, rt or QC(O)Cl, DIEA, DMF 0 °C to rt; (b) (i) $POCl_3$, 55 °C, 41% yield; (ii) NIS, DMF, rt to 55 °C, 53% yield; (iii) 1) 1,2,4-triazole, $POCl_3$, pyridine, rt, 2) 2M NH_3 in IPA, 0 °C to rt, 70% yield (c) $Pd(PPh_3)_4$, DME/H_2O, Na_2CO_3, 80 °C. For a detailed description of the synthesis of 7-chloro-2-phenylquinoline, utilized in the synthesis of boronates **9** and **15** (Schemes 1 and 2 respectively) see: An-Hu Li, Eilaf Ahmed, Xin Chen, Matthew Cox, Andrew P. Crew, Han-Qing Dong, Meizhong Jin, Lifu Ma, Bijoy Panicker, Kam W. Siu, Arno G. Steinig, Kathryn M. Stolz, Paula A. R. Tavares, Brian Volk, Qinghua Weng, Doug Werner, and Mark J. Mulvihill. *Organic & Biomolecular Chemistry* **2007**, *5(1)*, 61-64.

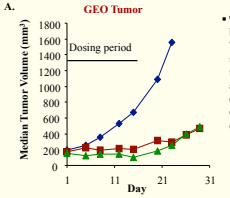
PK/PD correlation of OSI-906 at 60 mg/kg in a GEO tumor model



- Plasma and tumor levels of OSI-906 correspond with sustained inhibition of p-IGF-1R at a single 60 mg/kg dose.

Efficacy of OSI-906 in various xenograft models: significant TGI in GEO, Colo-205 and Cal-62 tumor model

A.



- %TGI (tumor growth inhibition) calculated by: (1-{Tt/T0 / Ct/C0} / 1-{C0/Ct}) × 100; Tt = median tumor volume of treated at time t, T0 = median tumor volume of treated at time 0, Ct = median tumor volume of control at time t and C0 = median tumor volume of control at time 0. Median % TGI was calculated during the entire dosing period for each treatment group.

B.

Xenograft Model	Median % TGI (60 mg/kg)	p value	T-C	p value
GEO	100	≤0.001	13	0.003
Colo-205	90	≤0.001	11	≤0.001
BxPC-3	53	≤0.001	5	≤0.001
Cal-62	89	0.015	0	NC

- OSI-906 is highly active in GEO (A) and Colo-205 colon carcinoma models and a Cal-62 anaplastic thyroid carcinoma model and moderately active in a BxPC3 model (B). Combination studies with small molecule EGFR inhibitor, erlotinib, demonstrated >100% TGI and regression in a GEO xenograft model as noted by sustained inhibition of both IGF-1R & EGFR respective downstream targets, p-AKT and p-ERK (data not shown).

SUMMARY & CONCLUSIONS

- Through structure based design and medicinal chemistry efforts, key pharmacophoric elements of the imidazopyrazine series were identified
- The benzyloxyphenyl moiety was replaced with the bioactive-conformationally constrained 2-phenylquinolinyl moiety resulting in a significant (13x) boost in cell-based mechanistic potency
- Installing a tertiary alcohol on the cyclobutyl substituent greatly improved *in vitro* metabolic stability
- A comparison of the imidazo[5,1-*f*][1,2,4]triazine series with the imidazopyrazine series indicated that, while the imidazotriazine showed similar SAR trends, it was generally less potent than the imidazopyrazine series
- Inhibition of phosphorylation of IGF-1R by OSI-906 showed downstream effects on AKT and 70S6K in GEO cells.
- OSI-906 demonstrated a good PK/PD correlation and significant TGI in several xenograft models.
- OSI-906 was identified as the best in series and has progressed into Phase I clinical trials.

ACKNOWLEDGEMENTS

The authors wish to thank Professors Stevan Hubbard and Todd Miller for cocrystallization studies, as well as OSI Leads Discovery, In Vitro Biology, In Vivo Pharmacology, CMC, Translational Research, and Medicinal Chemistry for their respective contributions to the overall success of the IGF-1R program.

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