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Discovery of novel imidazo[1,2-*a*]pyridines as inhibitors of the insulin-like growth factor-1 receptor tyrosine kinase

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ABSTRACT

We disclose a novel series of insulin-like growth factor-1 receptor kinase inhibitors based on the 3-(pyrimidin-4-yl)-imidazo[1,2-*a*]pyridine scaffold. The influence on the inhibitory activity of substitution on the imidazopyridine and at the C5 position of the pyrimidine is discussed. In the course of this optimization, we discovered a potent and selective inhibitor with suitable pharmacokinetics for oral administration.

The insulin-like growth factor-1 receptor (IGF-1R) has attracted much attention over the last few years as a therapeutic target for cancer.[1] Upon ligand binding, this receptor tyrosine kinase activates two important signaling cascades, namely the PI3K-AKT pathway and the Ras-Raf-MEK pathway. Deregulation of the IGF-1R axis has been implicated in a variety of solid cancers including lung, breast, colorectal and prostate. Such a deregulation can occur through overexpression of the receptor or its ligands (IGF-1 and IGF-2) or by down-regulation of binding proteins which modulate the signaling cascade by ligand sequestration.

Inhibition of the IGF-1R pathway has been and continue to be evaluated as a cancer therapy in clinical trials using either receptor antibodies such as CP-751,871 (figitumumab)[2] or the small molecule kinase inhibitors OSI-906[3] and BMS-754807[4] (Fig. 1). In addition, a number of other IGF-1R kinase inhibitors have been reported.[5–8] Herein, we report the discovery and the structure–activity relationships of a novel series of imidazo-pyridines which are potent IGF-1R kinase inhibitors.

Following a cellular high throughput screening campaign[9] of our compound collection at AstraZeneca, the imidazo[1,2-*a*]pyridine **1** (Table 1), a previously described CDK2 inhibitor,[10] was identified as a hit compound. When we measured the kinase inhibitory activities of **1** in biochemical assays, this compound appeared a 1000-fold more potent against CDK2 than IGF-1R. Recognizing the importance in avoiding inhibition of the Cyclin Dependant Kinases, which interfere with the cell cycle, shifting the strong CDK2 activity of **1** towards IGF-1R was a key element of our hit-to-lead strategy. We found that replacing the *para*-sulfonyl group, favorable for CDK2 inhibition, with a *N*-acetyl-piperidine (**2**) partially met this objective by improving IGF-1R potency by nearly 5-fold while significantly decreasing CDK2 activity. The addition of a methoxy group on the *ortho* position of the aniline (**3**) achieved an exquisite selectivity by abrogating CDK2 activity with little impact on the IGF-1R IC_{50}. This can be rationalized by a possible steric interaction between this methoxy group and the side chain of Phe_{82} of CDK2 (see crystal structure of **1** bound to CDK2 in Ref. 10a) whereas the corresponding residue in IGF-1R is Leu_{1078}. Interestingly, Emmitte et al.[6b] have observed a similar effect with their anilino-pyrimidines in which a methoxy group on the same position reduces activity against Aurora B without any detrimental effect on IGF-1R inhibition. They attributed this difference to the presence of a tyrosine (Tyr_{156}) in Aurora B in place of Leu_{1078} in IGF-1R.

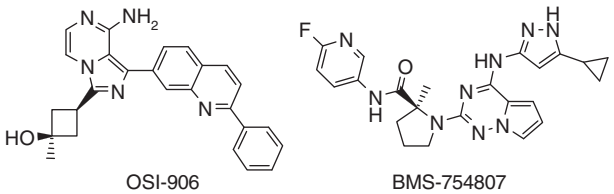

Figure 1. IGF-1R kinase inhibitors in clinical trials.

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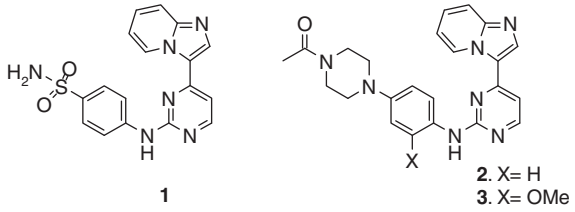
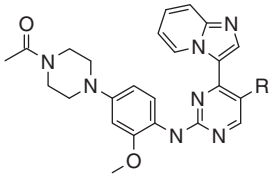
Table 1
IGF-1R and CDK2 inhibitory activity of compounds **1**–**3**



Compd	IGF-1R IC_{50}[a] (μM)	CDK2 IC_{50}[a] (μM)
1	1.26	0.001
2	0.27	0.078
3	0.45	>100

[a] IC_{50} values are means of at least three experiments, standard deviation is less than 2.

Table 2
Inhibition of IGF-1R and CDK2 kinase activity and inhibition of IGF-1R autophosphorylation in cell for compounds **3**–**10**

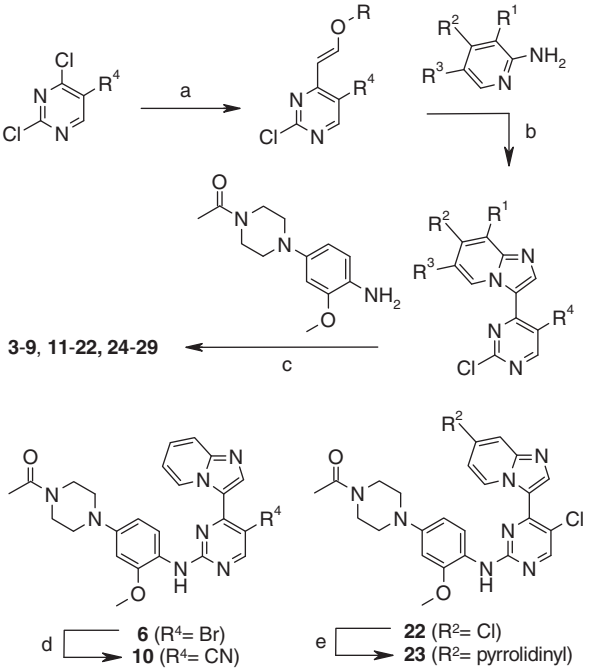


Compd	R	IGF-1R IC_{50}[a] (μM)	CDK2 IC_{50}[a] (μM)	Cell IC_{50}[a] (μM)
3	H	0.45	>100	1.34
4	F	0.089	2.7	0.13
5	Cl	0.010	1.1	0.017
6	Br	0.003	1.1	0.009
7	Me	0.047	10	0.092
8	CF3	0.015	16	0.058
9	OMe	0.090	53	0.19
10	CN	0.092	27	0.17

[a] IC_{50} values are means of at least two experiments, standard deviation is less than 2.5.

With the improved selectivity profile of compound **3**, we turned our attention to the optimization of the IGF-1R inhibition by modifying the 4-(imidazo[1,2-*a*]pyridin-3-yl)-pyrimidine core. Based on the binding mode of compound **1** within the CDK2 ATP binding site,[10a] we postulated that a small lipophilic substituent on the C5 position of the pyrimidine ring would favorably interact with the side chain of the methionine gatekeeper of IGF-1R. We were also interested in substituting the imidazopyridine ring in order to better understand the structure–activity relationship in this chemical series.

Modifications of the starting 4-(imidazo[1,2-*a*]pyridin-3-yl)-pyrimidine scaffold have been performed thanks to the general synthetic route outlined in Scheme 1,[11] which represents a more general and versatile route compared to the one published for compound **1**.[10b] Starting from the appropriate C5-substituted 2,4-dichloropyrimidine, a vinyl ethyl ether was prepared by a Suzuki coupling with tri-ethoxyvinyl-borane.[12] Alternatively, the corresponding *n*-butyl ether was prepared by a Heck coupling with *n*-butyl vinyl ether using phosphine-free conditions.[13] The (2-chloropyrimidin-4-yl)-vinyl ether was subsequently transformed into the desired imidazopyridine in a 2-steps one-pot procedure involving bromination with NBS followed by cyclocondensation with the relevant 2-aminopyridine. The resulting 2-chloropyrimidines were converted into the final products by acid mediated addition of the 2-methoxy-4-(*N*-acetyl-piperazin-1-yl) aniline.[14] The cyano derivative **10** was obtained from compound **6** via a palladium catalyzed cyanation and compound **23** was obtained by nucleophilic aromatic substitution of the chloro derivative **22** with pyrrolidine.

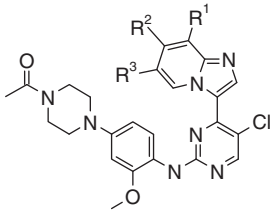


Scheme 1. General synthesis of compounds **3**–**29**. Reagents: (a) R = Et: ethynyl ethyl ether, BH_3, THF, then dichloropyrimidine, $Pd(PPh_3)_4$, NaOH; R = *n*-Bu: vinyl butyl ether, triethylamine, $Pd(OAc)_2$, PEG-400; (b) *N*-bromosuccinimide, dioxane/water, then aminopyridine; (c) 2-pentanol, *p*-toluenesulfonic acid, 130 °C; (d) $ZnBr_2$, Zn, Pd_2dba_3, xantphos, DMA; (e) pyrrolidine.

We started to explore the influence of the pyrimidine C5 substitution (compounds **4**–**10**, Table 2) on the IGF-1R and CDK2 inhibitory activities as well as on the inhibition of IGF-1R autophosphorylation in our cellular assay.[9] As expected from the postulated lipophilic interaction with the gatekeeper, anti IGF-1R activity was significantly improved by addition of a halogen atom, the chloro (**5**) and bromo (**6**) derivatives being more potent than the fluoro (**4**) compound. A methyl group (**7**), provided an activity between that of a fluoro (**4**) and a chloro (**5**). The more lipophilic trifluoromethyl derivative (**8**) displayed a similar kinase inhibition to chloro (**5**) but was slightly less potent in the cellular assay. Methoxy (**9**) and cyano (**10**) groups were tolerated, providing the same level of potency as the fluoro derivative **4**. Although the C5 substitution restored some weak CDK2 activity, especially for compounds **4**–**6**, the selectivity ratios remained excellent.

With little difference in activity between **5** and **6**, we decided to study the substitution of the imidazopyridine ring in the less lipophilic 5-chloropyrimidine series. Because a substituent on the position 2 or 5 of the imidazopyridine would presumably increase the torsion angle relatively to the pyrimidine, with a potential detrimental effect on the IGF-1R inhibition, we focussed our efforts on positions C6, 7 and 8 (respectively, R^3, R^2 and R^1 in Table 3). A fluorine atom was well tolerated at all three positions (see **11**, **17**, **24**, and **29**) with no significant impact on either enzymatic or cellular potency. Similarly a methyl group (see **12**, **18**, and **25**) did not affect potency compared to the parent compound (**11**). When a methoxy group was used (see **13, 19**, and **26**), a reduction in activity was observed, in particular with compound **13** in cells. The other substitutions evaluated on position 8 (see **14**–**16**) suggest

Table 3
Inhibition of IGF-1R kinase activity and inhibition of receptor autophosphorylation in cell for compounds **11**–**29**



Compds	R^1	R^2	R^3	IGF-1R IC_{50}[a] (μM)	Cell IC_{50}[a] (μM)
11	F	H	H	0.010	0.022
12	Me	H	H	0.013	0.038
13	MeO	H	H	0.040	0.11
14	NH2	H	H	0.035	0.054
15	CH_2OH	H	H	0.035	0.11
16	CN	H	H	0.14	0.77
17	H	F	H	0.011	0.025
18	H	Me	H	0.009	0.026
19	H	MeO	H	0.008	0.043
20	H	CH_2OH	H	0.049	0.13
21	H	CN	H	0.060	0.19
22	H	Cl	H	0.010	0.050
23	H	Pyrrolidin-1-yl	H	0.21	0.27
24	H	H	F	0.007	0.013
25	H	H	Me	0.010	0.013
26	H	H	MeO	0.055	0.066
27	H	H	CN	0.053	0.071
28	H	H	NMe_2	0.43	0.27
29	F	H	F	0.022	0.024

[a] IC_{50} values are means of at least two experiments, standard deviation is less than 2.5.

the activity is governed by size rather than electronic properties since the small but strongly donating amino group displays a similar IC_{50} to a methyl. The same trend is observed at C7 (see **19**–**23**), the pyrrolidine derivative being the least active, as well as position C6 (see **26**–**28**), a dimethyl amino group being disfavoured.

Since none of the modifications performed on the imidazopyridine ring led to a significant improvement over **5**, further evaluation of this lead compound was conducted. As expected from the high homology of their kinase domains, **5** inhibits the Insulin Receptor tyrosine kinase to the same extent as IGF-1R, as demonstrated by its activity in a kinase biochemical assay (IC_{50} = 9 nM) and in a cellular mechanistic assay[15] (IC_{50} = 12 nM). There is increasing evidence that the Insulin Receptor IR-A isoform is over-expressed in many cancers and potentially drives tumor progression upon IGF2 binding and/or hetero-dimers formation with IGF-1R.[1c] Therefore

Table 4
Pharmacokinetic parameters[16] of compound **5**

Species	CL [a]	V_{dss} [b]	iv $t½$ [c]	F[d] (%)
Mouse	15	0.8	0.9	36
Rat	6.5	0.7	4.6	49
Dog	4.7	1.9	5.5	30

[a] Plasma clearance expressed in mL/min/kg.
[b] Volume of distribution at steady state in L/kg.
[c] Terminal half-life in hour.
[d] Oral bioavailability (%).

Table 5
In vitro metabolic stability of compound **5**

	Mouse	Rat	Dog	Human
CL_{int} [a]	32	15	6.7	6.9

[a] Intrinsic clearance from incubation studies with hepatocytes, expressed in μL/min/10^6 cell.

a dual kinase inhibitor of IGF-1R and IR such as **5** may prove advantageous over specific IGF-1R targeting with antibodies.

The general kinase inhibition profile was assessed in a panel of 69 kinases and the results are shown as a heat map in Figure 2. Only three kinases belonging to the Mitogen-Activated Protein Kinase (MAPK) family were inhibited by more than 80% at a compound concentration of 1 μM, namely JNK1 (MAPK8), JNK2 (MAPK9) and ERK8 (MAPK15).

Finally, no significant inhibition of the human ether-a-go-go related gene (hERG) ion channel was seen in an electrophysiological assay (IC_{50} >30 μM).

The pharmacokinetic parameters of **5** in mouse, rat and dog are presented in Table 4. This compound displays a low plasma clearance and a low volume of distribution across the three species. Its oral bioavailability is good, especially in the rat, and its metabolic stability across species when incubated in the presence of hepatocytes (Table 5) predicts a low hepatic clearance in human. This provides evidence that compound **5** would be a suitable candidate for further evaluation as an orally active IGF-1R kinase inhibitor.

In summary, we have identified a novel series of imidazo [1,2-*a*]pyridines as inhibitors of the IGF-1R tyrosine kinase. Despite the CDK2 activity of the original hit, modification of the aniline provided a series with a good selectivity versus CDK2. The lead compound **5** demonstrates an appropriate cell potency and a good pharmacokinetic profile in pre-clinical species which enables further evaluation in in vivo efficacy models. In the following paper we report subsequent efforts towards the optimization of the aniline moiety in **5** and the resulting structure–activity relationship against hERG inhibition that emerged.

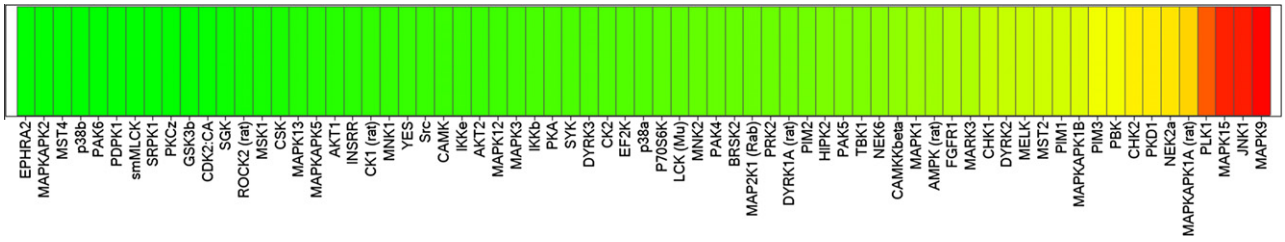


Figure 2. Heat map showing the inhibitory activity of compound **5** against a panel of 69 kinases at 1 μM. The percentage of inhibition is colored from green (0%) to yellow (60%) to red (90%).

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9. Our cellular assay is based on recombinant mouse fibroblasts expressing the human insulin-like growth factor-1 receptor (IGF-1R) stimulated with Insulin Growth Factor-1 (IGF1). For the high throughput assay, the cells were incubated with the compound before stimulation with IGF1. After fixing the cells with formaldehyde, the phosphorylation of IGF-1R is detected with a phospho-specific antibody and a fluorescent labeled secondary antibody. After co-staining of the nucleus with DRAQ5, the fluorescent signals are measured on the In Cell 3000 imaging system. The cellular IC_{50} values quoted in the present manuscript have been obtained using the same cell line and a similar protocol with a 12 points dose–response format on 384 well plates. In this case, the Acumen reader platform was used to detect the signal and nuclear co-staining was not required.
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14. Careful control of the reaction is necessary to avoid solvolysis of the acetamide. However, when de-acetylation occured, the piperazine product was easily converted back to the N-Ac compound by reaction with acetic anhydride.
15. Measuring inhibition of the Insulin Receptor auto-phosphorylation after insulin stimulation in CHO cells transfected with the human gene of IR.
16. Mouse PK: nude mice dosed at 4 μmol/kg iv and 10 μmol/kg po; rat PK: male Han Wistar rats dosed at 4 μmol/kg iv and 10 μmol/kg po; dog PK: mean values for one male and one female beagle dogs dosed at 0.8 μmol/kg iv and 10 μmol/kg po.