Indolin-2-Ones in Clinical Trials as Potential Kinase Inhibitors: A Review

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ABSTRACT

The kinases have been intensively studied because of their involvement in regulating essential cellular activation of signaling cascades in response to extracellular and intracellular stimuli to control cell growth, proliferation, and survival. Recent cancer genomic sequencing studies have revealed that many more kinases contribute to tumor genesis and are potential targets for inhibitor drug development intervention. Herein we review recent results that have helped to unravel the indolin-2-ones underlying the conflicting roles of the kinase inhibition regulation. This review focuses on the potential of kinases as a chemotherapeutic target in cancer treatment and highlights important recent advances in the development of indolin-2-ones as kinase inhibitors.

Keywords: Isatin; Enzyme Inhibition; Anticancer

1. Introduction

The kinases signify a large family of membrane-bound enzymes that play key roles in tumor growth, survival, and metastasis. Many kinases have been found to be closely involved in the processes leading to tumor cell proliferation and survival. Deregulation of kinase function has been implicated in other disorders, including neurological, immunological, metabolic and infectious disease. This has generated a great interest in the development of small molecule kinase inhibitors for the treatment of these disorders [1]. A number of small molecule kinase inhibitor like Sorafenib and Imatinib has been approved for the treatment of cancer. 3-Substituted indolin-2-ones have been reported in several literatures as potent and selective inhibitors of different kinases. Pyrrole-indoline-2-ones were among the first structures identified as kinase inhibitors and have been intensively studied for the inhibition of VEGFR, PDGFR α/β, c-kit, FLT3, and CSF1R [2]. Moreover the importance of indolin-2-one as kinase inhibitor is also reviewed by Underiner et al. [3]. The first compound of this type reaching the clinic was Sunitinib (Sutent; Pfizer) (Figure 1), has been approved and marketed for the treatment of renal cell carcinoma and Imatinib resistant GIST [4]. This review provides an overview of the indolin-2-one based molecules approved by USFDA and compounds under clinical trials as RTKIs.

2. Indolin-2-Ones Drugs in Market

The search for synthetic inhibitors of protein kinases as anti-tumor drugs has been invigorated by the successful approval of a number of molecules that target tyrosine kinases. The indolin-2-one based Sunitinib is a small-molecule inhibitor of multiple RTK involved in cancer, including VEGFR, PDGFR. It was approved by the USFDA for the treatment of GIST and advanced renal-cell carcinoma in January 2006 and European Union approval in January 2007 [5]. Sunitinib is the first anti-cancer drug which received simultaneous FDA approval for two different indications. It has potent anti-angiogenic effects and direct anti-tumor activities due to the selective inhibition of VEGFR-1 (also known as FLT1), VEGFR-2 (also known as FLK1/KDR), VEGFR3 (also known as FLT4), PDGFRα, PDGFRβ, c-kit, FLT3, RET, and CSF1R.

Toceranib Phosphate [6] (Palladia) an orally bioavailable compound is similar to Sunitinib, exhibiting potent inhibitory activity against members of the split-kinase receptor family, including VEGFR, PDGFR, and Kit, and was therefore predicted to have both anti-angiogenic and direct anti-tumor activity. Toceranib phosphate (Figure 2) is a small molecule with an indolinone chemical structure and approved by USFDA for the treatment of canine cu-
Figure 1. Sunitinib the first anticancer drug received FDA approval for dual indication.

Figure 2. Toceranib phosphate.

taneous (skin-based) mast cell tumors, a type of cancer responsible for about 1 out of 5 cases of canine skin tumors. The drug is approved to treat the tumors with or without regional lymph node involvement in June 2009. Toceranib an TKI and works in two ways: by killing tumor cells and by cutting off the blood supply to the tumor. In a random clinical trial study, Toceranib showed a statistically significant variation in tumor shrinkage when compared to placebo.

The field of anti-viral drug development has made dramatic, impressive strides over the past half century, since the discovery of the first-ever medicinal antiviral, Methisazone [7] (for use in the treatment of smallpox and other related viruses), in the 1950s. Methisazone (Figure 3), an N-methyl derivative of thiosemicarbazone, is active against poxviruses and appears to be effective in the prophylaxis of smallpox and treatment of the complications of vaccination.

3. Indolin-2-Ones That Have Entered Clinical Studies

3.1. SU5416

SU5416 (Semaxanib) (Figure 4) is a synthetic small molecule inhibitor of the tyrosine kinase domain of VEGFR-2. SU5416 is also a potent competitive inhibitor of KIT and less potently the PDGFR (indirectly involved in angiogenesis), but is not directly cytotoxic by itself. Recent work has also suggested that SU5416 may mediate some of its effects through inhibition of a second VEGFR [8]. Preclinical xenograft models have confirmed inhibition of tumor growth and a reduction in the number of metastases following treatment with SU5416, as well as a decrease in vascular density in treated tumors as...
measured by intravital video-microscopy. SU5416 recently underwent phase II clinical trials for its safety and efficacy in patients with recurrent or metastatic head and neck cancer [9].

3.2. SU5614

Karsten et al. [10] disclosed the expression of VEGFR-1/2 and its ligand VEGF in AML cell lines and characterized the inhibitory action of the protein TKI SU5614 (Figure 5) (5-Chloro-3-[(3,5-dimethylpyrrol-2-yl)methylene]-2-indolinone) on human endothelial and leukemic cells. The results of this study showed intracellular VEGF expression was detected in 9 of 10 leukemic cell lines. In contrast, VEGFR-1 and VEGFR-2 expression was restricted to 6 and 2 out of 10 cell lines, respectively. Although SU5614 was an effective inhibitor of the VEGF-induced endothelial cell sprouting in vitro, the sensitivity of leukemic cells toward the growth inhibitory activity of the compound was determined by the c-kit, but not by the VEGFR-1/2 expression. SU5614 induced growth arrest and apoptosis in c-kit expressing Kasumi-1, UT-7, and M-07e cells and inhibited the stem cell factor induced tyrosine phosphorylation of c-kit.

3.3. SU6668

SU6668 (Figure 6) is a selective inhibitor of the receptors for VEGFR, PDGFRβ and FGFR1 [11]. SU6668 inhibits the growth of tumor cell lines in vitro and has significant anti-tumor activity in xenografts after daily oral treatment. Laird and co-workers [12] determined the kinase inhibitory profile of SU6668, which is a more water soluble 2-oxindole derivative than SU5416. Using steady state enzyme kinetics with purified recombinant VEGFR-2, they reported that this compound is a competitive inhibitor with respect to ATP.

3.4. SU14813

SU14813 (Figure 7), an orally active indolinone multitargeted TKIs, blocks VEGFR-1, -2, and -3, PDGFRα, β, c-kit, and FLT3 at nanomolar concentrations [13]. In vitro, SU14813 inhibited VEGFR-2, PDGFRβ, and FLT3 internal tandem duplication phosphorylation, with cellular IC₅₀ values of 0.04, 0.02, and 0.05 µmol/l, respectively, and showed anti-tumor efficacy in tumor xenograft models. Preclinical studies in mice demonstrated a lack of major active metabolites, linear pharmacokinetics and oral bioavailability of 40%.

Shem et al. [14] reported a encouraging preclinical data for SU14813 and the clinical implications for its use based on a similar potency to Sunitinib but distinct pharmacokinetic profile, which may favorably affect tolerability and allow greater dosing flexibility, SU14813 warrants further clinical evaluation in human malignancies, both alone and in combination with cytotoxic chemotherapy.

3.5. SU11274

SU11274 (Figure 8) is an ATP competitive small molecule inhibitor of the catalytic activity of Met which exhibited selectivity for Met enzyme versus a panel of other tyrosine kinases [15]. Chemically SU11274 is a [(3Z)-N-(3-chlorophenyl)-3-{(3,5-dimethyl-4-[4-methylpiperazin-1-yl]carbonyl)-1H-pyrrol-2-yl} methylene]-N-methyl-2-oxindoline-5-sulfonamide]. Recent studies demonstrate that SU11274, a prototypic anti-MET small molecule, is an effective inhibitor of both HGF dependent and independent signaling, which results also from the presence of point mutations. Inhibition of the Met kinase activity by SU11274 led to time and dose dependent reduced cell growth and induced G1 cell cycle arrest and apoptosis. The bulk of clinical and experimental evidence has suggested that Met is a potential therapeutic target for development of inhibitors for treatment of cancers. Xueyan et al. [16] reported the identification and characterization of small molecule inhibitors SU11274 that potently and selectively inhibit the enzymatic activity of Met kinase. SU11274 displayed much lower IC₅₀ values for inhibition of Met, then towards other tyrosine kinases includes EGFR, FGFR1 or Src, thus, suggesting its high specificity. SU11274 belongs to a class of small molecule RTK inhibitors that exert their activity by competing for the Mg-ATP complex binding pocket, which results in
inhibition of kinase activity and subsequent downstream signaling.

3.6. SU12662

SU12662 (Figure 9) is an active N-desethyl metabolite of Sunitinib. SU12662 has receptor tyrosine kinase inhibitory profile similar to that of Sunitinib in vitro and has similar plasma protein binding [17].

3.7. SU5402

SU5402 (Figure 10) chemically 3-[(3-(2-carboxyethyl)-4-methylpyrrol-2-yl) methylene]-2-indolinone shown a selective inhibitory effect of tyrosine kinase with known anti-FGFR activity [18] against control Ba/F3 cells expressing the constitutively active ZNF198-FGFR1 fusion protein, and also against KMS-11 and OPM-2, t(4;14) positive MM cell lines that express activated FGFR3.

3.8. SU10944

Neela et al. [19] reported a novel small molecule inhibitor of VEGFR-2, SU10944 (Figure 11). This indolinone 2-one derivative is an effective, ATP-competitive inhibitor of VEGFR-2 biochemical activity and is active in the nanomolar range in cellular assays. SU10944 can be administered in vivo by the oral route and achieves sufficient exposure to inhibit nearly all VEGF stimulated ne-
ovascularization and vascular permeability. Robert disclosed steady state kinetic analysis of SU10944, he found that this compound is a potent ATP competitive inhibitor of VEGFR-2 \textit{in vitro} with an IC$_{50}$ value of 96 nM and a Ki value (a dissociation constant) of 21 nM [20]. They found that SU10944 is a more potent inhibitor of VEGFR-1 when compared with VEGFR-2. However, the compound is not an effective inhibitor of PDGFR$\beta$, KIT or FGFR1.

3.9. SU6656

SU6656 (Figure 12) is an inhibitor of the Src family of tyrosine kinases [21], a group of enzymes that regulate many important signaling pathways, including RAS/MAPK, PI3K/AKT and STAT, and which represent molecular targets for the treatment of various cancers. SU6656 showed cross reactivity with certain other kinases such as the Aurora B kinases, proteins that control cell ploidy and centrosome number. SU6656 can also significantly augment the anti-angiogenic effect of irradiation and may be a useful agent in the treatment of drug resistant cancers.

3.10. SU9516

3-substituted indolin-2-ones represent a potent and selective inhibitor for certain RTKs [22]. These small molecules typically act as adenine mimetics that occupy the ATP binding pocket in specific RTKs, with the specificity being governed by the substituents around the indoline core. Compounds of this class have reached clinical trials for the treatment of several cancers. One such compound is SU9516 (Figure 13) (3-[1-(3H-Imidazol-4-yl)-meth-(Z)-ylidene]-5-methoxy-1,3-dihydro-indol-2-one). From crystal structure of the inhibitor bound to cdk2 and kinetically characterized studies about the inhibition of cdks by SU9516 suggest that this compound could have usefulness as a core molecule for the study of cdk inhibition. Furthermore, information gained from the structural analysis should aid in the development of more potent and specific inhibitors in the future. SU9516 is also reported to inhibit GSK-3 which is involved in normal cell death [23].

3.11. SU4984

SU4984 (Figure 14) chemically 3-[4-(1-Formylpiperazin-4-yl)-benzylidenyl]-2-indolinone derivative inhibits the tyrosine kinase activity of FGFR1 by interacting with the catalytic domain of FGFR1. SU4984 also inhibits FGFR-
### Table 1. Summary of indolinones as kinase inhibitor.

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Structure</th>
<th>Targets</th>
<th>Disease Types</th>
<th>Clinical Stage</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunitinib</td>
<td><img src="image" alt="Sunitinib" /></td>
<td>VEGFR, PDGFR, FLT3, c-KIT, Ret, CSF1R</td>
<td>Renal cancer, Carcinoma of the oesophagus, Head and neck cancer, Liver cancer, Prostate cancer, Breast cancer, Brain metastasis, Glioblastoma, Colorectal cancer, Non small cell lung cancer, Melanoma, Ovarian cancer</td>
<td>Marketed</td>
<td>Pfizer</td>
</tr>
<tr>
<td>Toceranib</td>
<td><img src="image" alt="Toceranib" /></td>
<td>Kit, VEGFR, PDGFR, Flt-3</td>
<td>Mast Cell Tumours, sarcomas, carcinomas, melanoma, myeloma</td>
<td>Marketed</td>
<td>Pfizer</td>
</tr>
<tr>
<td>Semaxanib</td>
<td><img src="image" alt="Semaxanib" /></td>
<td>VEGFR-2, kit, PDGFR</td>
<td>Metastatic, Head and Neck cancer</td>
<td>Phase III</td>
<td>Pharmacia</td>
</tr>
<tr>
<td>SU5614</td>
<td><img src="image" alt="SU5614" /></td>
<td>VEGFR-1a, nd-2, PDGFR, c-kit</td>
<td>Cancer</td>
<td>Phase II</td>
<td>Sugen/Pfizer</td>
</tr>
<tr>
<td>SU6668</td>
<td><img src="image" alt="SU6668" /></td>
<td>VEGFR-1, PDGFR, FGFR1</td>
<td>Solid Tumors</td>
<td>Phase III</td>
<td>Pharmacia/Sugen</td>
</tr>
<tr>
<td>SU14813</td>
<td><img src="image" alt="SU14813" /></td>
<td>VEGFR-2, PDGFR, FLT-3, c-kit</td>
<td>Angiogenesis and tumour</td>
<td>Phase II</td>
<td>Pfizer</td>
</tr>
</tbody>
</table>
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Continued

SU11274

Met EGFR
FGFR1 Src
Cancer-controlling melanoma progression and metastasis
Pre clinical
Sugen/
Pfizer

SU11274

Met EGFR
FGFR1 Src
Cancer-controlling melanoma progression and metastasis
Pre clinical
Sugen/
Pfizer

SU12662

VEGFR
PDGFR/
FLT3 e-KIT
Ret CSF1R
Cancer
Pre clinical
Sugen/
Pfizer

SU5402

FGFR
ERK1/2
PDGFR
Cancer
Pre clinical
Sugen/
Pfizer

SU10944

VEGFR-2
Angiogenesis
Pre clinical
Sugen/
Pfizer

SU6656

Src Fyn
Yes Lyn
Aurora B
Cancer
Pre clinical
Sugen/
Pfizer
induced phosphorylation of ERK1 and ERK2 and tyrosine phosphorylation of PDGF and insulin receptors. SU4984 also effective against KIT with juxtamembrane activating mutations [24].

4. Conclusion

In less than a two decade, the field of chemical inhibitors of receptor tyrosine kinases has seen spectacular developments. Starting from “low throughput screens” the search for selective inhibitors has progressed to massive industrial High Throughput Screens (HTS). It has been accompanied by a large variety of studies ranging from kinase inhibitor interaction at the atomic level (crystallography), molecular modeling, in silico screening, thorough investigation of the inhibitor’s selectivity and cellular effects, and pre-clinical studies. These efforts have provided very useful pharmacological tools that can be used, amongst other applications, to synchronize cells, to scrutinize cell cycle phases, to link physiological/biochemical events with kinase activities. More challenging is the development of the application of these pharmacological compounds to multiple areas the scope of which is much wider than initially expected. So far, various kinase inhibitors have been tested in clinical trials resulting in great survival benefit for patients for whom no effective therapy was available. Indolinone a class of compounds displays very promising anti-tumor properties by inhibiting various kinase families. The anti-kinase activity of the indolinone set of kinase inhibitors was first reported in 1997 by Mohammadi et al. [25]. These small molecules have a low molecular weight and most of them bind to protein kinases competing with ATP for the ATP binding site. Moreover the apparent selectivity of the compound, its potency and favorable
physico-chemical properties render this molecule as an interesting lead structure for development of new kinase inhibitors. Because of that above mentioned reason a number of indolone based anti angiogenic TKIs currently under development may provide additional treatment options for patients in the future (A summary of indolinones as kinase inhibitors are shown in Figure 15). Preclinical as well as preliminary results of clinical trials suggest that this class of agent is promising for cancer treatment and may offer a promising alternative to classical anticancer drugs. Table 1 summarizes the current protein kinase inhibitors in the clinic and in development.

REFERENCES


[18] E. K. Grand, A. J. Chase, C. Heath, A. Rahemtulla and N. C. P. Cross, “Targeting FGFR3 in Multiple Myeloma: Inhibition of t(4;14)-Positive Cells by SU5402 and


**List of Abbreviations**

AKT = AKR mouse thymoma kinase  
AML = Acute myeloid leukemia  
ATP = Adenosine tri phosphate  
c-Kit = Stem cell factor receptor tyrosine kinase  
CDK = Cyclin dependent kinase  
CSF1R = Colony-stimulating factor 1 receptor  
EGFR = Epidermal growth factor receptor  
ERK = Extracellular signal regulated kinases  
FGF = Fibroblast growth factor  
FGFR = Fibroblast growth factor receptor  
FLT3 = fms-related tyrosine kinase 3  
GIST = Gastrointestinal stromal tumor  
GSK = Glycogen synthase kinase  
HGF = Hepatocyte growth factor  
IC₅₀ = Concentration required for 50% inhibition of a biochemical process *in vitro*  
KIT = Stem-cell growth factor receptor  
MAPK = Mitogen-activated protein kinase  
PDGF = Platelet-derived growth factor  
PDGFR = Platelet-derived growth factor receptor  
RTKs = Receptor tyrosine kinases  
RTKIs = Receptor tyrosine kinases inhibitors  
SAR = Structure-activity relationship  
Src = v-src sarcoma (Schmidt-Ruppin A-2) viral oncoprotein homologue  
STAT = Signal transducer and activator of transcription  
TKI = Tyrosine kinase inhibitor  
VEGF = Vascular endothelial growth factor  
VEGFR = Vascular endothelial growth factor receptor

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