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Inhibition of c-Kit, VEGFR-2 (KDR), and ABCG2 by analogues of OSI-930

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ABSTRACT

The quinoline domain of OSI-930, a dual inhibitor of receptor tyrosine kinases (RTKs) c-Kit and KDR, was modified in an effort to further understand the SAR of OSI-930, and the binding site characteristics of c-Kit and KDR. A series of 16 compounds with heteroatom substituted pyridyl and phenyl ring systems was synthesized and evaluated against a panel of kinases including c-Kit and KDR. Aminopyridyl derivative **6** was found to be the most active member of the series with 91% and 57% inhibition of c-Kit at 10 μM and 1 μM, respectively and 88% and 50% inhibition of KDR at 10 μM and 1 μM, respectively. The target compounds were also tested for their ability to inhibit efflux of mitoxantrone through inhibition of ATP dependent ABCG2 pump. Nitropyridyl derivative **5** and *o*-nitrophenyl derivative **7** exhibited complete inhibition of the ABCG2 pump with IC_{50} values of 13.67 μM and 16.67 μM, respectively.

Inhibition of receptor tyrosine kinases (RTKs) has emerged as a promising approach for the treatment of many types of human cancers. Epidermal growth factor receptor (EGFR) inhibition for the treatment of non-small cell lung cancer, c-Kit inhibition for the treatment of gastrointestinal stromal tumors (GIST), and Abl inhibition for the treatment of acute as well as chronic myeloid leukemia has found success in the recent years with introduction of several drugs in this arena.[1] Imatinib, a Bcr-Abl kinase and c-Kit kinase inhibitor; erlotinib, an EGFR inhibitor; lapatinib, an EGFR and vascular endothelial growth factor receptor (VEGFR) inhibitor; and OSI-930, a dual c-Kit and VEGFR-2 inhibitors are few of the many tyrosine kinase inhibitors, either currently available in market or in clinical trials for the treatment of cancer (Fig. 1).[1]

Studies have shown that c-Kit, a stem cell factor receptor, is over-expressed or mutated, in small cell lung cancer, mast cell leukemia, seminoma, acute myeloid leukemia and most commonly in GIST patients.[2] VEGFR-2 (KDR) has been shown to play an important role in the regulation of tumor angiogenesis.[3,4] Thus inhibition of both these RTKs can result in improved antitumor efficacy through inhibition of cell proliferation and anti-angiogenic effect.

A number of tyrosine kinase inhibitors including imatinib, AG1478, erlotinib, and lapatinib have been shown to interact with ATP-binding cassette (ABC) transporters such as the ABCG2 and block its efflux function, thus increasing the intracellular accumulation of anticancer drugs and reversing ABCG2-mediated multidrug resistance (MDR).[5–9] Thus tyrosine kinase inhibitors have the added potential to contribute to cancer chemotherapy through the reversal of MDR caused by the overexpression of ATP-binding cassette transporters.

OSI-930 (Fig. 1), a dual c-Kit and KDR inhibitor with IC_{50} values of 29 and 5 nM, respectively was chosen as a lead compound.[10,11] Target compounds exploring the structure–activity relationship of the southern part of OSI-930 were designed. Modifications involved replacement of the quinoline group with phenyl and pyridyl ring systems substituted with functional groups that

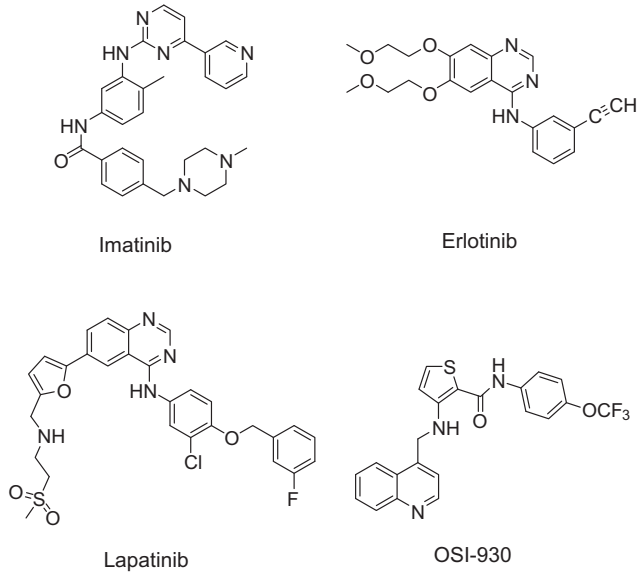

Figure 1. Examples of tyrosine kinase inhibitors on market or clinical trials.

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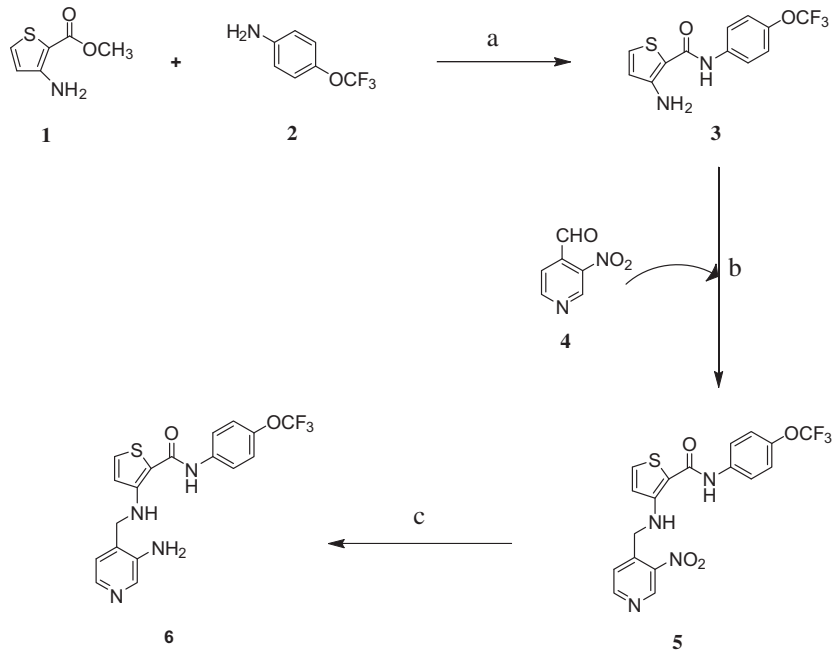


Scheme 1. Reagents and conditions: (a) $(CH_3)_3Al$, anhydrous toluene, rt (16 h) followed by reflux for 24 h; (b) $NaBH_4$, trifluoroacetic acid, EtOAc; (c) hydrazine monoformate Zinc Dust, MeOH.

impart different steric, electronic, solubility, and H-bonding characteristics. The functional groups chosen included those that were rich in heteroatoms with the potential to form H-bond interactions with the backbone cysteine residues in the hinge regions of c-Kit and KDR. In this report we describe the synthesis of compounds **5–20**, their activity against a panel of kinases including c-Kit and KDR, and the ABCG2 pump.

3-Amino-*N*-(4(trifluoromethoxy)phenyl)thiophene-2-carboxamide **3** was obtained by activation of 4-trifluoromethoxyaniline with trimethylaluminum, followed by reaction of the activated amine-trimethylaluminum adduct with methyl 3-aminothiophene-2-carboxylate under reflux conditions (Scheme 1).[12] The nitropyridyl derivative **5**, nitrophenyl derivatives **7–9** and hydroxyphenyl derivative **10** were obtained by the reductive alkylation of 3-aminothiophene carboxamide derivative **3** with 3-nitroisonicotinaldehyde **4** which was prepared according to the literature procedure[13] or the commercially available substituted benzaldehydes (Schemes 1 and 2).[14–16]

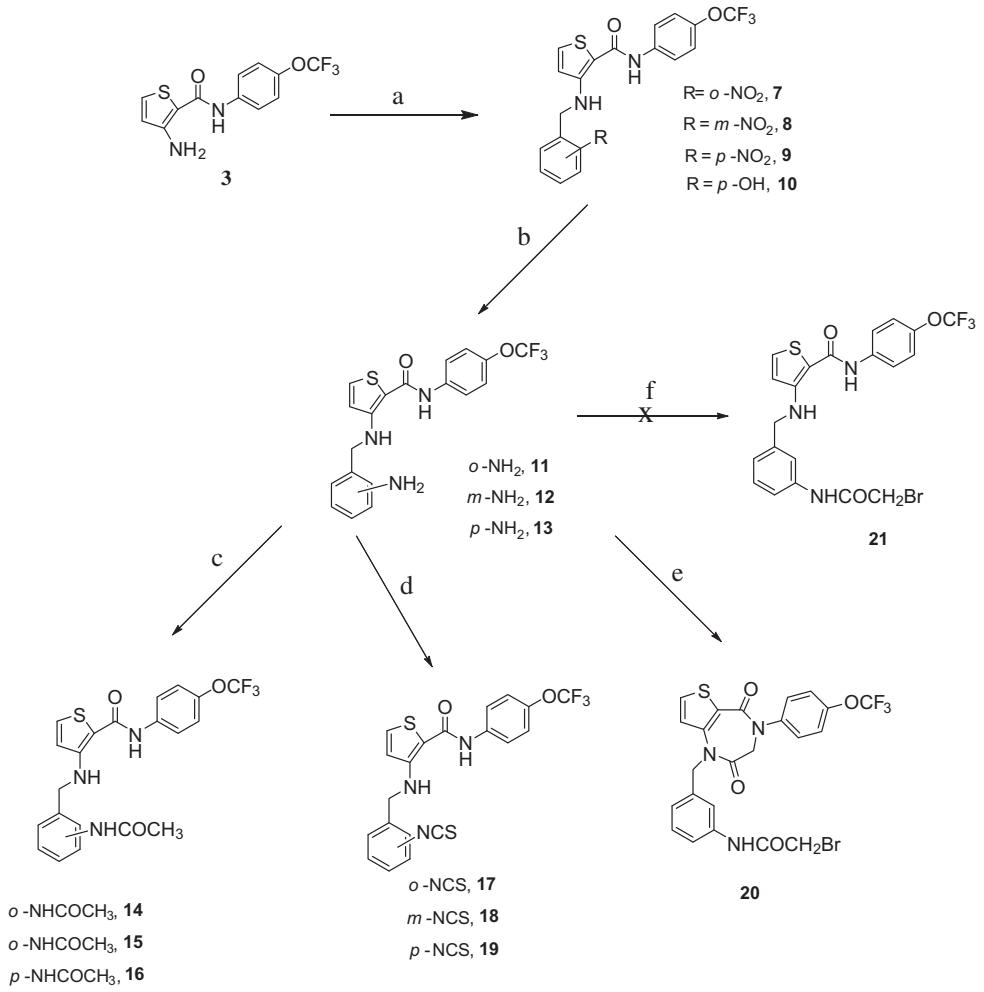
Nitro derivatives **5** and **7–9** were reduced to the corresponding amines **6** and **11–13** by using either Zn dust method[17] or PtO_2 catalyzed hydrogenation method.[18] The amine derivatives **11–13** were subsequently treated with acetic anhydride or di-2-pyridyl thionocarbonate in order to obtain acetamides **14–16**[19] and isothiocyanates **17–19**,[20] respectively (Scheme 2). Treatment of *m*-amino derivative **12** with bromoacetyl bromide to form the bromoacetamide derivative **21**[19] resulted in a product whose NMR spectrum showed all the expected protons including a sharp singlet for benzylic CH_2 protons at 5.16 ppm and a singlet for alkyl CH_2 protons of bromoacetyl group at 4.02 ppm. A singlet at 4.56 ppm integrating for two protons that did not correspond to any of the expected protons was also observed. LCMS data showed molecular ion peak of 568 instead of the expected molecular ion peak of 528. Based on this information, it was determined that the secondary amine at position 3 was also bromoacetylated which then underwent cyclization to yield compound **20**. The singlet at position 4.56 ppm presumably corresponds to the two protons of cyclic $-CH_2$ group.

Target compounds **5–20** were tested against c-Kit and KDR to evaluate their ability to inhibit these enzymes by Z'-LYTE assay.[21] Target compounds were also tested against other members of type III and type V kinase families to which c-Kit and KDR, respectively, belong to. All target compounds were tested against a panel of eight kinases which included c-Kit, PDGFRα, PDGFRβ, Flt3, KDR, VEGFR-1, VEGFR-3 and Tie-2 at 1 μM and 10 μM concentrations. Summary of c-Kit and KDR inhibition results of the target compounds exhibiting greater than 40% inhibition is presented in Table 1 and their selectivity versus other kinases is presented in discussion. Acetamidopyridyl derivative **22** was used as a reference.

As shown in Table 1, nitropyridyl derivative **5** and the aminopyridyl derivative **6** were among the best in inhibiting c-Kit and/KDR. Nitro analogue **5** showed 80% inhibition of c-Kit at 10 μM concentration. Amino analogue **6** exhibited 57% and 91% inhibition of c-Kit at 1 and 10 μM, respectively, whereas in case of KDR it showed 50% and 88% inhibition at 1 and 10 μM, respectively. Compound **6** weakly inhibited Flt3 (41%), VEGFR-3 (41%) and PDGFRβ (47%) at 10 μM concentration. Thus compound **6** is somewhat similar in potency to the reference compound **22** at 10 μM. Compound **22** displayed 81% inhibition of c-Kit at 1 μM and complete inhibition at 10 μM. It inhibits KDR completely at both concentrations. In addition, compound **22** also inhibited all other kinases in the test group completely at 10 μM. Compound **6** showed weaker inhibition of other kinases, and thus appears to be more selective for c-Kit and KDR than the reference compound.

Compounds in the phenyl series were generally less effective in inhibiting c-Kit and KDR, as well as the other kinases in the test group. *m*-Acetamide analogue **15** showed 58% inhibition of c-Kit at 10 μM, suggesting that acetamide group of compound **15** may be interacting with the c-Kit enzyme active site in a similar manner as the acetamide group of reference compound **22**. Lack of pyridyl N in **15** is contributing to its reduced activity compared to compound **22**.

Another compound in phenyl series which showed kinase inhibition was *p*-hydroxy analogue **10** which possesses H-bond acceptor group similar to the pyridyl derivatives **5, 6** and **22** except that it is one atom away from the aromatic ring. At 10 μM, compound **10** showed 68% and 54% inhibition of c-Kit and KDR, respectively. It showed 44% inhibition of PDGFRβ at 10 μM. *p*-Amino analogue



Scheme 2. Reagents and conditions: (a) $NaBH_4$/trifluoroacetic acid, EtOAc, substituted benzaldehydes; (b) PtO_2/H_2/MeOH, 40 psi/30 min; (c) acetic anhydride, chloroform; (d) di-2-pyridylthienocarbonate, chloroform; (e) $BrCH_2COBr$, $NaHCO_3$, distilled THF, 0 °C.

13 on the other hand showed less than 40% inhibition in all enzymes at both tested concentrations. Interestingly, cyclic bromoacetamide analogue **20** showed 41% inhibition of c-Kit at 10 μM. All other nitro (**7–9**), amino (**11–13**), acetamide (**14** and **16**) and isothiocyanate (**17–19**) analogues in phenyl series showed less than 40% inhibition of the kinases. Taken together, the kinase inhibition data reinforces the importance of H-bond acceptor group in the southern domain and in a precise location. Overlay of various target compounds with OSI-930 or the reference compound **22** as shown in Figures 2 and 3 demonstrates the relative placement of H-bonding groups in these molecules.

Table 1
Percent inhibition of c-Kit and KDR by OSI-930 analogues

Compound	X	-R	Concentration (μM)	% Inhibition	
				c-Kit	KDR (VEGFR-2)
5	N	2′-NO_2	1	*	*
			10	80	*
6	N	2′-NH_2	1	57	50
			10	91	88
10	C	4′-OH	1	*	*
			10	68	54
15	C	3′-$NHCOCH_3$	1	*	*
			10	58	*
20	C	Cyclized deriv.	1	*	*
		3′-$NHCOCH_2Br$	10	41	*
22	N	3′-$NHCOCH_3$	1	81	101
			10	102	101

* Indicates less than 40% inhibition. All experiments were performed in duplicates.

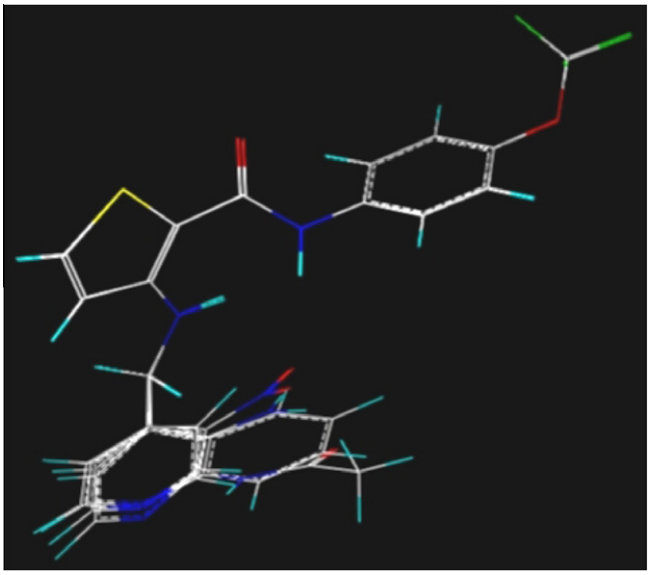


Figure 2. Overlay of OSI-930 and compounds **5, 6**, and **22**.

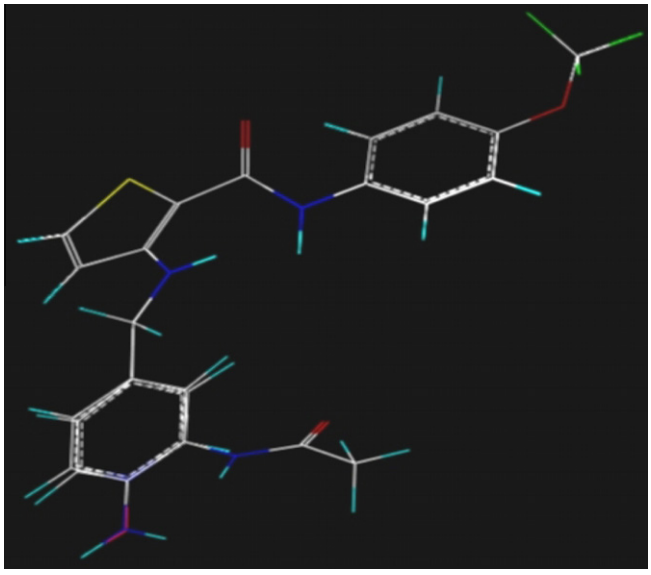


Figure 3. Overlay of compounds **10**, **13**, and **22**.

Table 2
Effect of analogs of OSI-930 on the cytotoxicity of mitoxantrone in the ABCG2-transfected multidrug resistant cells

Treatment	HEK293/pcDNA3.1		HEK/ABCG2-482-R2	
	IC_{50}	FR[a]	IC_{50}	FR[a]
Mitoxantrone	15.41 ± 4.68	1	240.8 ± 4.00	15.33
+**5** (10 μM)	7.46 ± 6.63	0.44	13.67 ± 10.44	0.82
+**6** (10 μM)	15.55 ± 2.64	1.09	51.67 ± 0.42	3.52
+**7** (10 μM)	7.66 ± 3.44	0.49	16.67 ± 14.17	0.99
+**8** (10 μM)	13.64 ± 2.13	0.91	51.27 ± 11.81	3.37
+**9** (10 μM)	13.31 ± 1.37	0.89	31.52 ± 21.4	1.92
+**10** (10 μM)	7.06 ± 1.42	0.47	29.57 ± 12.18	1.89
+**11** (10 μM)	9.38 ± 0.6	0.64	34.56 ± 29.4	2.05
+**12** (10 μM)	21.55 ± 12.4	1.59	57.7 ± 25.24	3.67
+**13** (10 μM)	20.44 ± 9.06	1.48	55.66 ± 12.89	3.65
+**14** (10 μM)	10.62 ± 2.59	0.7	35.89 ± 2.22	2.42
+**15** (10 μM)	9.12 ± 3.19	0.59	28.74 ± 10.58	1.85
+**16** (10 μM)	14.09 ± 0.36	0.96	46.28 ± 1.6	3.13
+**17** (10 μM)	11.84 ± 7.98	0.72	41.31 ± 1.83	2.79
+**18** (10 μM)	14.39 ± 3.63	0.94	89.9 ± 99.33	7.14
+**19** (10 μM)	150.82 ± 18.6	10.46	1967.29 ± 417.9	138.2
+FTC (2.5 μM)	12.94 ± 5.73	0.83	16.42 ± 7.69	1.03

Cell survival was determined by MTT assay. Data are the means ± SD of at least three independent experiments performed in triplicate.

[a] Fold-resistance was the IC_{50} value for mitoxantrone of HEK/ABCG2-482-R2 cells divided by IC_{50} value of HEK293/pcDNA3 cells in the absence or presence of analogs of OSI-930 or FTC.

The target compounds **5–19** were also screened to determine whether they could sensitize the transfected wild-type ABCG2-overexpressing cells to chemotherapeutic drugs such as mitoxantrone using the MTT assay and the IC_{50} values are reported in Table 2.[22] Nitropyridyl derivative **5** and *o*-nitrophenyl analogue **7** inhibited the efflux of mitoxantrone through the ABCG2 pump completely with IC_{50} values of 13.67 μM and 16.67 μM, respectively. With the exception of *p*-isothiocyanate derivative **18** all the compounds exhibited varying degrees of ABCG2 pump inhibition. Interestingly, the *p*-isothiocyanate derivative **19** appears to be promoting the efflux of mitoxantrone through the ABCG2 pump.

In conclusion, a series of pyridyl (**5–6**) and phenyl (**7–20**) analogues of OSI-930 were successfully designed, synthesized, characterized, and evaluated for their biological activity against c-Kit and KDR along with other kinases belonging to the same families to evaluate specificity in kinase inhibition. Nitropyridyl derivative **5** exhibited 80% inhibition of c-Kit at 10 μM concentration and was not effective against other kinases. Aminopyridyl derivative **6** showed 91% and 88% inhibition at 10 μM and 51% and 50% inhibition at 1 μM of c-Kit and KDR, respectively. Compound **6** exhibited weak or no inhibition of other kinases. Analogues in the phenyl series were generally less active. *m*-Acetamide derivative **15** with the structural resemblance to the reference compound **22** showed 58% inhibition of c-Kit at 10 μM. The significantly weaker activity of **15** versus **22** demonstrated the importance of the H-bond acceptor group in the ring. *p*-Hydroxy derivative **10** possesses the ability to serve as a H-bond acceptor similar to the reference compound **22** with a slightly altered location of the electronegative atom. Although it exhibited inhibition of c-Kit and KDR, it was weaker than the reference compound. The kinase assay results demonstrate the importance of a H-bond acceptor group in the southern region of the target compounds and further suggest that moving this H-bond acceptor functionality by even a one atom distance can significantly impact the activity. Although all target compounds were less active in inhibiting c-Kit and KDR than reference compound **22**, the most potent compound in the series **6** was found to be more selective for c-Kit and KDR. Nitropyridyl and nitrophenyl derivatives **5** and **7** significantly potentiate the sensitivity of anticancer drug mitoxantrone in ABCG2-overexpressing cells. These molecules do not influence the sensitivity of the parental cells.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.08.070.

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