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1,4-Dihydroindeno[1,2-*c*]pyrazoles as novel multitargeted receptor tyrosine kinase inhibitors

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Abstract—A series of 1,4-dihydroindeno[1,2-*c*]pyrazoles with a 3-thiophene substituent carrying a urea-type side chain were identified as potent multitargeted (VEGFR and PDGFR families) receptor tyrosine kinase inhibitors. A KDR homology model suggested that the urea moiety is able to interact with a recognition motif in the hydrophobic specificity pocket of the enzyme.

Receptor tyrosine kinases (RTKs) play a crucial role in signal transduction as well as in cellular proliferation, differentiation, and various regulatory mechanisms. They consist of an extracellular ligand binding domain, a transmembrane spanning region, and a cytoplasmic kinase domain.[1] After binding to their specific extracellular growth factors, RTKs undergo dimerization and autophosphorylation, initiating a cascade of downstream signaling events, which trigger a variety of cell responses. Genetic alterations and/or stimulation through autocrine/paracrine growth factor loops can result in RTKs that are constitutively active. These types of disturbances in the tightly regulated signal transduction pathways have been implicated in the development and progression of a variety of hyperproliferative diseases, in particular cancer.[2]

Members of the split kinase domain (class III RTK) subfamily include the vascular endothelial growth factor receptors KDR (VEGFR2, kinase insert domain-containing receptor tyrosine kinase) and FLT1 (VEGFR1, Fms-like tyrosine kinase 1), the platelet-derived growth factor receptors PDGFR α and β, the colony-stimulating factor-1 receptor (CSF-1R), and the stem cell factor receptor (cKit). KDR kinase is specifically expressed in vascular endothelial cells. It is the primary receptor for vascular endothelial growth factor (VEGF) and plays an essential role in tumor angiogenesis.[3] Inhibition of KDR blocks the neovascularization of tumors and has been shown to inhibit the growth of a variety of solid tumors in tumor xenograft models. Mutated cKit kinase on the other hand is a driving factor in mastocytosis and gastrointestinal stromal tumors.[4] Wild-type cKit is also considered to play a role in the progression of small cell lung cancer. Here it was proposed that cKit is involved in the formation of an autocrine/paracrine loop due to coexpression of ligand and receptor within the same tumor cell type.[5] Inhibition of both, KDR and cKit in the appropriate tumor types, has the potential to produce antitumor effects through two distinct mechanisms. Inhibition of cKit should result in direct effects on the tumor cell phenotype, while inhibition of KDR should produce indirect effects via disruption of endothelial cell function.[6]

In a previous report we have described our hit-to-lead efforts in establishing 1,4-dihydroindeno[1,2-*c*]pyrazoles as a novel class of KDR kinase inhibitors.[7] Two key compounds, **1** and **2** (Fig. 1), were identified with KDR $IC_{50} < 200$ nM. We now describe our

Keywords: Cancer; Receptor tyrosine kinase; KDR kinase; Inhibitor; 1,4-Dihydroindeno[1,2-*c*]pyrazole.
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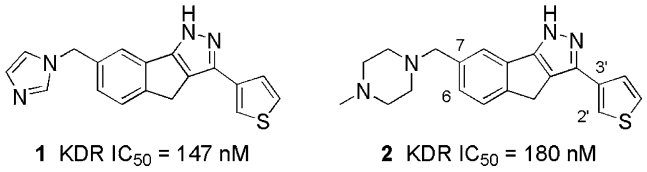


Figure 1. Structure and KDR inhibitory potency of early lead compounds **1** and **2**.

investigations toward extending those molecules into the hydrophobic specificity pocket of KDR kinase.

The imidazole-containing compound **1** showed excessive inhibition of human liver microsome (HLM)-derived CYP3A4[8] (IC_{50} = 254 nM), and so we focused on its 1-methylpiperazine-containing analogue **2** as our key structural unit (CYP3A4 (HLM) IC_{50} > 10 μM). Based on our earlier established structure–activity relationships (SARs), we continued to consider compounds with a basic side chain attached in either the 6- or 7-position. Previous studies, focused on accessing the hydrophobic specificity pocket of KDR kinase, identified a recognition motif for ureas in this site.[9–11] So in order to plan for the attachment of a urea functionality to our 1,4-dihydroindeno[1,2-*c*]pyrazoles, we utilized the published crystal structure of cKit kinase in its inactive conformation[12] to create a homology model of **2** bound into the ATP binding site of KDR kinase. Our studies revealed that either a 2′,4′- or a 2′,5′-linked thiophene would provide an acceptable trajectory for the urea side chain to access the pocket. A methylene spacer between the thiophene and the urea functionality was required to allow the urea to form two hydrogen bonds of 3.0 and 3.2 Å distance to Glu 885. In addition, we proposed that a small hydrophobic substituent in the 3-position off a terminal phenyl ring would be beneficial because it would project into a small hydrophobic groove formed by Ile 892, Ile 888, Leu 889, Val 898, and Leu 1019. Figure 2 shows compound **3** as the result of our design efforts and its predicted binding mode.

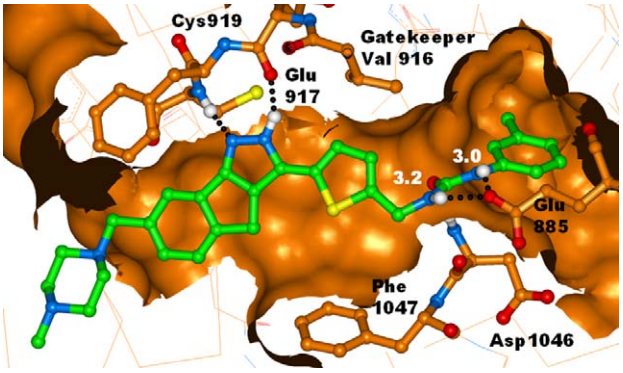
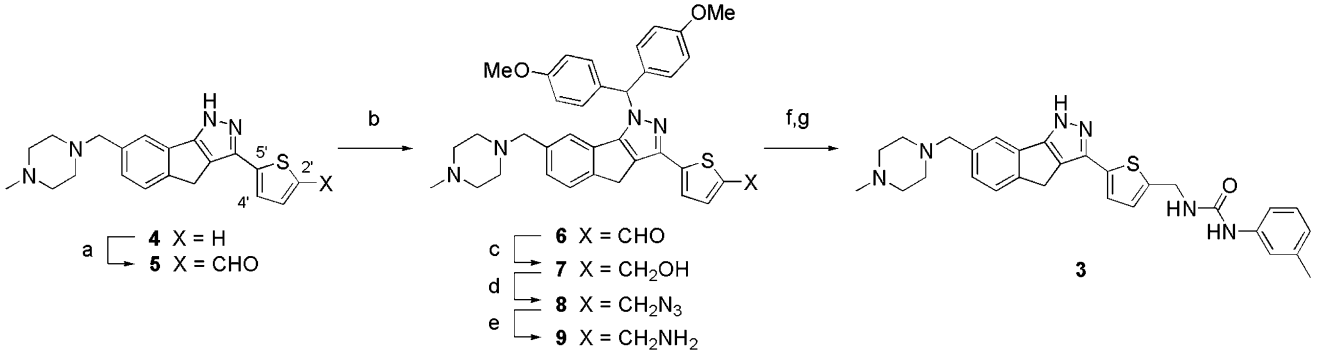


Figure 2. Model of compound **3** (green) bound to the active site of KDR (inactive conformation, homology model based on cKit PDB entry 1T46). Hydrogen bonds are shown in black dotted lines. H-bond distances from the urea to Glu 885 are shown in angstroms.

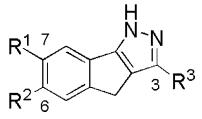
The synthesis of 1,4-dihydroindeno[1,2-*c*]pyrazoles with urea-type side chains is illustrated with the synthesis of **3** as a representative example (Scheme 1). Regioselective deprotonation of the thiophene portion of **4**,[7] followed by formylation with DMF, yielded aldehyde **5**. For the preparation of compounds containing 2′,4′-disubstituted thienyl groups, regioselectivity was achieved through halogen–lithium exchange of the corresponding bromides. After protection of the pyrazole moiety, the aldehyde functionality in **6** was reduced with sodium borohydride to give the hydroxymethyl compound **7**. Direct conversion of **7** to the azide **8** using diphenyl phosphorazidate (DPPA)[13] and subsequent Staudinger reduction afforded amine **9**. Reaction of this key intermediate with *m*-tolyl isocyanate followed by deprotection of the pyrazole led to the urea **3**.

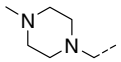
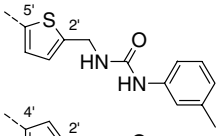
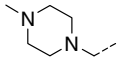
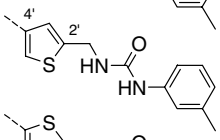
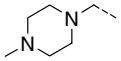
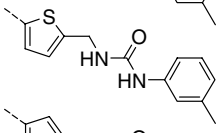
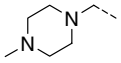
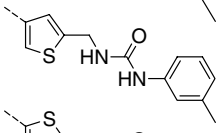
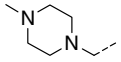
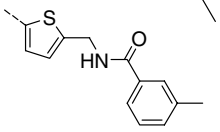
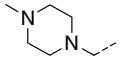
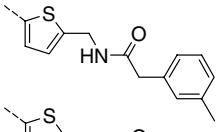
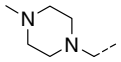
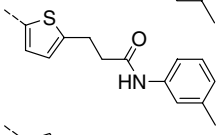
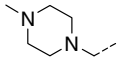
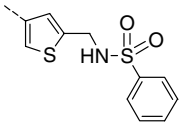
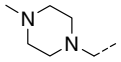
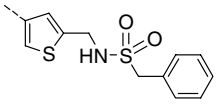
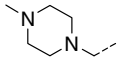
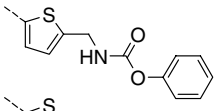
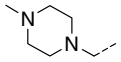
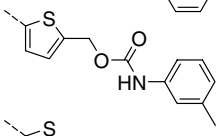
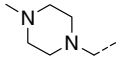
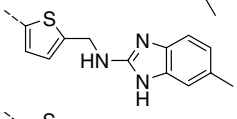
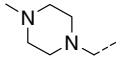
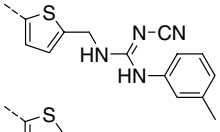
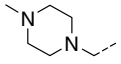
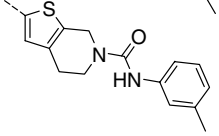
The activity of the target compounds to inhibit the phosphorylation of a peptide substrate (biotin-Ahx-AEEEYFFLFA-amide) by KDR kinase was assessed in an HTRF® assay at 1.0 mM concentration of adenosine-5′-triphosphate (ATP).[9] Table 1 shows that the urea linker indeed improved the potency for KDR inhibition. However, in contrast to our previous findings, attachment of the thienyl group to the 1,4-dihydroindeno[1,2-*c*]pyrazole core through its 5′-position (corresponding to the 2′-position in Fig. 1) (**10** and **3**) was now better tolerated than attachment through its 4′-position (corresponding to the 3′-position) (**11** and **12**). On the other hand, placing the basic side chain in the 7-position (**3** and **12**), again, was only slightly



Scheme 1. Reagents and conditions: (a) i—*n*-BuLi, THF, −78 °C; ii—DMF, THF, −78 °C to rt, 70%; (b) 4,4′-dimethoxybenzhydryl chloride, Et_3N, THF, 50 °C, 80%; (c) $NaBH_4$, MeOH/THF (2:1), 0 °C to rt, 65%; (d) DPPA, DBU, THF, 0 °C to rt, 95%; (e) i—PPh_3, THF, 40 °C; ii—H_2O, 40 °C, 96%; (f) *m*-tolyl isocyanate, CH_2Cl_2, rt, 62%; (g) HCl, EtOH/EtOAc (1:1), rt, 83%.

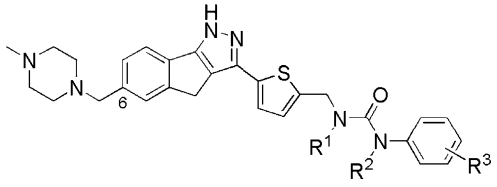
Table 1. KDR inhibitory activity of a selected set of 1,4-dihydroindeno[1,2-*c*]pyrazoles with various substituents in 3-position



Compound	R^1	R^2	R^3	KDR IC_{50}[a] (nM)
10	H			61
11	H			180
3		H		48
12		H		102
13[b]	H			12285
14[b]	H			2777
15[b]	H			212
16[b]	H			22795
17[b]	H			>50000
18[b]	H			24158
19[b]	H			42
20[b]	H			>50000
21[b]	H			>50000
22[b]	H			459

[a] Values are means of two experiments.
[b] Ref. 14.

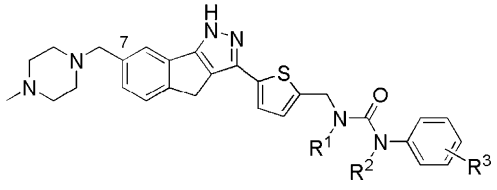
Table 2. Inhibitory activities of 1,4-dihydroindeno[1,2-*c*]pyrazoles with the basic side chain in 6-position and a selected set of substituents in the urea moiety



Compound	R^1	R^2	R^3	IC_{50}[a] (nM)		
				KDR	FLT1	cKit
23	H	H	2-CH_3	444	24996	1387
10	H	H	3-CH_3	61	2897	86
24	H	H	4-CH_3	308	11483	24
25	H	H	2-CF_3	2882	>50000	23829
26	H	H	3-CF_3	34	863	17
27	H	H	4-CF_3	281	4201	13
28	H	H	2-Cl	199	>50000	921
29	H	H	3,5-di-CH_3	63	1668	27
30	H	H	3,5-di-Cl	79	2275	38
31	CH_3	H	3-CH_3	159	4718	14735
32	H	CH_3	3-CH_3	4807	14581	139
33	CH_3	CH_3	3-CH_3	7458	27894	26845
34	CH_3	H	2-CH_3	122	4953	5710
35	CH_3	H	2-Cl	180	3842	>50000
36	CH_3	H	3-F, 5-CF_3	20	33	25
37	CH_3	H	3-OCF_3	27	19	56
38	Et	H	3-CH_3	45	131	2190
39	*n*-Pr	H	3-CH_3	35	59	75
40	*i*-Pr	H	3-CH_3	123	2824	9746
41	*c*-Pr	H	3-CH_3	21	38	136
42	*i*-Bu	H	3-CH_3	164	1590	17239
43	*i*-Amyl	H	3-CH_3	262	3615	4843
44	CH_3O	H	3-CH_3	259	1351	237
45	$CH_3OCH_2CH_2$	H	3-CH_3	171	2263	>50000

[a] Values are means of two experiments.

Table 3. Inhibitory activities of 1,4-dihydroindeno[1,2-*c*]pyrazoles with the basic side chain in 7-position and a selected set of substituents in the urea moiety



Compound	R^1	R^2	R^3	IC_{50}[a] (nM)		
				KDR	FLT1	cKit
46	H	H	H	184	3785	101
47	H	H	2-CH_3	159	2056	674
3	H	H	3-CH_3	48	592	69
48	H	H	4-CH_3	104	613	27
49	CH_3	H	3-CH_3	51	44	96
50	CH_3	H	3-CF_3	11	14	18
51	CH_3	H	3-Cl	12	20	38
52	CH_3	H	4-F	16	83	59
53	CH_3	H	4-OCF_3	17	25	19
54	CH_3	H	3,4-di-Cl	18	36	28
55	CH_3	H	4-Br	19	38	17
56	CH_3	H	4-Cl	21	64	36

[a] Values are means of two experiments.

favored over attachment to the 6-position (**10** and **11**). All efforts to identify a functionality, which could be superior to the urea moiety, resulted in compounds with reduced KDR potency. For instance, the acid amide **13** and the sulfonamide **16**, as well as the urea mimetics **20** and **21**, showed significantly diminished activities. The acid amides **14** and **15**, the sulfonamide **17**, and the carbamates **18** and **19** represent bioisosteric replacements for the urea functionality in the parent compounds **10** and **11**. While those modifications generally were detrimental, it should be noted that replacement of the external NH group of the urea (**14** and **18**) resulted in a larger drop in potency than replacement of the internal NH group (**15** and **19**). The activity of the carbamate **19** is particularly noteworthy because it is equipotent to its parent **3**; however, in the KDR whole cell assay[9] it only showed an IC_{50} of 2 μM and therefore was not pursued further. In comparison to **10**, an 8-fold loss in activity was observed for **22**, which contained a cyclized version of the urea.

An investigation of the substitution pattern in the urea moiety was initially conducted on 1,4-dihydroindeno[1,2-*c*]pyrazoles with the basic side chain in 6-position. The obtained results are summarized in Table 2. For comparison, the table also lists the activities for inhibition of FLT1,[9] as a closely related VEGFR family member, and cKit,[9] as a representative of the PDGFR subfamily, in addition to the KDR inhibitory potencies. As already predicted by our computer model, compounds **10** and **23**–**27** demonstrate that in mono-substituted phenyl ureas a small substituent in 3-position is preferred for KDR inhibition. This SAR carries over for FLT1, but for cKit the 4-substitution becomes more important. Substituents in the 2-position were the least favored, but gave rise to the most selective compounds, as demonstrated by **28** (>250-fold selectivity for KDR inhibition over inhibition of FLT1), **34** (about 45-fold selectivity for KDR over both, FLT1 and cKit), and **35** (>278-fold selectivity for KDR over cKit). In the case of disubstituted phenyl ureas, the 3- and 5-positions turned out to be optimal (**29** and **30**). Replacing the phenyl groups with aliphatic residues and aliphatic or aromatic heterocycles led to a complete loss of activity. Methylation of the internal urea nitrogen diminished the KDR potency only slightly (**31**: 2.6-fold), while alkylation of the external NH-group had a more detrimental effect (**32**: 79-fold and **33**: 122-fold loss). This corresponds with our earlier observation on bioisosteric replacements and confirms our computer model predictions, since the larger decrease in potency is observed when the more optimal urea hydrogen bond is blocked. The situation is reversed for the inhibition of cKit, where the internal hydrogen bond appears to play the more important role. The KDR whole cell IC_{50} of **31** and its parent compound **10** were found to be identical (146 nM), which prompted us to further investigate N-alkylated ureas. Re-optimization of the substitution pattern on the phenyl ring identified the ureas **36** and **37**, which for the first time displayed comparable potencies for all three kinases, but lost some potency in the KDR whole cell assay (IC_{50} = 233 and 218 nM, respectively). The modification of the *N*-alkyl substituent (**38**–**45**) revealed the *n*- and *cyclo*-propyl groups as the most promising residues, however, both variations again led to a slight loss in KDR whole cell activity (IC_{50} = 198 nM for **39** and 171 nM for **41**).

Turning our attention to 1,4-dihydroindeno[1,2-*c*]pyrazoles with the basic side chain in 7-position (Table 3), we found a few differences in reflection to previously established SAR trends. For compounds with a free urea moiety, the 3-substitution on the phenyl ring, again, is favored for KDR enzymatic activity, with the *m*-tolyl urea **3** being approximately 4-fold more potent than its unsubstituted parent compound **46**. Methylation of the internal NH-group of the urea now basically has no effect on the KDR inhibitory potency (**49** vs **3**), compared to a slight loss earlier. Surprisingly, this methylation has no effect on the cKit activity although it should prevent the formation of the more important hydrogen bond (**49** vs **31**). We speculate that the basic side chain in 7-position slightly re-orients the inhibitor in the cKit active site, such that the external hydrogen bond plays the more dominant role. Re-optimization of the phenyl substitution pattern then demonstrated that 4-substituents now become more important (**52**, **53**, **55**, and **56**), which then also shifted the preference for disubstitutions (**54**). Compounds **49** to **56** illustrate that at this point, our established SAR has enabled us to fairly routinely produce potent multitargeted RTK inhibitors. The trifluoromethyl analogue **50** stands out in this set, because in addition to its potency against KDR, FLT1, and cKit, it is the most active compound against Tie2 (IC_{50} = 259 nM), a more distant member of the RTK family, which also is a target for anti-angiogenic drug discovery.[15] The KDR whole cell IC_{50} of **50** was determined to be 195 nM.

In summary, 1,4-dihydroindeno[1,2-*c*]pyrazoles containing various substituents off a thiophene ring in 3-position were investigated to access the hydrophobic specificity pocket in KDR kinase. Phenylurea-type side chains were identified to be optimal. A homology model predicted that binding of those compounds into the ATP binding site of the inactive form of KDR would allow the urea moiety to interact with a specific recognition motif in the enzyme. Further optimization of the urea-type side chain and the position of a basic substituent on the core led to a series of multitargeted RTK inhibitors, which displayed potent inhibition against additional VEGFR family members (FLT1 and Tie2), as well as a member of the structurally related PDGFR family (cKit). Future work will focus on the evaluation of these compounds in various *in vivo* efficacy models.

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