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Synthesis and biological evaluation of 5-substituted 1,4-dihydroindeno[1,2-*c*]pyrazoles as multitargeted receptor tyrosine kinase inhibitors

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Abstract—We report the synthesis and biological evaluation of 5-substituted 1,4-dihydroindeno[1,2-*c*]pyrazoles as multitargeted kinase inhibitors. Initial efforts focused on the development of selective KDR inhibitors, while later strategies involved the improvement of potency toward multiple kinase targets. Thus, several compounds were identified as potent KDR, Flt1, Flt3, and c-Kit inhibitors.

Receptor tyrosine kinases (RTKs) are important regulators of cell survival, migration, and proliferation as well as angiogenesis and their over-expression or deregulation leads to uncontrollable cellular signaling and cancer. Platelet-derived growth factor receptors PDGFR (PDGFR-α, PDGFR-β, Flt3, CSF1R, and cKit) and vascular endothelial growth factor receptors VEGFR (KDR, Flt1, and Flt4) are RTK subfamilies that play key roles in tumor angiogenesis and therefore have been targeted for the development of anti-cancer therapies.

Initial strategies involved single target therapies and resulted in the FDA approval of Avastin (a humanized monoclonal antibody targeting VEGF, the growth factor that stimulates VEGFRs) for the treatment of metastatic colorectal cancer.[1] Gleevec (a Bcr-Abl inhibitor approved for the treatment of chronic myelogenous leukemia) is also a PDGFR and c-Kit inhibitor, and has been approved for the treatment of gastrointestinal stromal tumors (GIST).[2] Subsequent approaches involved the use of multitargeted inhibitors such as Sutent[3] (an inhibitor of KDR, Flt1, PDGFR-α, PDGFR-β, Flt3, CSF1R, cKit, and RET), which was approved by the FDA for the treatment of GIST and advanced renal-cell carcinoma (RCC), and Nexavar[4] (an inhibitor of KDR, Flt1, PDGFR-β, c-Kit, RET, and Raf isoforms) for

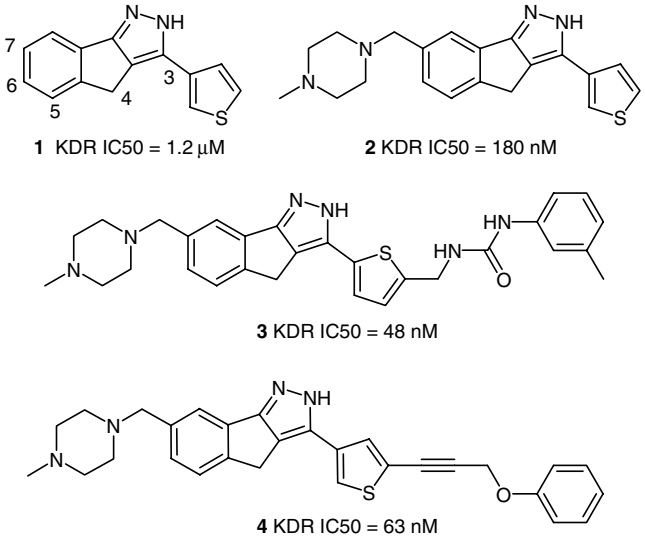

Figure 1. Structures and KDR inhibitory activity of various 1,4-dihydroindeno[1,2-*c*]pyrazoles.

Keywords: Tricyclic pyrazoles; 1,4-dihydroindeno[1,2-*c*]pyrazoles; Multitargeted kinase inhibitors; KDR inhibitors; Flt1 inhibitors; Flt3 inhibitors; c-Kit inhibitors; PDGFR inhibitors; VEGFR inhibitors.
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RCC. Whether the multitargeted inhibitors have the advantage of blocking more than one of the signaling pathways essential to tumor survival and growth,[5] the exact combination of activities against the various kinases, and which kinase target combination is required for efficacious therapeutics is still a fruitful area of investigation.

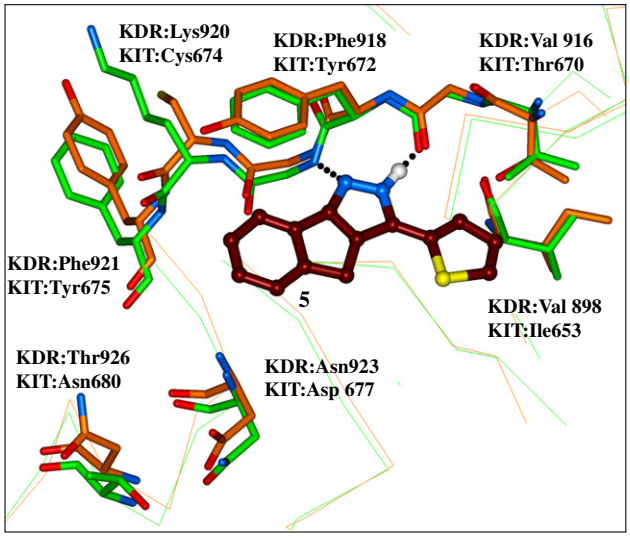
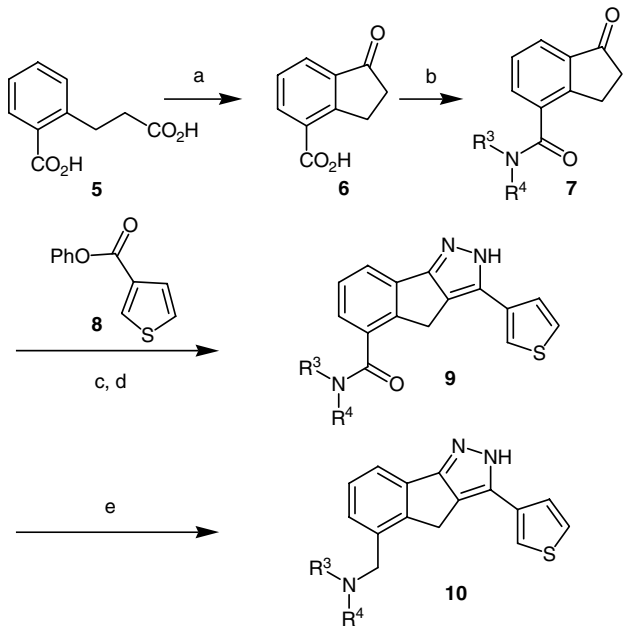


Figure 2. Overlay of KDR (green) and KIT (orange) protein crystal structures (PDB entries 1VR2 and 1T45, respectively) with a model of Compound 1 bound in ATP-site. Hydrogen bonds between the inhibitor and the hinge region are shown with black dotted lines. Select positions of residue difference between KIT and KDR kinases are indicated. The residue difference at KDR 923/KIT 677 was targeted with the compounds of this study utilizing substituents at position 5.

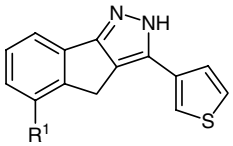


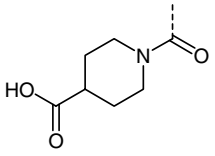
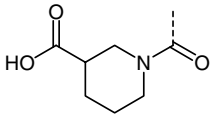
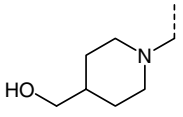
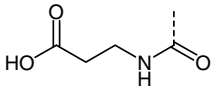
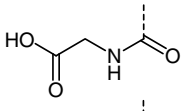
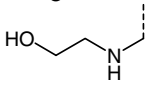
Scheme 1. Reagents and conditions: (a) $AlCl_3$, NaCl, 160 °C, 2 h, 44%; (b) R^3R^4NH, PS-DCC, HOBT, DMF; (c) 1—NaH, benzene, reflux, overnight; 2—$NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 1 h, 10–37% in three steps from compound **6**; (d) 1:1 TFA/CH_2Cl_2 for Boc protected acids or 2:2:1 1 M LiOH/MeOH/THF for esters, 11–55%; (e) LAH, THF, reflux, 33–50%.

We have previously described the discovery of 1,4-dihydroindeno[1,1-*c*]pyrazoles as a novel class of VEGFR and PDGFR inhibitors. Starting from the high throughput screening hit **1** (Fig. 1), compound **2** was identified as a lead compound with acceptable binding and whole cell activity.[6] Attachment of a urea side chain to access the hydrophobic specificity pocket in KDR led to a series of potent multitargeted (KDR, Flt1, cKit, Tie2) inhibitors such as **3**.[7] Introduction of an acetylenic side chain led to the discovery of **4**, which was efficacious in animal tumor models.[8]

In this report, we describe our efforts to explore the 5 position of compound **1**. Since in the early stages of the project one of the goals was to develop KDR selective inhibitors, we have attempted to improve the KDR over c-Kit selectivity by structure based drug design. A careful examination of the ATP binding sites of KDR and c-Kit revealed a prominent difference in the asparagine 923 residue in KDR versus aspartic acid 677 in c-Kit (Fig. 2). We reasoned that placement of acidic functionalities in close vicinity to these residues would

Table 1. Kinase inhibitory activity of 5-amide analogs

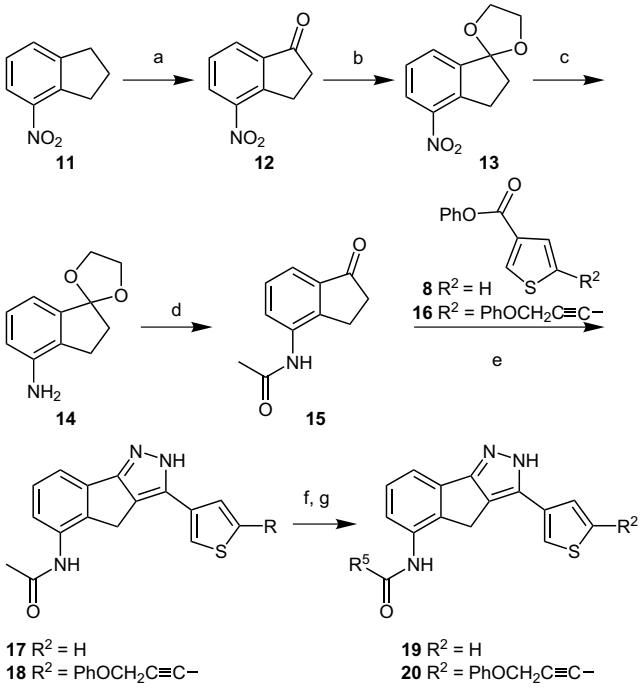


Compound	R^1	KDR IC_{50}[a] (μM)	Flt1 IC_{50}[a] (μM)	c-Kit IC_{50}[a] (μM)
9a		7.04	3.47	>50
9b		4.84	3.08	>50
10a		27.89	17.57	7.18
9c		0.58	0.37	5.61
9d		0.41	0.24	5.15
10d		0.44	0.27	1.67

[a] IC_{50} values are based on seven-point curves, performed in duplicate.

result in KDR selective molecules. Modeling of compound **1** in the active sites of KDR and c-Kit suggested that 5-substituted analogs were best in accessing these residues. Thus, we embarked in the development of new synthetic routes to access such molecules as shown in Scheme 1. Initial formation of indanone **6** was established with a Friedel-Crafts reaction of the neat reagents. Since direct formation of the pyrazole ring from compound **6** failed, we resorted in forming amides **7** first, using select amines containing a Boc or ester protected acid. Amides **7** readily reacted with phenyl thiophene-3-carboxylate **8** to provide, upon deprotection, the free carboxylic acid compounds **9**. Further LAH reduction yielded compounds **10**.

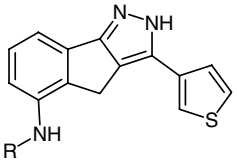
The compounds were tested against KDR, Flt1, and c-Kit in an HTRF assay format at 1 mM ATP.[9] We have selected a variety of cyclic and acyclic carboxylic acids in order to probe the KDR/c-Kit site. Table 1 summarizes some of our findings. The compounds were equipotent in Flt1 but were typically 10-fold less potent in c-Kit with the exception of compound **10a**. The presence of the carboxylic acid moiety seams to somewhat affect the selectivity, as shown in examples **9d** and its reduced version **10d**. However, the overall activity of these inhibitors was moderate at best and their challenging preparation prompted us to explore the 5-substituted reverse amides. These compounds were prepared from nitro indane **11** following Scheme 2. Oxidation of **11** yielded indanone **12**, which was protected, the nitro group was reduced, and the resulting amino group was protected with an acetyl group. Formation of pyrazoles with plain phenyl thiophene-3-carboxylate **8**, deprotection of the amine group, and amidation reactions provided the final products **19**.

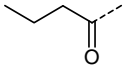
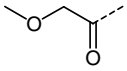
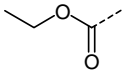
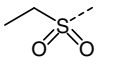
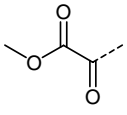
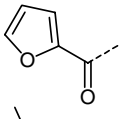
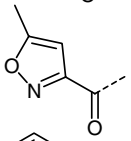
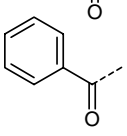
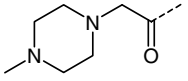


Scheme 2. Reagents and conditions: (a) Cr_2O_3, AcOH, rt, 5 h, 51%; (b) ethylene Glycol, cat *p*-TsOH, Benzene, Dean Stark, overnight, 96%; (c) H_2 60 psi, Raney Ni, 1:1 MeOH/EtOAc, 20 min, 96%; (d) 1—AcCl, pyridine, 30 min; 2—*p*-TsOH, acetone, H_2O, reflux, 1 h, 76% in two steps; (e) 1—NaH, Benzene, reflux, overnight; 2—$NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 1 h, 23% in two steps; (f) concd HCl, MeOH, reflux, 3 h, 82%; (g) R^5COCl, pyridine, 10 min, 2–58%.

Overall, reverse amides **19** were more potent and retained the same selectivity profile previously observed with compounds **9** (Table 2). Substituents carrying hydrogen bond acceptor groups such as the oxygen in **19b** were more potent than simple aliphatic chains such as in **19a**. However, the positioning of the oxygen group was crucial as observed in compounds **19b** and **19c**, and also in heterocycles **19f** and **19g**. Sulfonamides (**19d**) were also well tolerated as well as aromatic groups (**19h**), although in this case we observe an increase in c-Kit binding. The improved potency and selectivity of analogs bearing a hydrogen

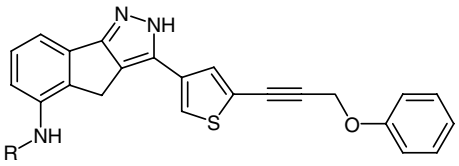
Table 2. Kinase inhibitory activity of 5-reverse amide analogs

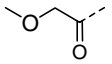
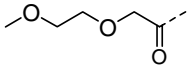
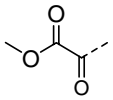
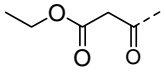
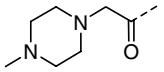


Compound	R	KDR IC_{50}[a] (μM)	Flt1 IC_{50}[a] (μM)	c-Kit IC_{50}[a] (μM)
19a		0.56	0.29	9.54
19b		0.07	0.03	1.58
19c		0.65	0.45	2.55
19d		0.31	0.29	4.39
19e		0.05	0.08	1.12
19f		0.11	0.09	1.10
19g		0.06	0.07	0.9
19h		0.10	0.05	0.18
19i		0.92	0.71	4.33

[a] IC_{50} values are based on seven-point curves, performed in duplicate.

Table 3. Kinase inhibitory activity of position 5-analogs containing acetylenic thiophene substituents



Compound	R	KDR IC_{50}[a] (μM)	CSF1R IC_{50}[a] (μM)	Flt1 IC_{50}[a] (μM)	Flt3 IC_{50}[a] (μM)	cKit IC_{50}[a] (μM)	Tie2 IC_{50}[a] (μM)	Lck IC_{50}[a] (μM)	Fyn IC_{50}[a] (μM)	Hck IC_{50}[a] (μM)	Lyn IC_{50}[a] (μM)	Src IC_{50}[a] (μM)
20a		0.053	0.113	0.016	0.153	0.175	40.595	16.038	13.681	30.27	20.806	43.311
20b		0.270	1.271	0.136	0.624	1.003	13.025	>50	>50	>50	>50	>50
20c		0.036	0.190	0.020	0.065	0.127	24.747	21.382	22.986	>50	30.587	>50
20d		0.263	2.975	0.153	1.108	0.619	>50	26.529	>50	>50	>50	>50
20e		0.010	0.045	0.006	0.022	0.049	4.877	30.963	>50	>50	>50	>50

[a] IC_{50} values are based on seven-point curves, performed in duplicate.

bond accepting group may be rationalized by the aminoacid difference cited above, that is, KDR has a nearby hydrogen bond donor (Asn 923) whereas c-Kit (Asp 677) cannot make an analogous hydrogen bond.

Encouraged by these results we selected several 5-reverse amide compounds to build more elaborate analogs incorporating the acetylenic moiety found in compound **4**. It was expected based on modeling analysis that the two substituents would have additive effect on activity as the 5-substituents were extending into the ribose pocket and the acetylenic moiety probed the hydrophobic pocket. Compounds **20** were synthesized according to Scheme 2 using the acetylenic thiophene benzoyl ester **16,**[10] instead of **8**. In direct comparisons of unsubstituted and acetylenic analogs such as **19b** versus **20a**, **19e** versus **20c**, and **19i** versus **20e**, the acetylenic analogs were more potent inhibitors across the same panel of kinases. However, the gains in potency resulting from interactions with the hydrophobic site also led to erosion of the c-Kit selectivity. One explanation for the lack of selectivity is possible shifting of the 5-substituent position in analogs bearing the acetylenic extension (Table 3).

All compounds **20** were also profiled against a larger panel of tyrosine kinases and proven to be selective for the PDGFR/VEGFR subfamilies. In addition, compounds **20a** and **20e** were also tested in KDR cellular assays and exhibited significant potency (cell KDR IC_{50} = 19 and 62 nM, respectively).[9]

In conclusion, we have discovered a series of 5-substituted 1,4-dihydroindeno[1,2-*c*]pyrazoles as potent multitargeted kinase inhibitors.

Acknowledgments

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9. For details on experimental assay protocols, see Ref. 8.
10. For the synthesis of compound **16**, see Ref. 8.