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Scaffold oriented synthesis. Part 1: Design, preparation, and biological evaluation of thienopyrazoles as kinase inhibitors

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Abstract—We report the synthesis of kinase targeted libraries based on the thienopyrazole scaffold. Several thienopyrazole analogs have been identified as submicromolar inhibitors of KDR.

As part of our group's ongoing effort to enhance the Abbott compound collection with novel and/or rare chemotypes, we have initiated an effort to design new kinase inhibitors for current and future kinase programs. Kinases have emerged in recent years as targets in a variety of therapeutic areas such as cancer,[1] diabetes,[2] inflammation,[3] cardiovascular,[4] and neurodegenerative[5] diseases, and several kinase inhibitors have been approved by the FDA for use in human.[6]

Much work has been directed toward the design and synthesis of kinase inhibitors,[7] including computational[8] and fragment-based[9] approaches. De novo design of new kinase inhibitors is greatly facilitated by the architecture of kinases having a mostly conserved catalytic site that binds ATP. ATP forms key hydrogen bond interactions in the hinge region of the catalytic site of protein kinases. Therefore, small and relatively flat heterocyclic molecules containing hinge-binding elements are expected to form weak interactions with the enzymes that can be amplified by functionalization to access additional pockets in the active site.

In the above context, we have decided to investigate the underexplored class of thienopyrazole structures as kinase inhibitors.[10] We expected to obtain the key donor/acceptor hydrogen bond hinge interactions from the amino groups of the pyrazole ring.[11,12] Subsequently, we were planning to explore the surrounding space by functionalizing the scaffold in different regions. A survey of the literature revealed very few references for their preparation, all of which were unsuitable for obtaining versatile scaffolds. Therefore, we developed a new route for the synthesis of thienopyrazoles and chemistries that provided access to all sites of the molecule.

We have prepared three small libraries around thienopyrazole scaffolds as well as various smaller groups of analogs to probe the various sites. Emphasis was placed on preparing a variety of structurally diverse analogs rather than closely following SAR trends. Our ultimate goal was to create a lead generation set that would provide good starting points for medicinal chemistry lead optimization exercises. The libraries were designed to have lead-like physicochemical properties.[13] In all, we have prepared a collection of 95 compounds with an average MW of 292, an average clog P of 2.5, and an average polar surface area of 71.

Scheme 1 illustrates the initial hurdles encountered with the synthesis of the basic thienopyrazole core. Reaction of the commercially available methyl dihydro-pyrazolone **1** with $POCl_3$ provided the intermediate 4-formyl-5-chloro Vilsmeier product, which was protected with *p*-methoxybenzyl chloride to yield two different regioisomers **2a** and **3a** in a two to one ratio, respectively. The identity of each regioisomer was confirmed by NMR experiments. An NOE was observed between the benzylic methylene protons and the meth-

Keywords: Thienopyrazoles; Kinase inhibitors; KDR inhibitors; Targeted libraries; De novo design.
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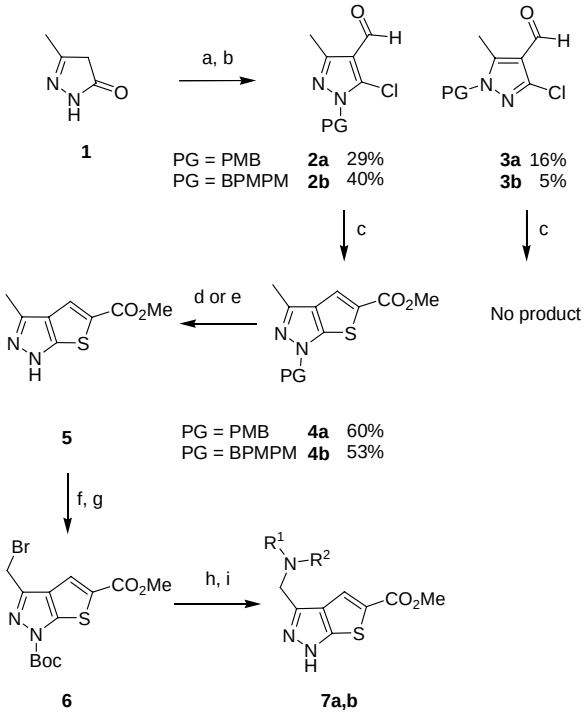
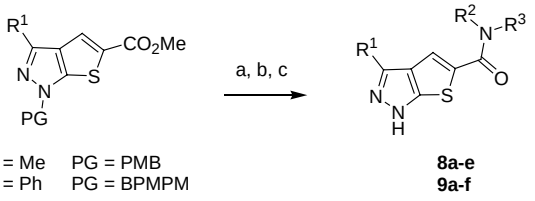
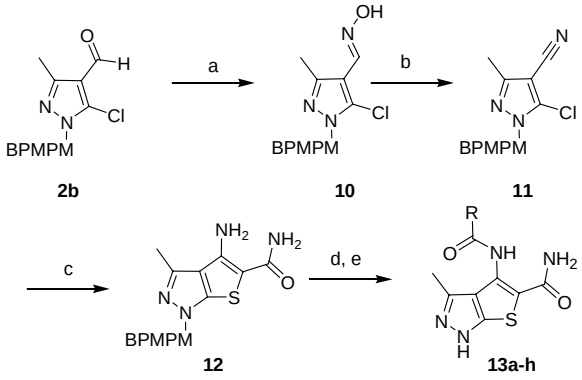


Scheme 1. Reagents and conditions: (a) $POCl_3$, DMF, 0 °C then reflux 2 h, 32%; (b) PG = PMB, PMBCl, K_2CO_3, DMF, 120 °C, 1 h; PG = BPMPM, BPMPMCl,[14] Et_3N, THF, rt, 1 h; (c) Mercapto-acetic acid methyl ester, Na_2CO_3, MeOH, reflux; (d) PG = PMB, 1:1 TFA/CH_2Cl_2, 120 °C, 20 min, microwave (CEM, 300 W, ramp time 1 min), 59%; (e) PG = BPMPM, 4 M HCl in dioxane, rt, 1 h, 60%; (f) Boc_2O, Et_3N, DMAP, MeCN, 3 h, 66%; (g) NBS, $(PhCO_2)_2$, CCl_4, reflux, 6 h, 24%; (h) R^1R^2NH, EtOH; (i) 1:1 TFA/CH_2Cl_2, 24–84% in two steps.



Scheme 2. Reagents and conditions: (a) 1:1 1 M NaOH/MeOH, 50 °C, R^1 = Me, 82%, R^1 = Ph, 44%; (b) R^1 = Me, 1:1 TFA/CH_2Cl_2, 120 °C, 20 min, microwave (CEM, 300 W, ramp time 1 min), 59%, R^1 = Ph, 4 M HCl in dioxane, 99%; (c) PS-carbodiimide, HOBt, DIEA, R^2R^3NH, DMA, microwave (Personal Chemistry, 300 W), 100 °C, 6 min, 2–12%.



Scheme 3. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, satd $NaHCO_3$, EtOH/H_2O, 7 h, 83%; (b) Ac_2O, reflux, 1 h, 73%; (c) 2-mercapto-acetamide, K_2CO_3, EtOH, reflux, 17 h, 77%; (d) RCOCl, Py, rt, 24 h; (e) 4 M HCl, dioxane, 6 h, 4–20% in two steps.

yl group in **3a** but not in **2a**. Interestingly, only regioisomer **2a** participated in the subsequent reaction with mercapto-acetic acid methyl ester to provide the fused thienopyrazole product, while **3a** remained completely unreactive.[15] Deprotection of the *p*-methoxybenzyl group was easily achieved by heating **4a** in the microwave with a one- to one-mixture of TFA/dichloromethane. Alternatively, the bulkier and more acid labile bis(4-methoxyphenyl)methyl group provided a better yield of the desired regioisomer **2b** over **3b** and can be readily removed at room temperature with 4 M HCl in dioxane. Thus, bis(4-methoxyphenyl)methyl became the protecting group of choice. Protection of **5** (Scheme 1) with Boc, subsequent NBS bromination of the methyl group, and reaction of the bromo intermediate **6** with amines provided final products **7** in good yields.

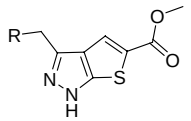
Similar sequences were followed starting from phenyl dihydro-pyrazolone[16] to provide the 3-phenyl thienopyrazole of Scheme 2. Both 3-methyl and 3-phenyl thienopyrazoles in Scheme 2 were first saponified with NaOH in MeOH to provide the carboxylic acids and then the pyrazole ring was deprotected. We have observed that this sequence was cleaner and higher yielding than first deprotecting the pyrazole and then saponifying. The final unprotected thienopyrazole acids were used for the synthesis of small amide libraries **8** and **9**.

An interesting diversion from the above synthetic protocol was the formation of 4-amino substituted analogs **13** (Scheme 3). Aldehyde **2b** was transformed in two steps to the corresponding cyano product **11**, which underwent reaction with 2-mercapto-acetamide to provide 4-amino thienopyrazole **12**. The 4-amino group could be further reacted with acyl chlorides in pyridine to yield the acylated products **13**.

All compounds were tested against a panel of 5 kinases of interest.[17] Although exploration of the 3-position (Table 1) was limited to a few analogs, low micromolar activity was already observed against some of the kinases. Simple analog **5** was active against KDR and CK2, while the benzylamine analog **7a** exhibited activity against Plk1, Pak4, CK2, and Akt. Piperidinyl analog **7b** was inactive indicating an initial preference of the site for benzylamine analogs. It should be noted that molecules such as **5**, which exhibit high binding efficiency (BEI = 24), i.e., strong binding in relation to their molecular weight,[18] represent excellent starting points for followup libraries to improve upon activity and selectivity.

Table 2 summarizes the activity of amides **8** and **9** against an extended panel of 9 kinases.[19] In general, amides **8** showed similar trends in activity as the ester **5** inhibiting

Table 1. Kinase inhibitory activity of position 3 analogs[a]



Compd	R	IC_{50} (μM)				
		KDR	Plk1	Pak4	CK2	Akt1
5	H	23[b]	>100	>100	38	>100
7a	NHPh	>100	21	37	5[b]	16
7b	Piperidinyl	>100	>100	>100	>100	>100

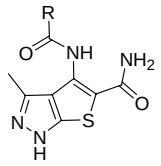
[a] IC_{50} values are based on an 11 point curves at 10 μM ATP; >100 indicates less than 50% inhibition at that concentration or an IC_{50} > 100 μM.
[b] Average of two values.

KDR and CK2 with low micromolar potency. However, replacement of the methyl group with a phenyl separated the KDR and CK2 activity, and resulted in submicromolar hits against KDR. Depending on the amide substitutions some 3-phenyl analogs have also showed activity against MK2 (**9c** and **9e**) and CDK2 (**9b**).

4-Amino analogs **13** (Table 3) also showed a preference for KDR and CK2 kinases. However, depending on the substitution on the 4-amino group, leads for Plk1 **13b** and **13e**, and Akt1 **13d** and **13h** were obtained. Urea analog **13h**[20] is an interesting case in that it inhibits most of the kinases with low micromolar potency.

In conclusion, we have prepared a diverse set of thienopyrazole-based lead-like molecules that were evaluated in a panel of kinase assays. This lead generation exercise was quite successful, as we have identified numerous leads with low micromolar potency against a variety of kinase targets. In addition, a subset of 3-phenyl substituted thienopyrazoles exhibited submicromolar potency against KDR.

Table 3. Kinase inhibitory activity of position 4-analogs[a]



Compd	R	IC_{50} (μM)				
		KDR	Plk1	Pak4	CK2	Akt1
13a	Me	19[b]	>20	>20	9[b]	>20
13b	CO_2Me	17	8	>100	3[c]	>100
13c	CH_2Oph	11	>100	>100	6	>100
13d	2-Thienyl	6	>100	>100	>100	17
13e	2-ClPh	8	23	>100	9[c]	>100
13f	3-ClPh	>100	>100	>100	>100	>100
13g	4-ClPh	>100	>100	>100	>100	>100
13h	NHPh	20	>20	13[b]	11	9[b]

[a] IC_{50} values are based on 11 point curves at 10 μM ATP; >100 indicates less than 50% inhibition at that concentration or an IC_{50} > 100 μM.
[b] Average of two IC_{50} values.
[c] Average of three IC_{50} values.

Acknowledgments

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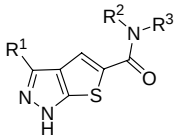
Table 2. Kinase inhibitory activity of analogs **8** and **9**[a]



Compound	R^1	R^2	R^3	IC_{50} (μM)								
				KDR	Plk1	Pak4	CK2	Akt1	CDK2[d,e]	p-38[d,e]	MK2[e]	COT[d,e]
8a	Me	H	H	17[b]	>20	>20	4[c]	>20	ND	ND	>20	>20
8b	Me	H	*n*-Bu	16	>20	>20	21	>20	ND	ND	>20	>20
8c	Me	Me	*n*-Bu	>20	>20	>20	24[b]	>20	ND	ND	>20	>20
8d	Me	H	Cyclohexyl	>20	>20	>20	>20	>20	ND	ND	>20	>20
8e	Me	H	Bn	12	16	>20	19[b]	>20	ND	ND	>20	>20
9a	Ph	Me	*n*-Bu	0.35	>20	>20	5	>20	>20	>20	>20	>20
9b	Ph	H	Cyclohexyl	0.49	12[b]	>20	>20	>20	11	>20	>20	>20
9c	Ph	H	Bn	0.5	ND	>20	2	15	ND	ND	17	>20
9d	Ph	H	CH_2Bn	2	17	>20	>20	>20	>20	>20	>20	>20
9e	Ph	H	*p*-MeOBn	0.51	>20	>20	>20	>20	>20	>20	16	>20
9f	Ph	-$CH_2CH_2N(Me)CH_2CH_2$-		4	>20	>20	>20	>20	>20	>20	>20	>20

[a] IC_{50} values are based on 11 point curves at 10 μM ATP; >20 indicates less than 50% inhibition at that concentration or an IC_{50} > 20 μM; ND, not determined.
[b] Average of two IC_{50} values.
[c] Average of four IC_{50} values.
[d] 100 μM ATP.
[e] IC_{50} values are based on seven point curves.

Jan Waters for the NOE experiments, and the Abbott High Throughput Purification and Structural Chemistry groups for their support with purification and analytical data, respectively.

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14. Prepared by refluxing for 3 h 4,4′-dimethoxybenzhydrol in $SOCl_2$ and then evaporating the reaction mixture to dryness.
15. Also, no reaction was observed with the unprotected 4-formyl-5-chloro Vilsmeier intermediate derived from **1**.
16. Prepared in one step by heating 3-oxo-3-phenyl-propionic acid ethyl ester, hydrazine hydrate, and acetic acid in ethanol at 90 °C for 2 h.
17. In vitro kinase assays. Recombinant CK2 (Calbiochem, San Diego, California) was commercially obtained. His-tagged AKT1[S378A, S381A, T450D, S473D] (139–480), His-tagged KDR (789–1354), His-tagged PAK4 (290–581), and GST-tagged PLK1 (1–331) were expressed using the FastBac baculovirus expression system (GIBCO BRL, Gaithersburg, MD) and purified using either nickel (His-tag) or glutathione (GST) affinity chromatography. Peptide substrates had the general structure biotin-Ahx-peptide with sequences: AKT, EELSPFRGRSRSAPPNLWA AQR; CK2, RRADDSDDDDD; KDR, AEEEYFFLFA-amide; PAK4, KEVPRRKSLVGTPYWMAPE; PLK1, AKMETTFYDDALNASFLPSEKKK-Amide. Inhibition of kinase activity was assessed using a radioactive Flash-Plate-based assay platform as previously described in Luo, Y.; Smith, R. A.; Guan, R.; Liu, X.; Klinghofer, V.; Shen, J.; Hutchins, C.; Richardson, P.; Holzman, T.; Rosenberg, S. H.; Giranda, V. L. *Biochemistry* **2004**, *43*, 1254.
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19. Additional in vitro kinase assays. The kinase assays were performed using the homogeneous time-resolved fluorescence (HTRF) method (G. Mathis, *Clin. Chem.* **1993**, *39*, 1953–1959). COT (made in-house) assay contained 13.7 nM COT, 0.5 μM biotin-MEK-peptide, 0.1 mM ATP, and compound in a buffer containing 50 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM EGTA, 2 mM DTT, 0.01% Brij 35, 5 mM β-phosphoglycerol. MK2 (made in house) assay contained 1.8 nM MK2, 1 μM biotin-cdc25-peptide, 10 μM ATP and compound in the MK2 Buffer (20 mM Mops, pH 7, 10 mM $MgCl_2$, 5 mM EGTA, 5 mM β-phosphoglycerol, 1 mM Na_3VO_4, 0.01% Triton X-100, and 1 mM DTT). p38α and CDK2 (UBI) assays contained either 7.8 nM p38α or 2.7 nM CDK2/cyclin A, and 0.5 μM biotin-MBP-peptide, 0.1 mM ATP, and compound in the MK2 Buffer. All assays were carried out at RT for 60 min and stopped by addition of EDTA. The products were detected by addition of revelation reagents containing Europium labeled phospho-specific antibodies and SAXL. The plates were incubated in dark at 4 °C overnight or RT for 10 min (for MK2) and read in the HTRF reader RUBYstar (BMG).
20. Synthesized from **12** upon reaction with phenyl-isocyanate in dichloromethane and subsequent deprotection with 4 M HCl in dioxane.