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Scaffold oriented synthesis. Part 3: Design, synthesis and biological evaluation of novel 5-substituted indazoles as potent and selective kinase inhibitors employing [2+3] cycloadditions

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ABSTRACT

We report the synthesis and biological evaluation of 5-substituted indazoles and amino indazoles as kinase inhibitors. The compounds were synthesized in a parallel synthesis fashion from readily available starting materials employing [2+3] cycloaddition reactions and were evaluated against a panel of kinase assays. Potent inhibitors were identified for numerous kinases such as Rock2, Gsk3β, Aurora2 and Jak2.

As part of our efforts to develop patentable, high quality lead molecules for kinase programs at Abbott,[1] we reported the discovery of underutilized unique heterocycles as hinge binders with potent inhibitory activity against kinase targets.[2] A second part of our strategy has been the implementation of underutilized but robust chemistries to engage known kinase hinge binding elements (Fig. 1). The rational behind this approach was the ability to rapidly explore the ATP binding site of numerous kinases, utilizing readily available starting materials, without compromising the novelty of the final molecules.

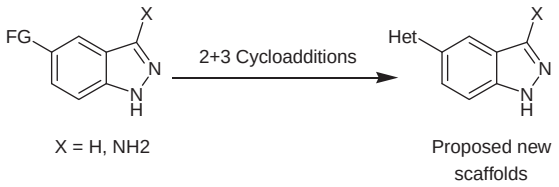
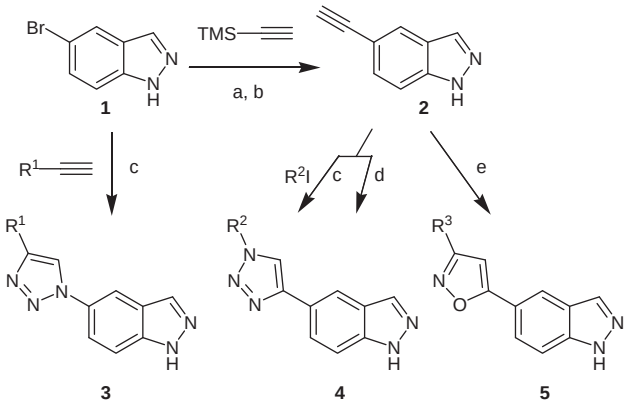


Figure 1. Design of novel molecules based on known kinase hinges.



Scheme 1. Reagents and conditions: (a) Boc_2O, DMAP, CH_2Cl_2, rt, quant; (b) CuI, $(Ph_3)_2Cl_2Pd$, DMF, Et_3N, 95 °C, 72%, then KOH, MeOH, 95%; (c) DMSO, NaN_3, L-proline, Na_2CO_3, sodium ascorbate, $CuSO_4{\cdot}5H_2O$, 65 °C, 6–39%; (d) R^2Cl, NaN_3, $CuSO_4{\cdot}5H_2O$, Cu(0), *t*-BuOH, H_2O, CEM microwave, 100 W, 125 °C, 10 min, 3–26%; (e) R^3CHO, $NH_2OH{\cdot}HCl$, 6 N NaOH, chloramine-T·$3H_2O$, $CuSO_4$, Cu wire, *t*-BuOH, H_2O, 50 °C, 4–5%.

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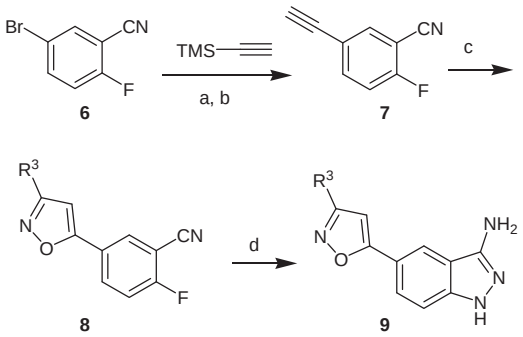


Scheme 2. Reagents and conditions: (a) CuI, $(Ph_3)_2Cl_2Pd$, DMF, Et_3N, 95 °C, 24 h, 93%; (b) TBAF, THF, 55%; (c) R^3CHO, $NH_2OH{\cdot}HCl$, 6 N NaOH, chloramine-T·$3H_2O$, $CuSO_4$, Cu wire, *t*-BuOH, H_2O, 50 °C, 33–69%; (d) NH_2NH_2, EtOH, 70 °C, 24 h, 53–78%.

In this report, we describe our efforts in applying the above strategy to indazoles and aminoindazoles, which are well known kinase hinge binding motifs.[3] We were pleased to discover that application of [2+3] cycloaddition reactions to suitably substituted indazoles rendered the compounds novel.

Starting with 5-bromo indazole **1** (Scheme 1), 5-(4-substituted-1,2,3-triazol-1-yl)-indazoles **3** could be obtained directly by cycloaddition reactions of in situ generated 5-azido-indazole with commercially available acetylenes following literature procedures.[4]

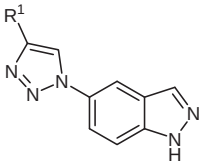
Alternatively, Sonogashira coupling of Boc protected **1** with trimethylsilylacetylene and subsequent base induced desylilation and Boc deprotection provided 5-ethynyl-indazole **2** which upon cycloaddition reactions with azides[4,5] yielded 5-(1-substituted-1,2,3-triazol-4-yl)-indazoles **4**. Intermediate **2** could also be utilized for the synthesis of isoxazoles **5** via the in situ formation of nitrile oxides.[6] Similarly, 5-substituted-3-amino indazoles could be prepared by Sonogashira coupling of trimethylsilylacetylene with commercially available 2-fluoro-5-iodobenzonitrile **6** (Scheme 2). Deprotected intermediate **7** was subjected to cycloaddition reactions, followed by treatment with hydrazine to afford the final products **9**.

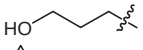
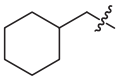
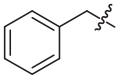
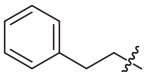
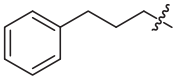
Following these procedures numerous analogs were prepared in a short period of time in a parallel synthesis fashion. The compounds were tested in a panel of kinase assays[7] covering all of the branches of the kinome tree.[8]

We were pleased to find that the majority of the compounds showed inhibitory activity in at least one of the kinase assays. In the case of triazoloindazoles **3** (Table 1) most of the compounds were potent inhibitors of Rock2 but they also inhibited Gsk3β, Aurora2 and Jak2. Depending on the substitution pattern the activity against the various kinases could be modulated. For example, although compounds **3g** and **3h** had similar potencies against Rock2, **3h** was more potent than **3g** against Aurora2 and Jak2. Furthermore, a cyclohexylmethyl substituent in compound **3f** greatly improved the Jak2 activity, while tethered phenyl groups in compounds **3g–3i** improved Gsk3β activity.

In the case of triazoloindazoles **4** (Table 2) we observed a different potency profile. A direct comparison of **3g** to **4b** showed that in contrast to **3g**, **4b** was more potent against Gsk3β and Aurora2 than Rock2 while the 3-amino indazole **4c** lost some of its Aurora2 and Gsk3β potency and gained Rock2 and Jak2 activity. Simple substitutions on the benzyl ring also have a dramatic effect on the potency and selectivity of the analogs. For example, although *meta* substituted benzyl ring derivatives improved potency against Aurora2, Gsk3β and Rock2 the methyl group appears to be the best substituent for Aurora2 activity, the fluoro for Gsk3β and the chloro for Rock2. For the dichloro analogs **4l**, **4m** and **4n** the

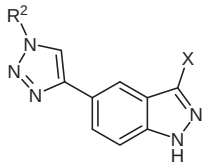
Table 1
Kinase inhibitory activity of 5-(4-substituted-1,2,3-triazol-1-yl)-indazoles **3**[a]

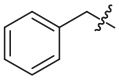
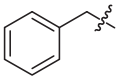
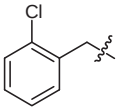
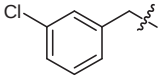
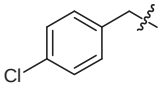
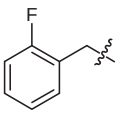
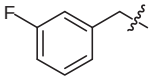
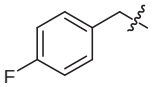
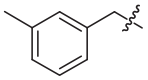
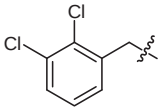
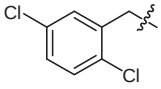
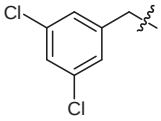


Compound	R^1	Aurora2 K_i (μM)	Egfr K_i (μM)	Gsk3β K_i (μM)	Jak2 K_i (μM)	Kdr K_i (μM)	Pak4 K_i (μM)	Pim1 K_i (μM)	Rock2 K_i (μM)
3a		>4.900	>1.800	2.026	0.756	>8.880	>3.750	8.359	0.108
3b		>4.900	>1.800	>5.450	>1.450	>8.880	>3.750	>8.570	0.635
3c		>4.900	>1.800	5.216	1.453	>8.880	>3.750	>8.570	0.230
3d		>4.900	>1.800	3.466	1.453	>8.880	>3.750	>8.570	0.356
3e		>4.900	>1.800	1.298	0.791	>8.880	>3.750	>8.570	0.044
3f		>4.900	>1.800	0.891	0.281	>8.880	>3.750	>8.570	0.100
3g		1.577	>1.800	0.542	1.282[b]	>8.880	>3.750	>8.570	0.018
3h		0.524	>1.800	0.446	0.898	>8.880	>3.750	>8.570	0.019
3i		1.930	>1.800	0.528	0.644	>8.880	>3.750	>8.570	0.062

[a] K_i values are based on six point curves unless otherwise noted.
[b] K_i value is based on an eleven point curve done in triplicate.

Table 2
Kinase inhibitory activity of 5-(1-substituted-1,2,3-triazol-4-yl)-indazoles **4**[a]



Compound	R^2	X	Aurora2 K_i (μM)	Egfr K_i (μM)	Gsk3β K_i (μM)	Jak2 K_i (μM)	Kdr K_i (μM)	Pak4 K_i (μM)	Pim1 K_i (μM)	Rock2 K_i (μM)
4a	H	H	0.493	ND	0.448	1.453[b]	3.554	69.911	2.219	0.209
4b		H	0.073	>1.800	0.039	0.284[b]	>8.880[c]	>5.450	0.923	0.097
4c		NH_2	0.252	>1.800	0.265	0.091[b]	3.978	>3.750	>8.570	0.064
4d		H	0.553	ND	0.045	>1.453[b]	ND	ND	4.153	0.097
4e		H	0.160	ND	0.023	1.450	13.142	ND	ND	0.039
4f		H	0.186	ND	0.197	ND	2.710	ND	37.846	0.068
4g		H	0.016	ND	0.091	ND	>0.915[b]	13.090	10.153	0.285
4h		H	0.042	ND	0.026	ND	ND	ND	12.000	0.180
4j		H	ND	ND	0.070	ND	ND	ND	165.230	0.285
4k		H	0.015	ND	0.070	ND	>0.915[b]	ND	ND	0.375
4l		H	0.066	ND	0.048	ND	ND	67.636	20.307	0.015
4m		H	ND	ND	0.016	ND	126.500	ND	36.000	0.018
4n		H	0.240	ND	0.028	ND	>0.915[b]	ND	41.538	0.041

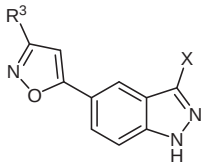
[a] K_i values are based on six point curves unless otherwise noted.
[b] K_i value is based on an eleven point curve done in triplicate.

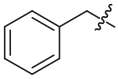
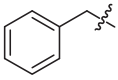
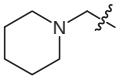
position of the second chloro group modulates the Gsk3β and Rock2 activity with **4l** being more potent in Rock2, **4m** being equipotent against Gsk3β and Rock2 and **4n** being more potent against Gsk3β.

When the heterocyclic ring at the 5-position of the indazole ring is an isoxazole (Table 3) the compound **5a** maintained its Rock2 and Gsk3β potency but it was also more potent against Aurora2 and Jak2 as compared to **3g**. Addition of an amino group at the 3-position of the indazole improved significantly its Aurora2 and Jak2 activity. Aliphatic substituents on the isoxazole ring maintained moderate Rock2 activities.

In conclusion, we have discovered multiple series of easily accessible 5-substituted indazoles as potent kinase inhibitors. Although the compounds showed high potency against multiple kinases we have already observed selectivity trends based on substitution patterns of very close analogs. In addition to the panel of kinase assays reported here, numerous compounds were also tested in a large panel of more than 100 kinases. Many interesting

Table 3
Kinase inhibitory activity of 5-(3-substituted-isoxazol-5-yl)-indazoles **5**[a]



Compound	R^3	X	Aurora2 K_i (μM)	Egfr K_i (μM)	Gsk3β K_i (μM)	Jak2 K_i (μM)	Kdr K_i (μM)	Pak4 K_i (μM)	Pim1 K_i (μM)	Rock2 K_i (μM)
5a		H	0.323	>1.800	0.572	0.351	>8.880	>3.750	>8.570	0.028
5b		NH_2	0.026	ND	0.450	0.016[b]	3.291	3.588	5.155	0.016
5c		H	2.366	>1.800	2.530	0.482[b]	>8.880	>3.750	>8.570	0.113
5d		H	>4.900	ND	4.272	ND	>8.880	>3.750	3.685	0.402
5e		H	>4.900	>1.800	3.810	>1.453	>8.880	>3.750	>8.570	0.315

[a] K_i values are based on six point curves unless otherwise noted.
[b] K_i value is based on an eleven point curve done in triplicate.

activity profiles emerged out of these activities and the results will be reported in due course.

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7. Using a Tecan Gemini robot, compounds were diluted in 100% DMSO into six points and five-fold dilutions into a 384 well polypropylene plate, starting at 500 μM. The plate was diluted 17-fold in assay buffer to reduce the DMSO concentration, then 8 μl was transferred to black HTRF assay plates. Low controls were prequenched with EDTA. ATP, enzyme and biotinylated peptide were added in sequence for a total volume of 24 μl and the reaction run for 1 h at room temperature.
The receptor tyrosine kinase (RTK) reaction buffer consisted of 50 mM HEPES pH 7.5, 1 mM DTT, 10 mM $MgCl_2$, 2 mM $MnCl_2$, 0.01% BSA, and 100 μM Na_3VO_4.
The serine/threonine (SK) kinase reaction buffer consisted of 20 mM HEPES pH 7.5, 1 mM DTT, 10 mM $MgCl_2$, 100 μM Na_3VO_4, and 0.0075% Triton X 100. Jak2 was run at 5 μM ATP, Gsk3β, Pak4, Aurora2, Rock2, Kdr and Pim1 were run at 10 μM ATP and Egfr at 50 μM ATP.
Peptide substrates used were: 2 μM pGS (Gsk3β), L15 (Pak4), kemptide (Aurora2), longS (Rock2), AL1 (Pim1), 0.5 μM PDKtide (Jak2), 0.5 μM FGFR (Egfr, Kdr).
The final compound concentration run from 10 μM to 3.2 nM and the final DMSO concentration was 2%.
The RTK enzyme reactions were stopped with an equal volume of Revelation Quench buffer containing 0.04 M Hepes 7.4, 0.48 M KF, 0.01% Tween20, 0.1% BSA, 0.06 M EDTA, Cisbio anti-phospho serine/threonine antibody labeled with Eu 3+-Cryptate and Streptavidin–Allophycocyanin. After equilibration for 1 h the plates were read on an Envision plate reader optimized for HTRF: 320 nm excitation and 665/615 nm emission.
The S/T enzyme reactions were stopped with 30 μM final EDTA in PBS transferred to a PerkinElmer Streptavidin labeled Flashplate, washed three times with PBS/0.05% Tween20 and counted on a Packard Topcount.
Results were analyzed utilizing an in house MS Excel macro to calculate % inhibition, IC_{50} and K_i values.
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