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Oxindole derivatives as inhibitors of TAK1 kinase

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ABSTRACT

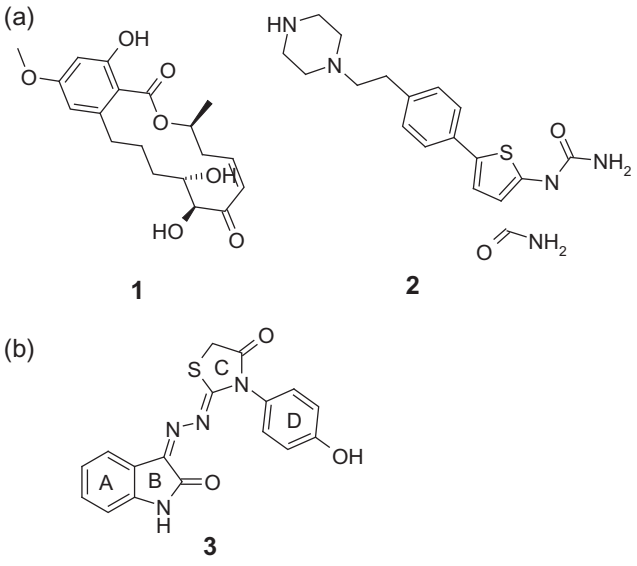
Several series of oxindole analogues were synthesized and screened for inhibitory activity against transforming growth factor-β-activating kinase 1 (TAK1). Modifications around several regions of the lead molecules were made, with a distal hydroxyl group in the D region being critical for activity. The most potent compound **10** shows an IC_{50} of 8.9 nM against TAK1 in a biochemical enzyme assay, with compounds **3** and **6** showing low micromolar cellular inhibition.

Inflammation is a crucial process for wound healing, but if not done in an orderly fashion it can lead to unregulated cell proliferation and tumor growth.[1,2] A key cellular factor linking inflammation and cancer is transcription factor NF-κB. Knockout of upstream NF-κB regulator IKKβ leads to a significant reduction in tumor volume and number in a murine model of hepatocellular carcinoma[3] as well as a dramatic reduction in tumor incidence in a colitis-associated cancer mouse model.[4]

A critical component of the NF-κB-activating pathway is transforming growth factor-β-activating kinase 1 (TAK1, MAP3K7, EC 2.7.11.25). TAK1 associates with TAB1 and TAB2, and this complex activates IKK after ubiquitination mediated by both TRAF6 and Ubc13-Uev1A.[5–7] Kinase-dead TAK1 has been shown to inhibit the activation of NF-κB via both the TNFα[8] and IL-1[9] pathways.

Small molecule inhibitors of TAK1 have been described (Scheme 1a). The natural product 5Z-7-oxozeaenol **1** is a potent, irreversible ATP-competitive inhibitor of TAK1, with and IC_{50} = 8 nM. It acts to block NF-κB activation in cells and prevents proinflammatory signaling. Heterocyclic small molecule inhibitors of TAK1 have also been described. The most potent of these thiophenecarboxamide compounds, **2**, has an IC_{50} = 50 nM and sub-micromolar cytotoxicity across a panel of leukemia and lymphoma cell lines.[10]

High-throughput screening and elimination of promiscuous hits led to the elucidation of one hit class: oxindoles as exemplified by **3**. The molecule was subdivided into regions and SAR explored (Scheme 1b).



Scheme 1. Reagents: (a) literature inhibitors 1, IC_{50} = 8nM; 2, IC_{50} = 50nM; (b) lead molecule 3, IC_{50} = 46 nM.

Synthesis was undertaken using variations on literature methods (Scheme 2).[11–13]

Isatins were condensed with thiosemicarbazides using catalytic sulfuric acid in ethanol at 120 °C for 15 min under 300 W of microwave heating. The resulting semicarbazones were cyclized with various α-bromoesters in the presence of sodium acetate in

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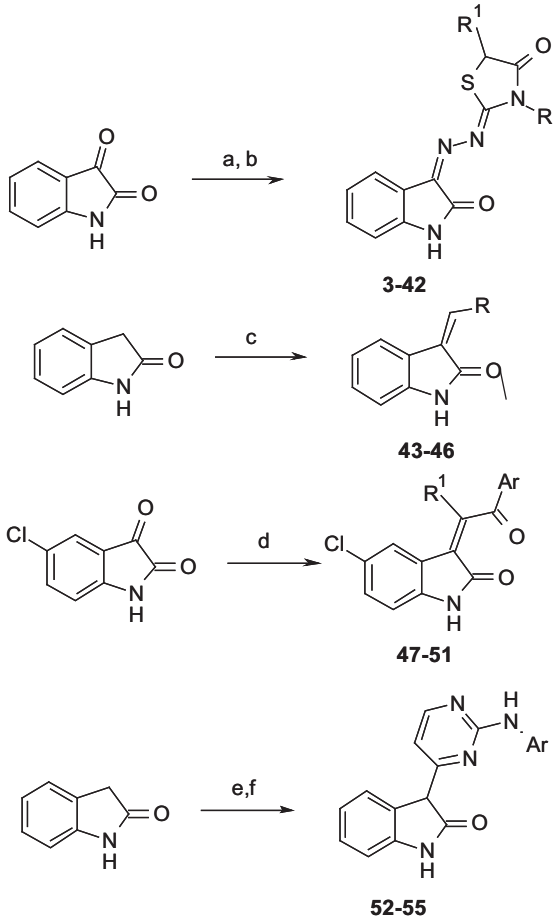


Scheme 2. Reagents and conditions: (a) R-NHCONHNH$_2$, H_2SO_4, EtOH; (b) Br(R1)CHCOOEt, NaOAc, EtOH; (c) ArCHO, NaOMe, MeOH; (d) R1COAr, AcOH; (e) (i) NaH, DMF; (ii) 2,4-dichloropyrimidine; (f) K_2CO_3, 2-ethoxyethanol.

ethanol to yield the oxindole products. Variations in the isatin lead to changes in zones A and B, variations in the α-bromoester led to change in zone C, and variations in the thiosemicarbazide led to changes in zone D. After purification, an *E* and *Z* isomer was observed in many cases and assigned on the basis of 1-D and 2-D ^{1}H NMR. It could not be unambiguously identified which double bond had changed in conformation, only that the D-ring was positioned above the A-ring (defined as Z) or not (defined as E).

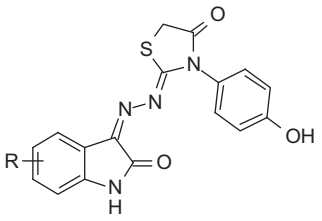
Different routes were employed to generate diversity around the linker. Aldol condensations were utilized to generate alkenyl-derivatives **43**–**46** and enones **47**–**51**. Deprotonation of oxindoles with NaH followed by addition of 2,4-dichloropyrimidine and subsequent amination with various anilines led to compounds **52**–**55**.

The primary in vitro assay used was a ^{33}P-incorporation assay utilizing a TAK1-TAB1 fusion protein.[14] The potencies of active compounds were confirmed with an IP kinase assay.[15]

Zone A tolerated changes at the 5-position of the oxindole (Table 1). Similar biochemical activities were shown by the 5-methyl, -bromo, -chloro, and -fluoro compounds, while the larger 5-CF_3 derivative showed reduced inhibition and no further substitution was tolerated. Both disubstituted derivatives and compounds differently substituted around the A-ring were not active. For most E/Z pairs there was little difference in the potencies of the different conformations: only the 5-bromo compounds **10** and **11** showed greater than 2-fold difference in IC_{50}.

The effects of varying substitution were similarly stark when modifications were made to the D region (Table 2): a D-region

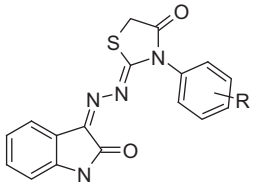
Table 1
In vitro activities of compounds **3**–**19**: zone A modifications



Compound	E/Z[a]	R	IC_{50} (μM) or% inhibition at 10 μM
3	E	H	0.046
4	Z	5-Me	0.098
5	E	5-Me	0.042
6	Z	H	0.100
7	E	5-F	0.099
8	Z	5-F	0.099
9	Mix	5-Cl	0.021
10	E	5-Br	0.0089
11	Z	5-Br	0.032
12	Amb	5-OCF_3	3.0
13	Amb	5,7-Cl_2	9%
14	Mix	5-Cl-7-Me	4%
15	Mix	4-Br-5-Me	35%
16	Mix	4-Cl	4%
17	Amb	4,7-Cl_2	10%
18	Mix	4-Cl-7-OMe	9%
19	Mix	6-Cl-7-Me	0%

[a] Assignment of stereochemistry around C=N bond: E, Z, mix, or ambiguous.

Table 2
Activity of compounds **20**–**36**: D-region modifications

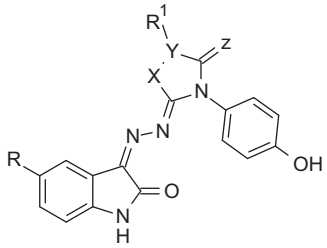


Compound	E/Z[a]	R	IC_{50} (μM) or% inhibition at 10 μM
20	Amb	5-OPh-Ph	0%
21	Amb	4-Me-Ph	0%
22	Amb	4-OMe-Ph	>5
23	E	H	>5
24	Z	H	>5
25	Amb	4-CF_3-Ph	4%
26	Amb	4-COOEt-Ph	17%
27	Amb	3,4-OCH_2O-Ph	17%
28	E	4-OBn-Ph	6%
29	Z	4-OBn-Ph	19%
30	Amb	4-$N(Me)_2$Ph	>50
31	Amb	CH_2(2-pyrrolidine)	30
32	Amb	CH_2(4-pyridine)	8.2
33	Amb	4-OH(3-pyridine)	25
34	Amb	2-OH-Ph	5.8
35	E	3-OH-Ph	29
36	Z	3-OH-Ph	25

[a] Assignment of stereochemistry around C=N bond: E, Z, mix, or ambiguous.

4-phenol was critical to TAK1 inhibition. In order to determine the function of this distal hydroxyl residue compounds were made to specifically mimic possible modes of action (compounds **33**–**36**). These analogues suggest that the irreplaceable element of the hydroxyl group is its hydrogen-bond donating ability: compounds containing substituents with similar electron donating abilities or hydrogen-bond accepting capabilities are completely inactive without the hydroxyl hydrogen. Additionally, the placement of

Table 3
Activity of compounds **37**–**42**[a]: C-region modifications



Compound	R	X	Y	Z	R1	IC_{50} (μM) or% inhibition at 10 μM
37	H	S	CH	O	H	0%
38	H	N	N	H	H	0%
39	Cl	S	CH	O	Me	0.63
40	Cl	S	CH	O	Et	>5
41	Cl	S	CH	O	*i*-Pr	>5
42	Cl	S	CH	O	Ph	4%

[a] All compounds other than **38** showed ambiguous E/Z stereochemistry.

the hydroxyl substituent is key to the compounds' inhibition: movement of the group away from the 4-position leads to inactive compounds. Lastly, the lack of activity for **33** suggests that it exists predominantly as the pyridone rather than the 4-hydroxypyridine.

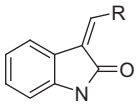
Limited SAR was conducted around the C region (Table 3). The carbonyl of the thiazolone was critical, as the analogues of **3** replacing the 5-thiazolone with a thiazoline, **37**, or a 1,2,4-triazole, **38**, were both inactive. Methyl substitution was tolerated at the 4-position of the thiazolone, however larger substituents were not potent.

Efforts were also targeted towards oxindole compounds with greater diversity in the linker, C, and D regions (Table 4). Due its critical nature within the SAR of **21**–**36**, all analogues in these series included a hydroxyl group or alternative hydrogen-bond donor in the area replacing the D region. Styrene-type compound **44** demonstrated weak activity, although the activity was eliminated when the trihyroxybenzene group was replaced by a disubstituted ring (**43**) or an imidazole (**45**–**46**).

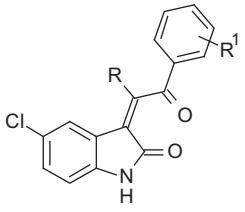
More success was found when the styrene-like core was homologated to form an enone structure. The pattern of substitution about the distal phenyl ring was found to be critical, with 3-OH (**48**, 4.1 μM) showing slight activity, 3,4-OH_2 showing significantly more activity (**47**, 0.56 μM), and both the 2,4-OH_2 (**49**, 20% inhibition) and unsubstituted compound (**50**, >5 μM) showing little activity. Unexpectedly, conversion of the trisubstituted enone to a tetrasubstituted compound dramatically decreased potency (**51**, 39% inhibition). Additional screenings on these compounds, however, showed them to not be competitive with ATP. Compounds were also made utilizing a 2-aminophenyl-4-oxindoylpyrimidine core. These compounds (**52**–**55**) showed weak activity.

After the identification of potent TAK1 inhibitors in a biochemical assay, efforts were shifted to determining the cellular activities of these compounds. Due to the dramatic decreases in aqueous solubility upon 5-substitution (PBS solubility at pH 7.4: **3** = 65 μM, **10** = 3.1 μM), **3** and **6** were chosen for cellular work despite their lessened potencies compared to **9**–**11**. Cellular TAK1 inhibition was measured utilizing an ELISA assay to detect TAK1 autophosp-

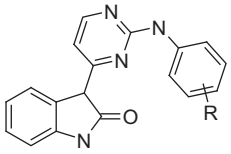
Table 4
Activity of compounds **43**–**55**: Thiazolone replacements



Compound	R	IC_{50} (μM) or% inhibition at 10 μM
43	3,4-$(OH)_2$Ph (E)	>5
44	2,3,4-$(OH)_3$Ph (E)	1.7
45	4-imidazoyl (E)	32%
46	4-imidazoyl (Z)	20%



Compound	R	R1	IC_{50} (μM) or% inhibition at 10 μM
47	H	3,4-$(OH)_2$	0.56
48	H	3-OH	4.1
49	H	2,4-$(OH)_2$	21%
50	H	H	>5
51	Me	3,4-$(OH)_2$	39%



Compound	R	% inhibition at 10 μM
52	4-OH	39%
53	3-OH	36%
54	2-OH	52%
55	2,4-$(OH)_2$	43%

orylation on Thr187.[16] Both **3** and **6** inhibited TAK1 in cells with an IC_{50} of 11–12 μM. This cellular shift typical is for ATP-competitive inhibition of protein kinases, as the biochemical assay was conducted at an ATP concentration of 25 μM (twice substrate K_M) and intracellular ATP is approximately 1–10 mM. Unexpectedly, treatment with neither compound **3** nor **6** leads to significant cytotoxicity in HeLa cells (TC_{50} >100 μM). Due to the unexpected lack of cytotoxicity exhibited by compounds **3** and **6**, no additional biochemical work was done to determine the selectivity of any tested compounds for TAK1 over other kinases.

In conclusion, several series of oxindole-based inhibitors of TAK1 were synthesized and screened. Only 5-subsitution on the oxindole was tolerated and the compounds required the hydrogen-bond donating ability of a free hydroxyl group at the terminus of the D region. Compounds **3** and **6** showed low micromolar cellular inhibition of TAK1, although no significant cytotoxicity. These data raise doubts to the role TAK1 plays in cellular viability and consequently its attractiveness as an oncology therapeutic target, although the possibility that compounds with higher solubility and potency will affect cellular viability cannot be completely discounted.

Acknowledgments

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13. All synthesized compounds were characterized by reverse phase-HPLC/MS and ^{1}H NMR.
14. Inhibition of recombinant TAK1-TAB1 enzymatic activity was determined with a ^{33}P filter plate assay using 25 μM ATP and histone H1 as substrate.
15. HeLa cells overexpressing TAK1 and TAB1 were stimulated with TNFα in the absence or presence of TAK1 inhibitors and TAK1 kinase activity was measured by immunoprecipitating TAK1–TAB1 from the cell lysates and performing an in vitro kinase reaction.
16. Cells overexpressing TAK1 and TAB1 were stimulated with TNFα in the absence or presence of TAK1 inhibitors and TAK1 autophosphorylation was measured with an in-cell ELISA assay. Cells were fixed and stained with anti-phospho-TAK1 (T187) antibody followed by HRP-conjugated secondary antibody and HRP activity was measured.