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Discovery and in vitro evaluation of potent TrkA kinase inhibitors: oxindole and aza-oxindoles

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Abstract—The discovery, synthesis, potential binding mode, and in vitro kinase profile of 3-(3-bromo-4-hydroxy-5-(2′-methoxyphenyl)-benzylidene)-5-bromo-1,3-dihydro-pyrrolo[2,3-*b*]pyridin-2-one, 3-[(1-methyl-1*H*-indol-3-yl)methylene]-1,3-dihydro-2*H*-pyrrolo[3,2-*b*]-pyridin-2-one as potent TrkA inhibitors are discussed.

The survival rate for patients with pancreatic ductal adenocarcinoma is among the poorest for all cancers.[1] The aggressive behavior and poor prognosis of this cancer is associated with an increased expression of many growth factors and their respective receptors. Pancreatic cancer invades the nerve sheathing and results in pressure on the nerve and pain. Strong evidense exists for the observation that NGF and TrkA are over-expressed in pancreatic cancer and may be causative for the neural invasion and pain associated with that disease.[2] There is also evidence that Trk tyrosine kinases play a role in the development of a variety of other cancers including breast and prostate cancer.[3,4]

TrkA is a receptor tyrosine kinase that belongs to a subfamily that includes TrkB and TrkC. TrkB and TrkC are structurally similar to TrkA, but respond to different ligands in the neurotrophin family.[5] NGF signaling through TrkA has been best characterized in the PC12 system and is similar to signal transduction mechanisms of other tyrosine kinase receptors.[6] NGF exists as a homodimer and its binding promotes dimerization and autophosphorylation of TrkA. Phosphorylation of TrkA increases the catalytic activity of the kinase domain and creates binding sites for SH2 domain containing cytoplasmic proteins. These proteins initiate the activation of several signal transduction pathways such as PLCγ, ras, PI3 kinase/AKT, and Raf/MEK/ERK. Data has emerged which suggests that mediation of the Trk kinase signaling could provide beneficial biological effects.[7,8] Further, there is some evidence that blocking the NGF receptors could improve the effectiveness of erbB2 inhibitor drugs[9] (Fig. 1).

The expression of TrkA and the dependence of downstream signaling on its activities (proliferation, differentiation, apoptosis) vary with cell type and grade of transformation.[10] Therefore, modulating disease via inhibition of TrkA kinase activity using small molecules in pre-clinical models may provide complex results. Very few TrkA kinase inhibitors are known, and none are reported to be selective.[11] However, encouraging preclinical data for Staurosporine derivatives, CEP701 and related analogues, have been reported.[12] CEP701 is an orally active compound that inhibits prostate cancer growth in 10 different animal models independent of the tumor growth rate, androgen sensitivity, metastatic

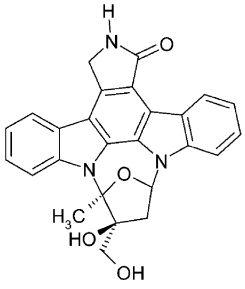

Figure 1. CEP701: non-selective ATP competitive TrkA kinase inhibitor.

Keywords: TrkA kinase inhibitors.
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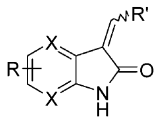


Figure 2. General class of active compounds discovered through a TrkA kinase enzyme screen. X=CH or N, R = small substitutions, R′ = aryl or anilino.

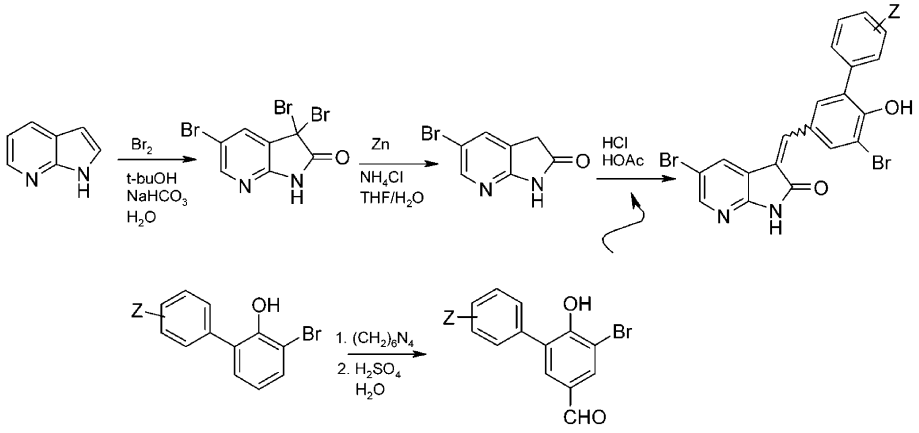
ability or state of tumor differentiation.[13] CEP701 and CEP2563, a prodrug designed to improve physicochemical properties, is reported to be under study in clinical trials to evaluate inhibition of TrkA kinase activity in pancreatic cancer.[14]

While good evidence was supplied to link the efficacy of CEP701 and its close analogues to the inhibition of TrkA, we sought more selective compounds than the Staurosporine and Tyrphostin series. Herein we report the identification and selectivity of potent, more selective TrkA kinase inhibitors. Focused screening of a proprietary kinase pharmacophore set of compounds in a TrkA catalytic enzyme assay revealed related oxindole and aza-oxindole active compounds (Fig. 2).[15,16]

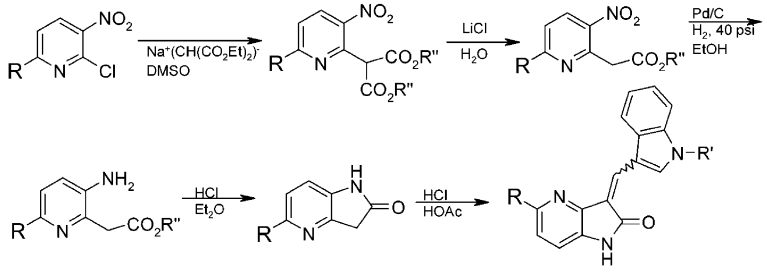
The syntheses of the active TrkA inhibitors are shown in Schemes 1–4.

The 3-arylidene-7-azaoxindole derivatives were synthesized beginning with commercially available 7-azaoxindole. Treatment of 7-azaindole with Br_2 under basic aqueous conditions, using saturated sodium bicarbonate in water, in *t*-butanol affords the tribrominated intermediate. The selective reduction of the 3,3-dibromide can be achieved with activated zinc in the presence of saturated ammonium chloride in THF. The mixed aldol condensation reaction was effected with HCl in acetic acid at 100 °C. The 3-halo-4-hydroxy-5-phenyl benzaldehyde can be generated by treating 2-halo-6-substituted phenyl phenols with excess hexamethylenetetramine in acetic acid followed by aqueous acid hydrolysis using sulfuric acid in water.[17]

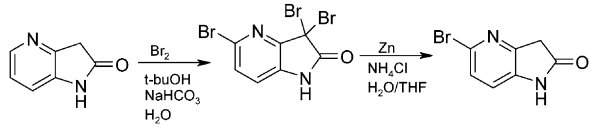
The synthesis of the 4-azaoxindole analogues began with the treatment of 2-chloro-3-nitropyridine with the anion of diethylmalonate prepared using sodium hydride in DMSO. Decarboxylation was effected with LiCl/water and DMSO to provide the 3-nitropyridylmethylcarboxylate. The reduction of the nitro group was carried out using an atmosphere of H_2 under 40 psi of pressure in the presence of a catalytic amount of Pd/C in ethanol. The mixed aldol condensation reaction with a substituted indole-3-carboxaldehyde with HCl in acetic acid was performed at elevated temperatures in a range of 50–100 °C.



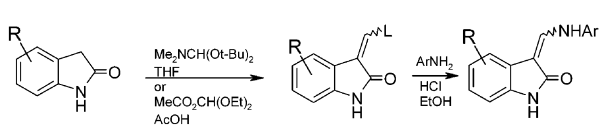
Scheme 1. Synthesis of 3-arylidene-7-azaoxindole derivatives.



Scheme 2. Synthesis of 3-arylidene-4-azaoxindole derivatives.



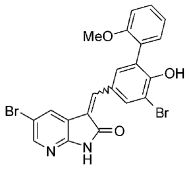
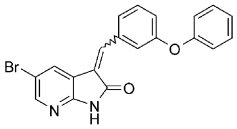
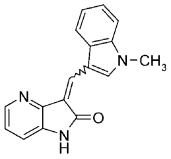
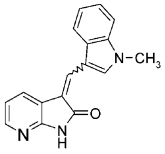
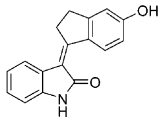
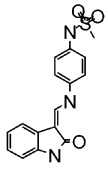
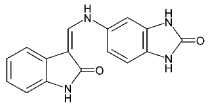
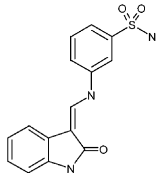
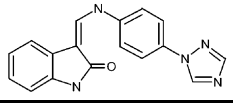
Scheme 3. Synthesis of 5-bromo-4-azaoxindole.



Scheme 4. Synthesis of vinylogous urea oxindoles.

The 'R' groups can be incorporated in the beginning of the synthesis as in Scheme 2, or alternatively, one could introduce substitution via a key 5-bromo derivative generated from the unsubstituted 4-azaoxindole as shown in Scheme 3. Treatment of 4-azaoxindole with Br_2 in saturated aqueous sodium bicarbonate in *t*-butanol afforded the tribrominated intermediate. The selective reduction of the 3,3-dibromide was achieved as described above. The aryl bromide was further elaborated uisng Pd catalyzed reactions, such as Suzuki and Heck couplings, before conducting the mixed aldol condensations or formylations described in Scheme 4.

Table 1. TrkA kinase enzyme inhibition expressed as IC_{50} values in μM[13]

Compd	Structure	TrkA	CRaf1	CDK2
1		0.008	0.70	10.4
2		0.061	> 50	> 50
3		0.002	> 12	> 7
4		0.012	7.2	> 6
5		0.006	> 10	8
6		0.006	> 10	4.8
7		0.007	> 10	2.5
8		0.063	> 10	> 10
9		0.008	> 10	7.2

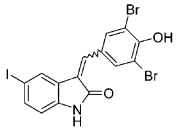
For derivatives **6–9**, a straightforward two-step synthesis beginning with commercially available oxindole was used as shown in Scheme 4. The formylation of the 3-position and displacement with wide diversity of substituted anilines is well described in the literature.[18] Many of these reactions could be conducted in solution parallel array format.

Examples of potent TrkA inhibitors are listed in Table 1. Well over 750 substituted oxindole and azaoxindole compounds were evaluated in a TrkA kinase enzyme assay, but only a few compounds demonstrated potent inhibition. Previously, we had reported potent and selective inhibitors in the oxindole series for cRaf1 inhibition and CDK2 inhibition.[19,20] It is interesting to note the substitutions that confer extraordinary selectivity between these kinases whilst retaining a largely similar scaffold (Fig. 3).

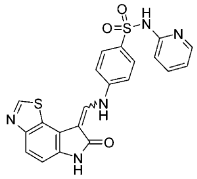
The typical donor/acceptor binding motif for kinase small molecules can easily be achieved in the lactam portion of the oxindole ring system.[21] However, multiple binding modes can be envisioned with the additional binding potential of the 7-position nitrogen.

cRaf1 kinase inhibition appeared dependent on an acidic phenol in the 4-position of the benzylidene group with two flanking substituents, while little, if any, inhibition of CDK2 catalytic activity was observed with 3-benzylidene substitutions. The TrkA ATP binding pocket appeared to tolerate a variety of substitutions, but potency enhancements occurred with *m*-aryl groups. With identical 5-bromo-7-azaoxindole scaffolds, the inhibition data for **1** and **2** demonstrate greater than 1000-fold selectivity for TrkA inhibition over CDK2 and cRaf1. From SAR, we know that the phenol substitution was essential for cRaf1 inhibition, so it was expected that the removal would confer greater selectivity for TrkA. Unfortunately, there was a concomittant 8-fold reduction in potency for TrkA inhibition.

An analysis of the SAR suggests that the hinge region interaction includes the carbonyl oxygen as depicted in Figure 4. Comparison of the inhibition profile of **3** and



GW5074[15]
cRAf1 IC_{50} 0.011 uM
TrkA IC_{50} 4.7 uM



GW8510[14]
CDK2 IC_{50} 0.010 uM
TrkA IC_{50} 0.009 uM

Figure 3. Literature compounds in the oxindole series.

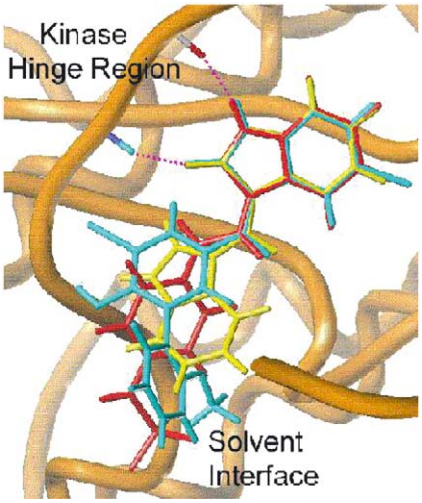


Figure 4. Structure overlap of **1**, **3**, and **6**.

4 suggests that the 7-position nitrogen is not the H-bond acceptor in the hinge region since a large reduction in potency would be expected with the removal of this key interaction. Further SAR can be derived with the potency and selectivity of **5**, suggesting that there may be multiple binding modes possible within the oxindole and azaoxindole series.

The homologation of the linker between the benzylidene aryl ring and the oxindole scaffold provides further SAR and unique inhibitors of TrkA. While GW8510 is not selective between TrkA and CDK2, removal of fused ring substituents from the oxindole portion of the molecule seemed to greatly reduce the inhibitory activity of CDK2, while very little reduction in activity for TrkA was observed. Reversing the connectivity of the sulfonamide, as in **6**, afforded nearly 10,000-fold improvement in selectivity. Other substituents that confer potency for TrkA inhibition are phthalimide, 3-sulfonamide, and a 4-triazole shown by **7**, **8**, and **9**.

To provide some insights for potential binding modes, **1**, **3**, and **6** were over-layed by aligning the oxindole rings (Fig. 4). The substituted vinyl aniline and benzylidene groups seem to fit the ATP binding pocket with a *Z*-isomer configuration orientated toward the solvent interface. While other binding modes are feasible, this binding mode can accommodate the diversity of groups that are tolerated in the 3- and 4-position of the terminal aryl ring. The available crystal structure information can explain how to reduce CDK2 activity,[19] but the increase in TrkA inhibition activity remains empirical.

As with all kinase inhibitors, the absolute selectivity profile is difficult to determine. However, several of these TrkA inhibitors did show significant selectivity over a range of kinase enzymes examined. Figure 5 is a compilation of representative kinase inhibition data for **1**, **3**, and **6**. There is >10-fold selectivity observed for all of the kinases tested, and in many cases >100-fold selectivity is achieved. These data need to be taken into account when interpreting cellular activity.

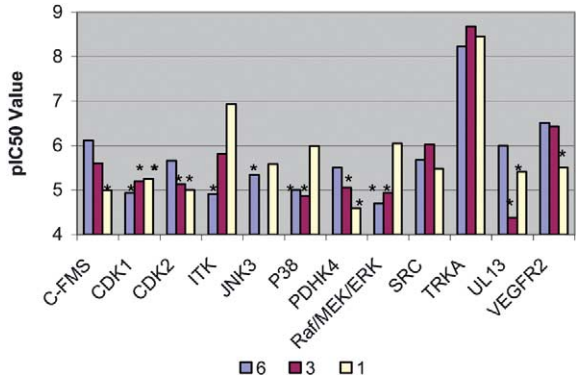


Figure 5. Kinase enzyme inhibition results against a representative set of kinase enzyme assays. *Denotes maximum concentration tested, not IC_{50} value determined.

In conclusion, we have discovered potent TrkA kinase inhibitors in the oxindole series that are significantly more selective than the staurosporine analogue(s) reported in pre-clinical studies and clinical trials. We included synthetic details, kinase enzyme data for trkA plus representative kinase selectivity data, SAR and a proposed binding mode in the ATP pocket of the kinase catalytic region. It is also noteworthy that these compounds are significantly more selective than published trkA inhibitors (Staurosporines and Tyrphostins). These compounds may offer the scientific community useful target validation and signaling tools. Further biological evaluation of these compounds is necessary to determine if the TrkA activity is sufficient to achieve anti-cancer effects.

Acknowledgements

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15. Screening format: TK activity is being measured using a synthetic peptide substrate (H_2N-RRRAAAEEIYGEI-NH_2). The enzyme is a GST-fusion of the intracellular domain expressed in SF9 cells. The enzyme is pre-incubated with cold ATP and Mg to allow autophosphorylation prior to running the screen which increases the initial rate of catalysis ~3-fold. The assay is performed in microtitre plates, and reaction products are detected following filtration through Millipore p81 phosphocellulose plates. Peptide K_m, 60 μM; ATP K_m 30 uM; k_{cat}/K_m (peptide) 10^4.
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