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Discovery of wrightiadione as a novel template for the TrkA kinase inhibitors

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ABSTRACT

Enzymatic kinase assays and docking simulation studies have shown that the natural product wrightiadione displays inhibitory activity toward TrkA and PLK3. In this study, the template of wrightiadione served as a starting point for Trk inhibitor development campaigns. Molecular simulation provided structural insights for the design of derivatives that were efficiently generated by our recently developed 3-step tandem synthetic approach, resulting in the discovery of compound **2h** with biochemical potency at the single-digit micromolar level.

Since the approval of imatinib by the FDA for the treatment of chronic myelogenous leukemia in 2001, the development of kinase inhibitors has been a central topic in the field of drug discovery for a variety of therapeutic applications.[1] As a result, 26 kinase inhibitors have been approved as drugs, and 266 human kinases have been targeted using small molecule inhibitors.[2] However, kinase inhibitors are unevenly distributed across the human kinome: approximately 50% of human kinases have largely been unexplored using small molecules.[2b] Thus, the development of small-molecule new chemical entities (NCEs) remains a high priority because structurally new inhibitors spanning diverse structures and properties can provide ample opportunities to expand chemical spaces and to develop selective molecular probes.[3] Moreover, novel molecular frames of inhibitors may present different and improved profiles of potency and selectivity.[4] Natural products or synthetic compounds based on natural-product pharmacophores broaden and diversify the current chemical space and provide privileged molecular templates from which a variety of biologically active compounds can be generated. Recognition and utilization of the potential of natural products as novel inhibitor platforms has been one of the most powerful methods used to produce new classes of inhibitors in terms of molecular targets, potency, selectivity profile, and physiochemical properties.[5]

We recently reported a highly efficient one-pot synthetic route consisting of 3-step tandem dehydrogenation/oxidation/oxidative cyclization reactions using a Pd/Cu catalytic system, which enabled the straightforward preparation of wrightiadione (**1**) and its derivatives from easily accessible starting materials.[6] With the goal of identifying a new class of potent Trk inhibitors,[7] we investigated the binding modes of wrightiadione in the active site of Trk and extensively modified the structure of wrightiadione. Herein we report that wrightiadione revealed high affinity toward tropomyosin receptor kinase A (TrkA) as assessed using enzymatic kinase assays. Subsequent synthesis of wrightiadione derivatives guided by structure-based drug design demonstrated that wrightiadione served as a promising starting point for the development of TrkA inhibitors, resulting in the discovery of compound **2h** with single-digit micromolar potency.

Wrightiadione, isolated from the dried bark of *Wrightia tomentosa*, is a natural product that exhibits a wide range of biological activities, including cytotoxicity against leukemia cell lines (Fig. 1).[8] However, the molecular mechanisms underlying its anticancer activities are unknown. Our docking simulation studies indicated that the tetracyclic isoflavone moiety of wrightiadione could fit into the ATP binding sites of kinases, and provide a new molecular platform for the development of potential kinase inhibitors (vide infra). These observations led us to hypothesize that the anticancer activity of wrightiadione may be attributed in part to the inhibition of several different kinases.[9] Thus, wrightiadione was subjected to enzymatic kinase assays to test the hypothesis

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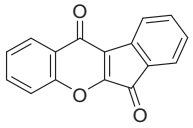


Wrightiadione (**1**)

Figure 1. Structure of naturally occurring wrightiadione.

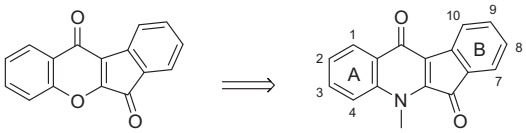


Figure 3. Kinase activities of wrightiadione (**1**) and its derivative **2a**.

by performing a high-throughput binding assay at 10 μM against a panel of representative panel of 97 cancer-related kinases.[10] As shown in Figure 2, these preliminary data indicated that wrightiadione inhibits TrkA and PLK3 with remarkable selectivity compared to other kinases (Fig. 2 and Supporting information). The activity of wrightiadione was further confirmed using radiometric kinase assays where wrightiadione exhibited good potency against TrkA (IC_{50} = 55.8 μM) and PLK3 (IC_{50} = 9.0 μM).[11] To the best of our knowledge, the tetracyclic isoflavone scaffold of wrightiadione has not been reported as kinase inhibitors thus far.

As a strategy to further increase potency over TrkA employing the wrightiadione scaffold, we envisioned that the enaminone derivatives of wrightiadione would potentially provide additional handle to manipulate potency by modifying the *N*-substituents. A representative compound **2a** was obtained by employing our efficient 3-step tandem synthetic method (vide infra)[6] and subsequently subjected to IC_{50} measurement. Switching to the enaminone scaffold resulted in an enhancement of the potency against TrkA: compound **2a** was found to be more potent (IC_{50} of 28.9 μM) than wrightiadione (Fig. 3). Importantly, we observed that the replacement of an enolone system with an enaminone had resulted in drastic loss of activity for PLK3 (e.g., **2a**, IC_{50} > 200 μM). Considering its low molecular weight (~260), compound **2a** is anticipated to serve as a new Trk inhibitor scaffold from which more potent inhibitors can be derived. Tropomyosin receptor kinases (Trks), which are mainly expressed in neuronal tissues, serve as receptors for neurotrophins and play an important role in the development and maintenance of the central and peripheral nervous systems.[12] In addition to their role in chronic pain and inflammation,[13] numerous reports have indicated that Trks are involved in malignant transformation, metastasis, survival, migration and invasion signaling in a variety of human cancers, including prostate, colorectal, pancreatic, breast and lung cancers and neuroblastoma.[14] Thus, the development of Trk kinase inhibitors has received considerable attention from the field of drug discovery.[15] However, most Trk kinase inhibitors have been derived from relatively well-known kinase scaffolds, and the development of Trk inhibitors based on new scaffolds is highly desired to identify improved selectivity profiles and physiochemical properties.

To obtain structural insight into the inhibitory mechanisms of compound **2a**, its binding mode was investigated by employing the TrkA crystal structure (PDB ID: 4AOJ) as a simulation template.[15f] Figure 4 shows the lowest energy conformation of compound **2a** as calculated using Discovery Studio software. Docking simulations indicated that the carbonyl group at C11 forms key hydrogen bonding with the backbone N–H of Met592 in the hinge region of the ATP binding site. The binding of compound **2a** can be further stabilized via hydrophobic interactions with residues Leu516, Val524, Ala542, and Leu657. We also observed that the ring B of compound **2a** is located adjacent to the polar residues Arg593 and Arg599. Based on these observations, we envisaged that installation of hydrophobic or hydrophilic groups on appropriate positions of ring B would further strengthen the binding of the resulting derivatives of compound **2a**.

Based on the structural analysis, we planned to install a variety of substituents around the wrightiadione scaffold to expand the structure–activity relationship (SAR) profiles. Our synthetic strategy for wrightiadione derivatives is illustrated in Scheme 1. Recently, our group reported an efficient route for the three-step tandem reaction process employing a Pd/Cu catalytic system.[6] Thus, the required starting materials, 2-benzyl substituted chromanones or enaminones **3** were conveniently prepared by 1,4-addition using appropriate benzyl cuprate reagents.[16] Next, a tandem dehydrogenation/oxidation/oxidative cyclization process from 2-benzyl dihydroquinolinones successfully provided the desired wrightiadione derivatives in moderate to good yields.

The IC_{50} values of the synthesized derivatives were then determined for TrkA (Table 1). Our early investigation on the effects of the substituents at the C3 position of ring A suggested that substituents on the Ring A might not be beneficial to enhance the potency (**2b** and **2c**), which prompted us to study the effect of substituents on the Ring B. Again, installing a methyl group on various

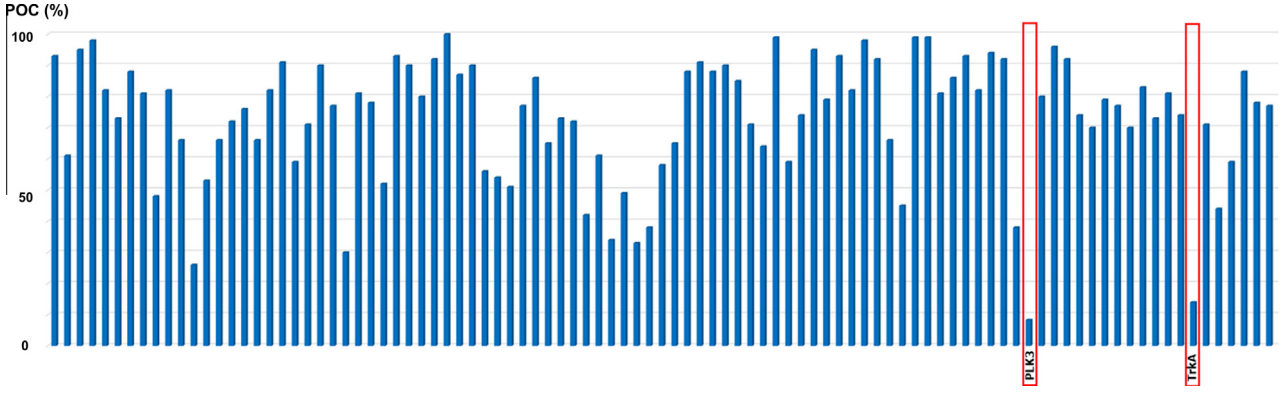


Figure 2. Selected results of the KINOMEscan profile of wrightiadione (**1**). Panel of 97 kinases were tested at 10 μM in a high-throughput binding assay (KINOMEscan). POC = percent of control; values are the average of duplicate measurement; lower values indicate stronger hits (see Supporting information for details).

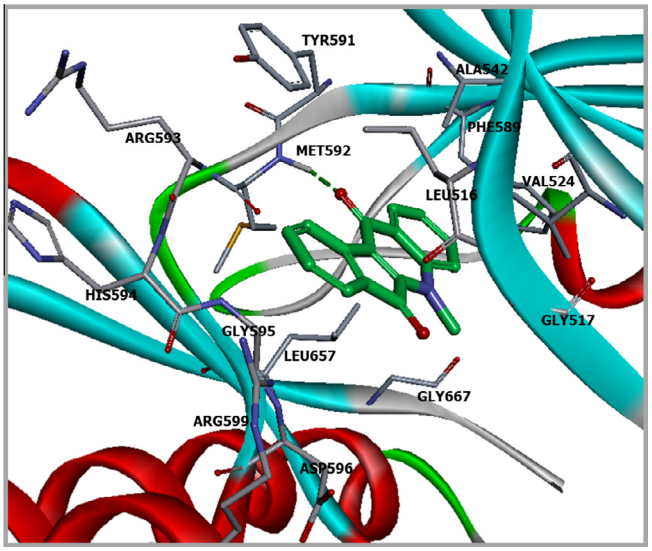


Figure 4. Calculated binding mode of compound **2a** in the ATP binding pocket of TrkA (PDB ID: 4AOJ).

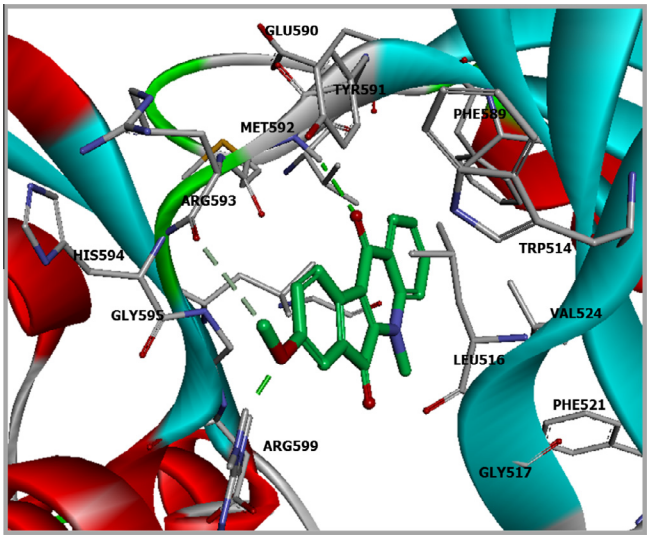
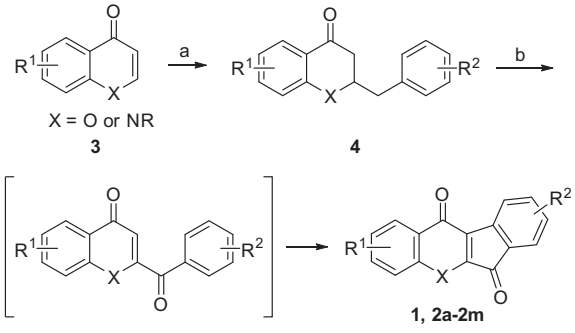


Figure 5. Calculated binding mode of compound **2h** in the ATP binding pocket of TrkA (PDB ID: 4AOJ).

positions of the Ring B was detrimental to the overall activity (**2d**–**2f**). We then speculated that the introduction of substituents on Ring B that can form molecular interactions with polar residues, such as Arg593 and Arg599, might be helpful to enhance the potency against TrkA. Importantly, as presented in compounds **2h** and **2i**, a methoxy substituent at C8 or C9 of Ring B was advantageous in improving TrkA activity: compound **2h** showed an IC_{50} of 6.6 μM, featuring our most potent TrkA inhibitor at this stage. Placing substituents at proper locations appeared to be important, as compounds **2g** and **2j** were not active at all. We also tested different groups on the nitrogen atom. Interestingly, replacement of the methyl group with either the benzyl group or ethyl ester was well tolerated, and as a result, compounds **2k** and **2l** was approximately two-fold more potent than compound **2a**. However, when we evaluated compound **2m**, which was obtained by hybridizing compounds **2h** and **2k**, only a modest increase was observed.

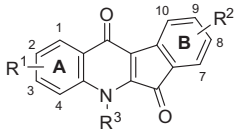
The most potent derivative **2h** exhibited similar configurations with comparable interactions with the amino acid residues in the active site (Fig. 5). Moreover, docking simulations clearly indicated that the methoxy group at C8 of **2h** appeared to form two additional hydrogen bonds with the backbone carbonyl oxygen of Arg593 and guanidine group of Arg599 (Fig. 5). Presumably, these additional molecular interactions of **2h** might be attributed to the improved potency, which was approximately 4 times greater than that of **2a**. Compound **2h** could also be further stabilized in the active site of TrkA via a key hydrogen bond with the backbone N–H of Met592 and hydrophobic interactions with residues Leu516, Val524, Ala542 and Leu657.

In conclusion, we discovered that the natural product wrightiadione can serve as a new template for the development of kinase inhibitors. The wrightiadione scaffold could be easily equipped with an additional substituent using the newly developed synthetic approach and we successfully identified compound **2h** with potent activity (IC_{50} = 6.6 μM), thereby expanding the chemical space of TrkA inhibitors. The molecular simulation results provided insights into the generation of potent TrkA inhibitors. Overexpression or activating mutation of TrkA has been implicated in human acute myeloid leukemia (AML) cells.[17,18] As wrightiadione displayed cytotoxicity against murine leukemia cell lines,[8] we envision that our wrightiadione-based TrkA inhibitors may show promising anticancer effects for human AML. Further studies



Scheme 1. Synthetic route of wrightiadione derivatives. Reagents and conditions: (a) R^2MgX (X = Cl, Br), CuI, TMSCl, THF/DCM, −78 °C, 41–89%; (b) $Pd(OAc)_2$, Cu $(OAc)_2$, Ag_2O, NaOAc, PivOH, O_2, 120 °C, 50–76%.

Table 1
Structure and activity relationship (SAR) of wrightiadione derivatives



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Compd	R^1	R^2	R^3	TrkA IC_{50}[a] (μM)
2a	H	H	Me	28.9
2b	3-CF_3	H	Me	117
2c	3-Cl	H	Me	88.5
2d	H	7-Me	Me	73.3
2e	H	8-Me	Me	47.2
2f	H	9-Me	Me	—
2g	H	7-OH	Me	141
2h	H	8-OMe	Me	6.6
2i	H	9-OMe	Me	14.4
2j	H	10-OMe	Me	209
2k	H	H	Bn	10.3
2l	H	H	CO_2Et	16.5
2m	H	8-OMe	Bn	16.4

[a] The IC_{50} measurements were performed at the Reaction Biology Corp (Malvern, PA, USA).

aimed at further increasing the potency and evaluation of anticancer activities are currently in progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.09.070.

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