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Synthesis, determination of absolute configuration, and biological evaluation of spiro-fused thiadiazoline inhibitors of kinesin spindle protein (KSP)

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ABSTRACT

A facile and highly convergent synthesis of biologically active spiro-fused thiadiazoline KSP inhibitors is reported. The highlights of the synthesis include the Michael reaction and cyclization of thiosemicarbazone to 1,3,4-thiadiazoline. This chemistry lends itself to the preparation of (+)-**2**, a potent and orally bioavailable anti-cancer agent, and to the development of a structure–activity relationship program.

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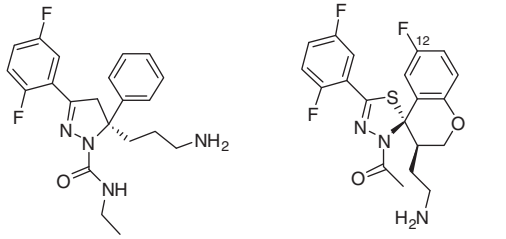
Several strategies have been pursued in the discovery of small molecules as viable cancer chemotherapeutic agents, which may function through a range of biological mechanisms. A clinically validated approach is the development of anti-mitotic agents (e.g., taxanes and vinca alkaloids) that target tubulin, the major component of the mitotic spindle.[1] However, since tubulin also functions in post-mitotic nerve cells, these microtubule-targeting drugs have been shown to cause peripheral neuropathy in patients.[2] One of the most desirable and promising settings for the successful application of anti-mitotic agents would be the one in which the mechanism of action involves targeting an enzyme that primarily functions in the regulation of mitosis.[3] As a result, these agents have the potential to preclude the undesired mechanism-based side effects that are common to microtubule-targeting drugs.[4]

The identification of kinesin spindle protein (KSP) has attracted attention due to its inherent representation as a novel mitotic target, that is, not present in post-mitotic neurons.[5] KSP belongs to the kinesin-5 family and is necessary for the formation of the bipolar mitotic spindle.[6] Inhibition of this motor enzyme has been validated to result in mitotic arrest and apoptosis.[7] We sought to undertake a program directed toward the synthesis of KSP inhibitors primarily due to its unique mechanism of action where they are expected to act specifically on proliferating cells. Many reported KSP inhibitors are currently being tested in clinical trials for the treatment of cancer.[8] However, these potential anti-cancer agents are delivered intravenously utilizing a variety of dosing schedules. Accordingly, the development of an orally bioavailable small molecule KSP inhibitor represents a promising means of providing a more convenient dosing schedule.

A medicinal chemistry effort to discover small molecule inhibitors of KSP was initiated following the disclosure of 3,5-diaryl-4,5-dihydropyrazole **1** and related analogs (Fig. 1).[9] Inspired by its potent biological activity, we were encouraged to envision compound **1** to serve as our initial structural platform from which to access a range of spiro-fused-five-membered ring scaffolds.[10]



1
IC_{50} = 44 nM

(+)-**2**
EC_{50} (p-HH3) = 2 nM

Figure 1. Structures of dihydropyrazole **1** and spiro-fused thiadiazoline **2**, inhibitors of KSP.

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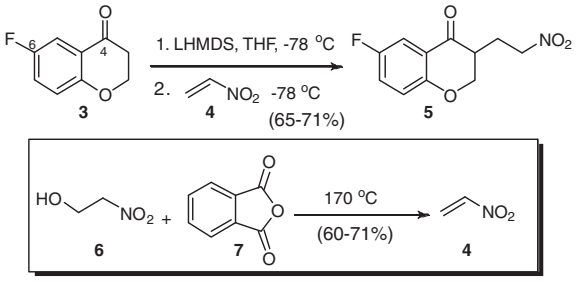
We report herein the use of a spiro-fused-1,3,4-thiadiazoline bearing an acetamide substituent as a viable surrogate to the dihydropyrazole core possessing a urea moiety. The key transformations performed in the design of analog (+)-**2** were the fusion of the phenyl ring and the propyl amino side-chain connected via an ether linkage leading to the dihydrochromane motif of congener (+)-**2**. Moreover, it was anticipated that perhaps the ether oxygen of the dihydrochromane motif would serve as an electron donating group resulting in an electron rich π-system. Thus, a fluoro substituent was incorporated at C(12) (Fig. 1) in order to block a potential oxidation site. It was expected that these strategies may generate KSP inhibitors with more favorable phamacokinetic properties.[11]

The central feature of the synthetic strategy entailed the application of a method whereby acylation of ketone thiosemicarbazones favors cyclization to construct 1,3,4-thiadiazolines.[12] The starting material employed for our synthesis was the commercially available 2,3-dihydro-6-F-chroman-4-one (**3**). Nitroethylene (**4**), obtained from the dehydration of 2-nitroethanol (**6**) with phthalic anhydride (**7**), was selected as the Michael acceptor (Scheme 1).[13] The expectation was that at a suitable time, the nitro group would lend itself to conversion into the amino function without the need of any protecting group manipulations. Conjugate addition of **3** with electron deficient alkene **4** proceeded to afford the Michael adduct **5**.

It was in the course of performing this sequence that we took note of the limited commercial availability of a number of dihydrochromanone derivatives. Though it is not directly relevant to the synthesis of the target structure at hand, it warrants further study, since these compounds would serve as precursors in the development of SAR that would deliver analogs of value in elucidating the key structural elements responsible for the potential anti-mitotic and pharmacological properties. Thus, we evaluated two synthetic methods for the preparation of related 2,3-dihydrochroman-4-one derivatives.



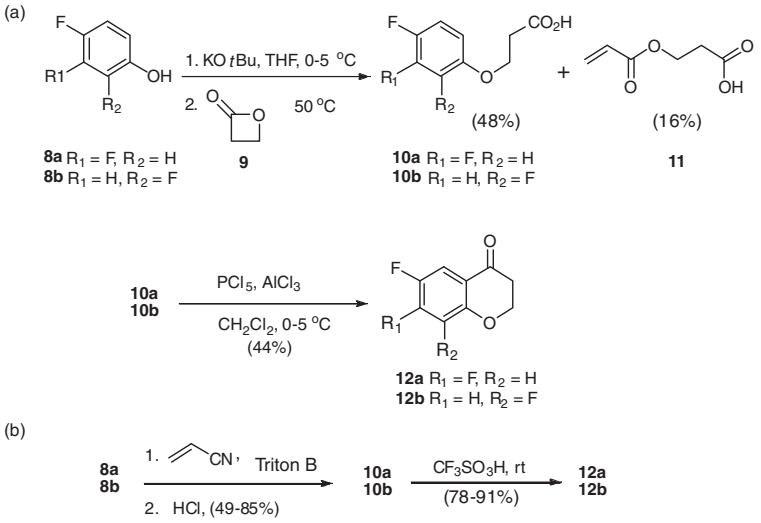
Scheme 1. Synthesis of 6-fluoro-3-(2-nitroethyl)-chroman-4-one **5**.

The initial method employed toward the preparation of dihydrochromanones **12a–b** involved an alkylation of phenols **8a–b** with β-propiolactone to afford, as the principal product (in 48% yield), carboxylic acids **10a–b** (Scheme 2a). Also produced in this reaction was a 16% yield of enone **11**.[14,15]

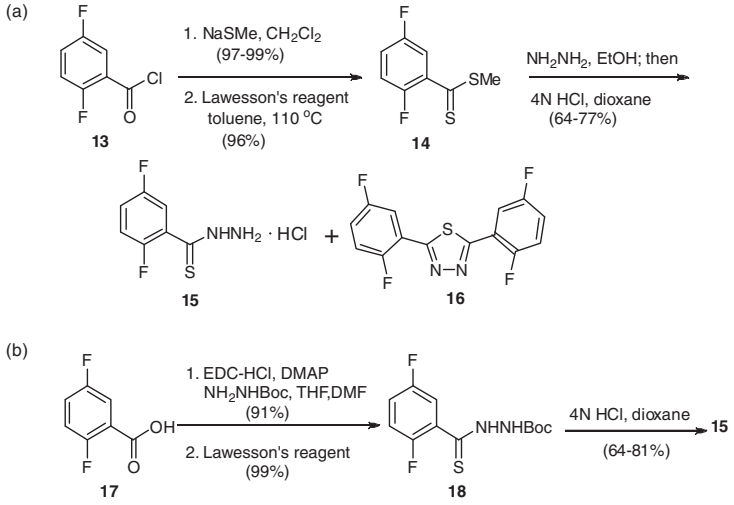
Due to the unexpected problems encountered on the initial reaction condition and for efficiency purposes, we sought a means by which to suppress the formation of the undesired byproduct **11**. Pleasingly, Triton B catalyzed conjugate addition of phenols **8a–b** with acrylonitrile followed by acid hydrolysis of the resultant nitrile generated carboxylic acids **10a–b** in situ as the sole product (Scheme 2b). Subsequent intramolecular Friedel–Crafts acylation with either neat triflic acid or PCl_5 and $AlCl_3$ furnished dihydrochromanone derivatives **12a–b**.

With an efficient route to intermediate **5** and derivatives thereof in hand, attention was next turned to the preparation of thiohydrazide **15**. A three-step process was devised commencing with 2,5-difluorobenzoyl chloride (**13**) as shown in Scheme 3a. Addition/elimination reaction with sodium thiomethoxide gave rise to the corresponding benzothioate and subsequent thionation with Lawesson's reagent generated benzodithioate **14**.[16] Treatment of compound **14** with hydrazine followed by the addition of HCl resulted in the formation of the desired thiohydrazide **15**.[17] A weak aspect in the translation of Scheme 3a to practice arose in the conversion of thiohydrazide **15** in its free base form to the corresponding HCl salt. A revealing finding was that when free form **15** was heated following work up, a significant amount (>50%) of thiadiazole **16** was observed, presumably arising from the dimerization of the unstable free-base form of **15**.[18]

Ultimately, a set of conditions was devised to minimize the formation of the dimerized product **16**. The extent of the unwanted dimerization was greatly suppressed when high temperatures during work up were eliminated and immediate conversion of the free base form of **15** to the more stable HCl salt was critical. We also exploited a modified synthetic route for the preparation of hydrazide **15**. As shown in Scheme 3b, a direct incorporation of *tert*-butyl carbazate to 2,5-diflourobenzoic acid (**17**) was accomplished via



Scheme 2. (a) Preparation of intermediates **12a–b** (method A). (b) Improved synthesis of **12a–b** (method B).



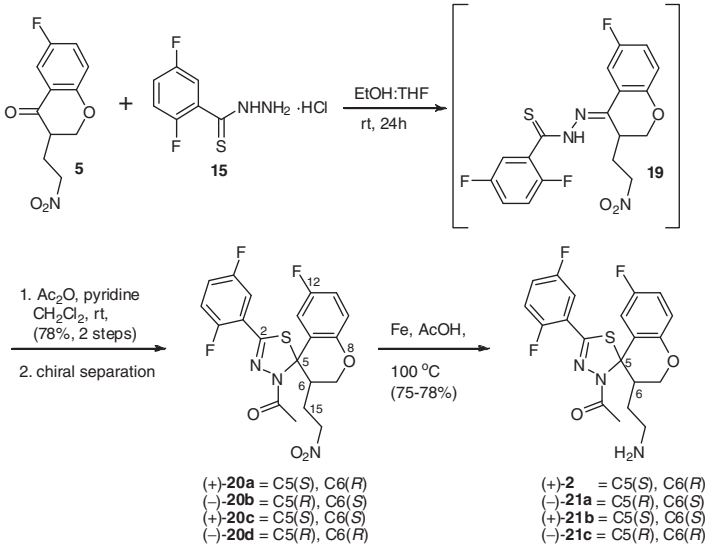
Scheme 3. Synthesis of thiohydrazide **15**.

standard EDC hydrochloride conditions, then the corresponding Boc-protected hydrazide[19] reacted readily with Lawesson's reagent to afford **18** in good yield. Removal of the Boc group under acidic conditions provided the desired thiohydrazide **15**.

With both coupling partners in hand, efforts were next directed toward the union of ketone **5** and thiohydrazide **15** to afford the fully functionalized thiosemicarbazone **19** (Scheme 4).[20] Acetylation of **19** with acetic anhydride and pyridine also effected cyclization to afford a mixture of all four possible isomers **20a–d** (6:6:1:1, favoring the *trans*-isomers). The mixture was resolved via preparative chiral HPLC using a Chiralcel OD column and all four isomers were carried forward. Finally, reduction of the nitro moiety with iron powder under acidic media furnished (+)-**2** and its derivatives in good yield.

The absolute stereochemistries of the major *trans*-isomers (+)-**2** and (−)-**21a** were identified via X-ray crystallography (Fig. 2). A single crystal structure determination of compound (−)-**20b** was performed and the absolute stereochemistry was determined by full matrix refinement of a Flack Parameter utilizing the anomalous signal from a single sulfur atom. The spiro ring juncture at C(5) is in the *R* configuration and the attachment point of the nitroethyl substituent at C(6) is in the *S* configuration. The crystals exhibited twinning, however, the dominant twin component could be successfully indexed and the intensities extracted. Flexional ring disorder is displayed in the dihydrochromane ring, with C(7) and O(8) atoms adopting two discrete conformations. The C(7)/O(8) and C(7)/O(8′) pairs have sites occupancies of 53% and 47%, respectively. The nitroethyl substituent at the C(6) position also displays conformational disorder. This is due to the flexional ring disordering causing the first atom of the nitroethyl chain at C(15)/C(15′) to lie either axially C(15) or equatorially C(15′). The C(16) atoms are nearly coincident but the nitro groups are oriented differently. The disordering within the dihydrochromane ring and nitroethyl side chain is purely conformational and is not enantiomeric or diastereomeric in nature.

The biochemical activities of compounds (+)-**2** and **21a–c** were determined by assessing their abilities to inhibit the ATPase activity of KSP using microtubules as a substrate (Table 1). Pleasingly, (+)-**2** potently inhibited KSP ATPase activity with an IC_{50} value $\leqslant$5 nM (assay detection limit), supporting our design rationale. On the other hand, the antipode, (−)-**21a**, is not an inhibitor. The *cis*-isomers, (+)-**21b** and (−)-**21c** displayed IC_{50} values 675 nM and 23 nM, respectively. Furthermore, (+)-**2** potently induced



Scheme 4. Syntheses of congeners **2** and **21a–c**.

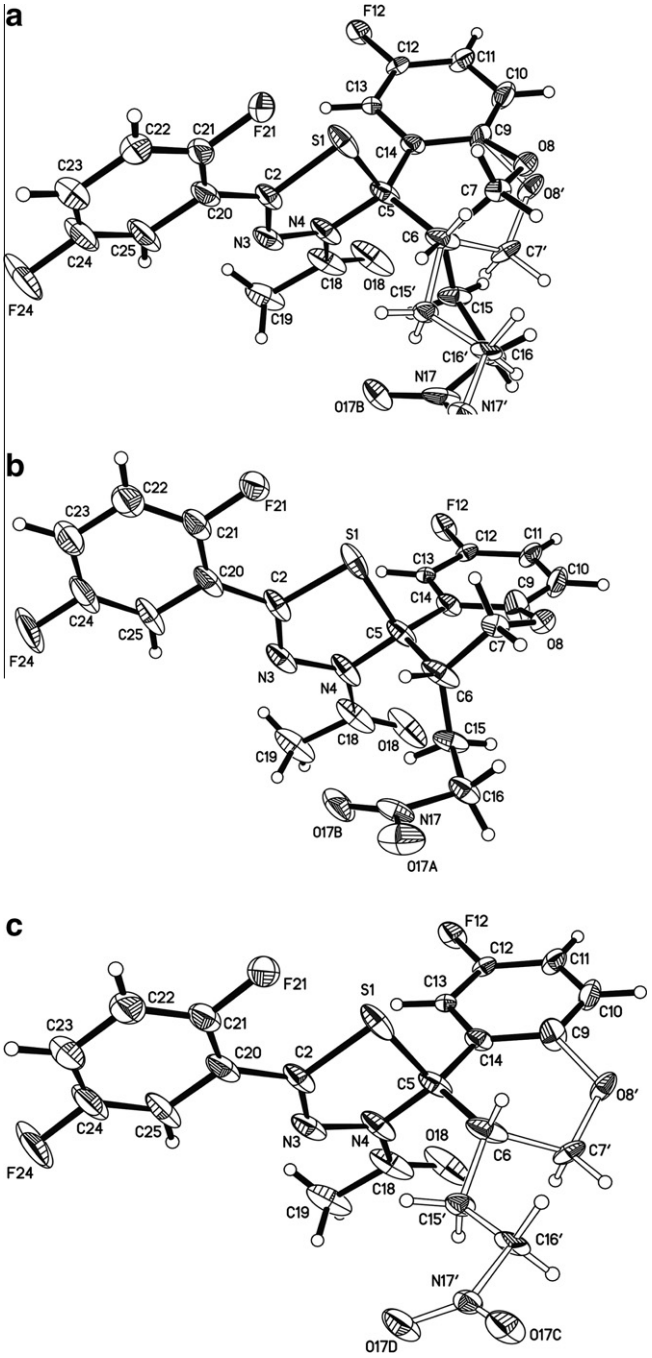


Figure 2. (a) Asymmetric unit of compound (−)-**20b** in ORTEP representation, with its assigned numbering system. Ellipsoids are set at the 30% probability level. The minor conformer of the disordered regions is shown in open bonds. (b) Conformation 1: 53% site occupancy, nitroethyl group axial. (c) Conformation 2: 47% site occupancy, nitroethyl group equatorial. Figures a and b show the two conformations adopted by the molecule, separated for clarity.

Table 1
Biological data of compounds (+)-**2** and **21a–c**

Compound	KSP IC_{50} (nM)	EC_{50} (*p*-HH3) (nM)
(+)-**2**	⩽5	1.0
(−)-**21a**	>3000	>1000
(+)-**21b**	675	267
(−)-**21c**	23.0	28.0

pharmacodynamic marker, Histone H3 (*p*-HH3 EC_{50} = 1 nM), in A2780 human ovarian carcinoma cells.

Taken together, the data described herein suggest that the inhibitory activity of (+)-**2** in vitro is enantiospecific and was therefore selected as one of the promising candidates for further evaluation as an anti-cancer agent. Thus, the initial pharmacokinetic properties of (+)-**2** in rats were evaluated. Happily, the oral bioavailability was 21% and the oral dosing of 50 mg/kg yielded an AUC value of 3.9 μM h.

In summary, a highly convergent synthesis of (+)-**2**, a potent and orally bioavailable anti-mitotic agent has been accomplished. Furthermore, an improved method for the preparation of 2,3-dihydrochroman-4-one derivatives that will serve as precursors to structurally related compounds has been developed. This chemistry lends itself to the development of a SAR program that will be reported in due course.

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Supplementary data

Supplementary data associated (CCDC 791341 contain the supplementary crystallographic data for compound (−)-**20b**. This data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.) with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.09.066.

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