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Novel azulene-based derivatives as potent multi-receptor tyrosine kinase inhibitors

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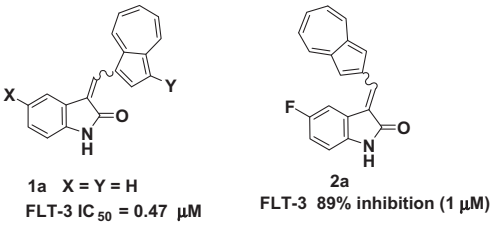
ABSTRACT

A series of azulene-based derivatives were synthesized as potent inhibitors for receptor tyrosine kinases such as FMS-like tyrosine kinase 3 (FLT-3). Systematic side chain modification of prototype **1a** was carried out through SAR studies. Analogue **22** was identified from this series and found to be one of the most potent FLT-3 inhibitors, with good pharmaceutical properties, superior efficacy, and tolerability in a tumor xenograft model.

Receptor tyrosine kinases (RTKs) are a large family of transmembrane receptors with diverse biological activity. At least 19 RTK subfamilies have been identified. One example is the platelet-derived growth factor receptor (PDGFR) subfamily. Members of this family include PDGFRα, PDGFβ, colony-stimulating factor-1 receptor (CSF-1R), FMS-like tyrosine kinase 3 (FLT-3), and c-KIT, and these are believed to promote angiogenesis and tumor cell growth. The constitutive activation of FLT-3 and c-KIT by mutation is directly associated with acute myeloid leukemia (AML) and gastrointestinal stromal tumors (GIST). FLT-3 is expressed on blast cells in most patients with AML, and internal tandem duplication (ITD) of the FLT-3 mutation has been found in up to 30% of all AML patients. The important role played by FLT-3 in the survival and proliferation of blast cells and its overexpression in most patients with AML make FLT-3 an attractive therapeutic target.[1–8] Rational design efforts in our laboratory identified an azulene-oxindole lead compound, designated **1a**, as a submicromolar inhibitor of FLT-3 (Fig. 1). Modifications of this lead structure, either by adding a side chain to an azulene moiety or by altering oxindole into azaoxindole, were investigated in this study.

The synthetic route for preparing azulene-oxindole-based derivatives is shown in Scheme 1. Treatment of commercially available tropolone with *p*-toluene sulfonyl chloride and triethyl amine yielded *p*-(tosyloxy)tropone **4**, whereas treatment of *p*-(tosyloxy)tropone with dimethyl malonate in the presence of sodium methoxide in toluene resulted in **5**.[9] Treatment of **5** with tetrabutyl ammonium hydroxide in alkaline solution produced **6**, which was then treated with dibromoalkane in acetonitrile to yield **7a** ($n = 1$) and **7b** ($n = 2$). Compound **7a** underwent [8+2] cycload-



1a X = Y = H
FLT-3 IC_{50} = 0.47 μM

2a
FLT-3 89% inhibition (1 μM)

Figure 1. Novel azulene-based compounds.

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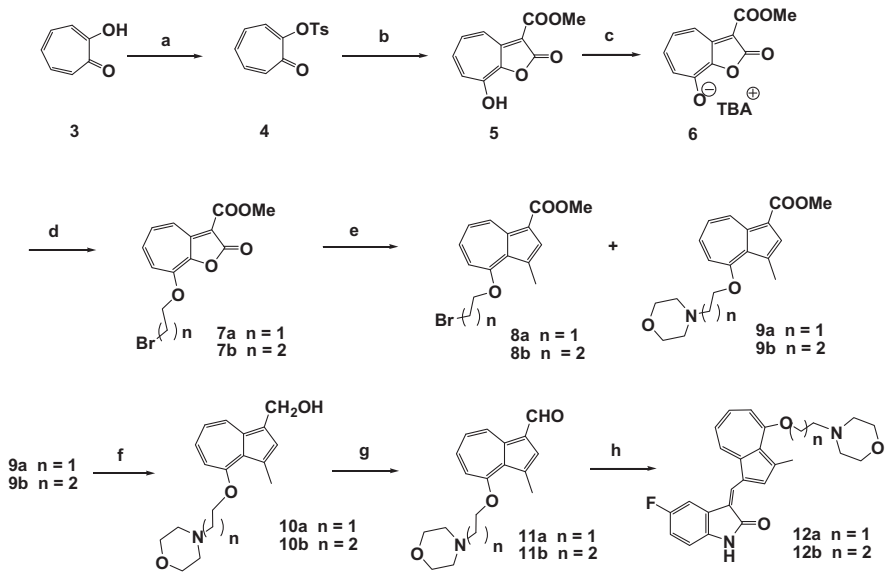


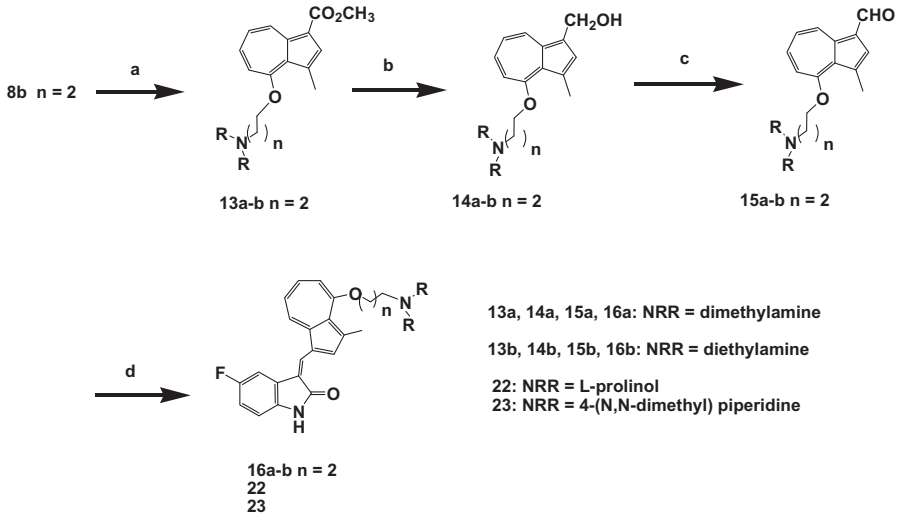
Scheme 1. Synthesis of compounds **12a** and **12b**. Reagents: (a) TsCl, Et_3N/CH_2Cl_2; (b) CH_2 $(COOMe)_2$, MeONa/MeOH, toluene; (c) $NaOH/H_2O$, tetrabutyl ammonium hydroxide; (d) 1,2-dibromoethane ($n = 1$), 1,2-dibromopropane ($n = 2$)/CH_3CN; (e) EtCHO, morpholine/toluene; (f) DIBAL/ether; (g) MnO_2/CH_2Cl_2; (h) 5-fluoro-2-oxindole/ethanol, piperidine.

dition with morpholine and propionyl aldehyde in toluene to produce the bromo compound **8a** and morpholine-substituted compound **9a**. Reduction of the methyl ester **9a** with DIBAL/heptane in ether formed the primary alcohol **10a** and subsequent oxidation with MnO_2/CH_2Cl_2 resulted in the aldehyde **11a**. The subsequent reaction of **11a** with 5-fluoroxindole yielded the desired compound **12a** in the single isomer (*Z*) form. Compound **12b** could be prepared by a procedure similar to that described for **12a**.

The bromo compound **8b** ($n = 2$) was treated with a selective amine to produce **13a–b**, and procedures b, c, and d were used to produce the desired products **16a–b** (Scheme 2). All these final products could be obtained in the single isomer form or as a mixture of the *E*/*Z* isomers. The latter could be separated by chromatography and were assigned by ^1H NMR experiments.[10]

The compounds were assayed for their activity against a panel of receptor tyrosine kinases, including FLT-3, c-KIT, and KDR. Given that FLT-3 plays a pivotal role in AML, our emphasis was on optimizing the potency of the compound against FLT-3. Compound **1a** was used as the lead compound in the series, and it showed moderate inhibitory activity against the FLT-3 enzyme in the submicromolar range (0.47 μM). The results of the SAR studies on **1a** are shown in Tables 1–3. We investigated the effects of different substituents on the oxindole and azulene rings. As shown in Table 1, fluoro substitution at the 5-position of oxindole and incorporation of a fluoro or methyl group at the 3-position of the azulene core moiety was essential for potency in the series (**1h**–**1j**). Replacement of the methyl group of **1i** (*Z*) with hydrogen resulted in loss of potency (**1b** (*Z*) >1000 nM). Removal of the fluoro group from **1i** (*Z*) yielded **1g** (*Z*), which had significantly lower inhibitory activity. This trend was also observed in the case of **1h** (*E*).

To improve the inhibitory activity against FLT-3, we explored the effects of attaching an alkylaminoalkyl ether link to the seven member ring (Table 2). Various terminal alkylamino groups were investigated, including cyclic and noncyclic tertiary amine groups. In most cases (X = 5-F, Y = C), a spacer length of 2–3 methylene units was tolerated (except **12b** (*Z*)) for FLT-3 inhibitory activity.



Scheme 2. Synthesis of compounds **16a-b**, **22**, and **23**. Reagents: (a) Amine/CH_3CN; (b) DIBAL/ether; (c) MnO_2/CH_2Cl_2; (d) 5-fluoro-2-oxindole/ethanol, piperidine.