Bioorganic & Medicinal Chemistry 22 (2014) 3171–3179

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Optimization of diaryl amine derivatives as kinesin spindle protein inhibitors

Tomoki Takeuchi [a], Shinya Oishi [a,*], Masato Kaneda [a], Ryosuke Misu [a], Hiroaki Ohno [a], Jun-ichi Sawada [b], Akira Asai [b], Shinya Nakamura [c], Isao Nakanishi [c], Nobutaka Fujii [a,*]

[a] Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan
[b] Graduate School of Pharmaceutical Sciences, University of Shizuoka, Suruga-ku, Shizuoka 422-8526, Japan
[c] Faculty of Pharmacy, Kinki University, 3-4-1 Kowakae, Higashi-osaka 577-8502, Japan

ARTICLE INFO

Article history:
Received 12 March 2014
Revised 5 April 2014
Accepted 5 April 2014
Available online 13 April 2014

Keywords:
Diaryl amine
Kinesin spindle protein
Aqueous solubility

ABSTRACT

Structure–activity relationship studies of diaryl amine-type KSP inhibitors were carried out. Diaryl amine derivatives with a pyridine ring or urea group were less active when compared with the parent carboline and carbazole derivatives. Optimization studies of a lactam-fused diphenylamine-type KSP inhibitor revealed that the aniline NH group and 3-CF_3 phenyl group were indispensable for potent KSP inhibition. Modification with a seven-membered lactam-fused phenyl group and a 4-(trifluoromethyl)pyridin-2-yl group improved aqueous solubility while maintaining potent KSP inhibitory activity. From these studies, we identified novel diaryl amine-type KSP inhibitors with a favorable balance of potency and solubility.

1. Introduction

Kinesins constitute a superfamily of molecular motor proteins to move along microtubules.[1] Mitotic kinesins are involved in cell division.[2] Non-mitotic kinesins are principally involved in intracellular transport of organelles and vesicles.[3] The kinesin spindle protein (KSP; also known as Eg5) is the mitotic kinesin that belongs to the kinesin-5 family. The structure of KSP is comprised of three parts: an N-terminal motor domain, a central α-helical coiled coil stalk domain, and a C-terminal tail domain.[4] The N-terminal motor domain contains a catalytic site for ATP hydrolysis and microtubule binding region. KSP moves toward the plus end of the microtubule, just like other kinesins with an N-terminal motor domain, using the energy generated from the hydrolysis of ATP.[5] The KSP movement is required for centrosome separation and bipolar spindle formation during cell division. Inhibition of KSP leads to mitotic arrest in the prometaphase with the formation of the monopolar spindle and subsequent apoptotic cell death.[6–9] Therefore, KSP inhibitors are expected to be favorable agents for cancer chemotherapy without neurotoxic side effects.[10–13]

Recently, we reported that carbazole derivative **1** with the 2-CF_3 group showed potent KSP inhibitory activity (Fig. 1).[14] Carbazole derivatives, with a lactam ring (**2**) or urea group (**4c**), and the β-carboline derivative **3a** were also identified as highly potent KSP inhibitors by structure–activity relationship studies of **1**.[15] However, these inhibitors exhibited limited solubility in the aqueous solvents employed for in vivo studies. To satisfy the potent inhibitory activity requirements as well as better solubility in aqueous solution, we have designed diphenylamine derivatives such as **5a** by modification of the planar carbazole-type inhibitor **2**.[16] Diphenylamine **5a** exhibited better solubility than carbazole **2** while maintaining potent KSP inhibitory activity. Structural analysis by single crystal X-ray diffraction studies and free energy

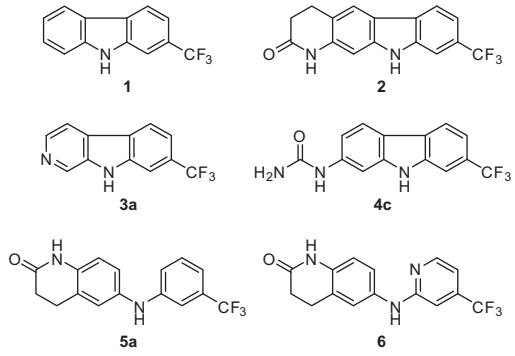


Figure 1. Structures of carbazole- and carboline-type (**1**–**4**) and diaryl amine-type (**5**, **6**) KSP inhibitors.

* Corresponding authors. Tel.: +81 75 753 4551; fax: +81 75 753 4570.
E-mail addresses: soishi@pharm.kyoto-u.ac.jp (S. Oishi), nfujii@pharm.kyoto-u.ac.jp (N. Fujii).

http://dx.doi.org/10.1016/j.bmc.2014.04.008
0968-0896/

calculations demonstrated that the improved solubility of **5a** is attributed to fewer van der Waals interactions in the crystal packing, as well as a hydrogen-bond acceptor nitrogen in the aniline moiety for favorable solvation. Interestingly, compound **5a** possibly binds to the interface of the α4 and α6 of KSP in an ATP-competitive manner, whereas most KSP inhibitors (e.g., monastrol, *S*-trityl-L-cysteine) bind to the allosteric pocket formed by helices α2, α3 and loop L5 to show ATP-uncompetitive behavior.[17–19] Replacing the right-hand 3-CF_3-phenyl group in **5a** with a pyridine ring provided a more soluble KSP inhibitor **6**; however, this compound showed slightly lower potency than **5a**.[16] In this article, we describe the structure–activity relationship study for novel diaryl amine-type KSP inhibitors with high potency and aqueous solubility. For this purpose, we performed: (i) modification of ring-fused indoles such as **3a** and **4c** using the same approach employed for the development of **5a** and (ii) intensive optimization studies of diphenylamine **5a**.

2. Results and discussion

2.1. Investigation of diaryl amine-type KSP inhibitors by modification of ring-fused scaffolds

We speculated that the poor solubility of carboline and carbazole derivatives would be attributable to the significant intermolecular interactions in the crystals (e.g., π–π stacking interactions) as seen for compound **2**.[16] To disrupt the possible crystal packing of compounds **3a** and **4c**, the design of less planar analogs was expected to be a promising approach.[20] Therefore, we designed diaryl amine derivatives **7** and **8**, in which the pyrrole C—C bond in the central part of carbolines **3** and carbazoles **4** was cleaved (Fig. 2). Diaryl amines **7a**,**b** with a pyridine ring were designed based on carbolines **3a**,**b** with potent KSP inhibitory activity (Fig. 2A). Diphenylamines **8a**–**f** with a nitro, amino or urea group at the 3- or 4-position on the left-hand phenyl ring were similarly investigated, which represent the cleaved analogs of carbazoles **4b**–**f** (Fig. 2B). Diaryl amine derivatives **7a**,**b** and **8a**,**d** were prepared by palladium-catalyzed *N*-arylation using aryl bromides **9** and substituted anilines **10** (Scheme 1).[21] For the preparation of compounds **8c**,**f** with a urea group, nitro derivatives **8a**,**d** were reduced to the corresponding amines **8b**,**e** using Pd/C and ammonium formate, which were converted to the expected compounds **8c**,**f** by KOCN.

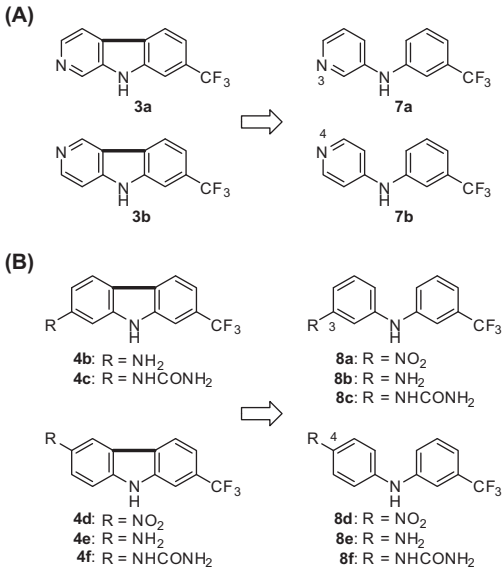
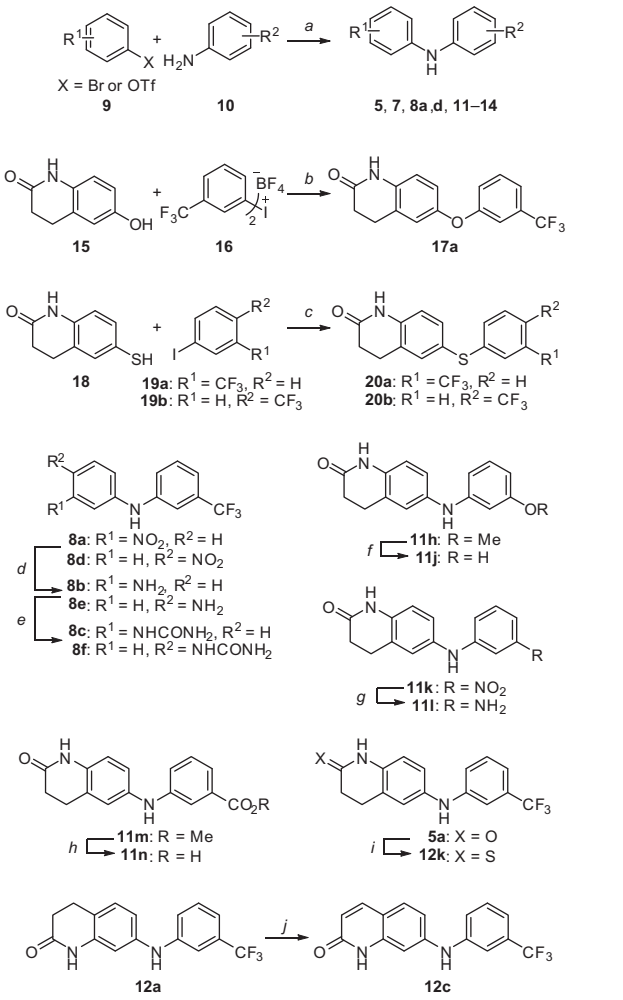


Figure 2. Design of novel KSP inhibitors **7**, **8** with diaryl amine scaffolds.



Scheme 1. Synthesis of diaryl amine derivatives. Reagents and conditions: (a) $Pd_2(dba)_3$, biaryl phosphine ligand, NaO*t*-Bu, toluene, 100 °C; (b) KO*t*-Bu, DMF, 40 °C; (c) CuI, ethylene glycol, K_2CO_3, 2-propanol, 80 °C; (d) Pd/C, HCO_2NH_4, EtOH, reflux; (e) KOCN, AcOH, H_2O, rt; (f) BBr_3, CH_2Cl_2, rt; (g) Zn, AcOH, rt; (h) LiOH·H_2O, MeOH, H_2O, 50 °C; (i) Lawesson's reagent, toluene, reflux; (j) $Pd(OAc)_2$, O_2, AcOH, 115 °C.

First, KSP inhibitory activity of compounds **7a**,**b**, with a pyridine ring in the left-hand part, was comparatively assessed with the parent carboline-type inhibitors **3a**,**b** (Table 1). Unfortunately, the cleavage of the pyrrole C—C bond in carboline led to loss of KSP ATPase inhibitory activity at 6.3 μM. The solubility of these compounds was evaluated by a thermodynamic method.[22] A mixture of EtOH–phosphate buffer (pH 7.4) (1:1) [50% EtOH] and phosphate buffer (pH 7.4) were employed as aqueous media.[22] In these solutions, the parent carbazole-type inhibitor **1** was moderately soluble (0.424 mg/mL) and insoluble (<1 μg/mL), respectively. *N*-(Pyridin-3-yl)amine **7a** showed the anticipated improvement in thermodynamic solubility, being 30 times more soluble in 50% EtOH (14.3 mg/mL) compared with the corresponding carboline **3a**. *N*-(Pyridin-4-yl)amine **7b** also exhibited approximately 14 times greater solubility in 50% EtOH (24.0 mg/mL) than the parent carboline **3b**. Of note, compound **7b** had moderate solubility (264 μg/mL) even in phosphate buffer, which was 80 times or more soluble compared with **3b** and **3a**, respectively. Although these pyridinylamine derivatives **7a**,**b** were inert in KSP inhibition, it was suggested that cleavage of the pyrrole C—C bond in carboline