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Original article

Design, synthesis and biological evaluation of indole derivatives as novel inhibitors targeting B-Raf kinase

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ABSTRACT

A series of novel indole derivatives were designed and synthesized and their inhibitory activity against B-Raf and HepG2 cell were also described. Among them, compounds **7a** and **7b** exhibited excellent potency, which showed the potential for further research as lead compounds.

1. Introduction

The RAS-RAF-MEK-ERK signal transduction pathway plays a critical role in tumor biology. This pathway is controlled by extracellular signals through membrane receptors such as receptor tyrosine kinases and is activated by oncogenic mutations in many types of cancer [1–4]. The most common mutation, V600E mutant (B-Raf^{V600E}) has been demonstrated *in vitro* to be constitutively active in carcinoma cells and to increases cell proliferation and survival [5–7]. Over the past decade extensive efforts have been devoted to developing B-Raf kinase inhibitors and there were three successful examples (Fig. 1). Sorafenib (Bayer/Onyx) was approved by the US Food and Drug Administration (FDA) for the treatment of hepatocellular carcinoma and renal cell carcinoma; Regorafenib was also approved for the treatment of advanced colorectal cancer several months ago and marketed by Bayer and Onyx, and its structure is very similar to that of Sorafenib. Different from Sorafenib and Regorafenib as multi-kinase inhibitors, Vemurafenib (Plexxikon/Recho) is a selective B-Raf^{V600E} kinase inhibitor, which was approved to treat patients with advanced or metastatic melanoma. So the B-Raf kinase deserves more attention.

Employing receptor-based drug discovery approaches, our group found that there was a small hydrophobic pocket in B-Raf kinase beside the spot where the center ring of Sorafenib binds in a binding model, so a methyl or ethyl group was attempted at this position to increase the activity. The results evaluated by Discovery Studio were shown in Fig. 2a. They both superimpose well with Sorafenib. Then, we discovered that using an indole to replace the phenyl ring could better occupy the hydrophobic pocket, because the volume of pyrrole was similar to that of an ethyl group. Therefore, the compound **A** (Fig. 3) was synthesized. Furthermore, we found that if a methylene was introduced between the indole ring and the pyridine ring, the overlay of our hit compound and Sorafenib was better compared with compound **A** (Fig. 2b), though only one rather than two NH in the urea moiety formed hydrogen bond with side residue in Glu501. Moreover, many articles reported that only two hydrogen bonds were sufficient to produce the targeted binding affinity [8]. The design procedure was exhibited in Fig. 3.

2. Experimental

The commercially available ethyl isonicotinate **1** (5.00 g, 33.08 mmol) was dissolved in *N*-methylformamide (20 mL), and

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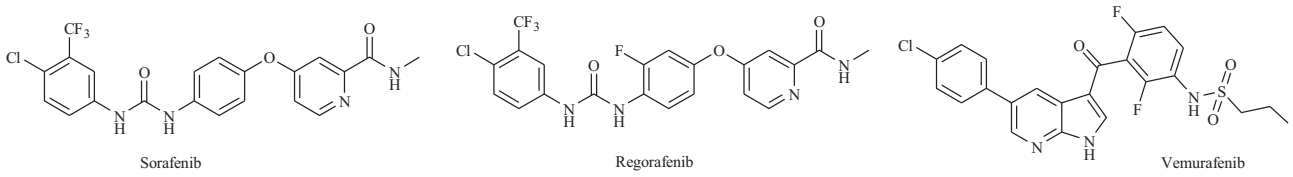

Fig. 1. Structures of Sorafenib, Regorafenib and Vemurafenib.

cold 98% H_2SO_4 (3.24 g, 33.08 mmol) was added drop wise and the temperature was kept below 5 °C. After the addition of H_2SO_4, 30% H_2O_2 and a saturated solution of $FeSO_4$ were added to the reaction mixture at 10–15 °C [9] until compound **1** disappeared. After extraction, washing, recrystallization, the pure compound **2** (4.04 g, 19.40 mmol, 58.64%) was obtained.

A mixture of compound **2** (2.08 g, 10 mmol) and sodium borohydride (1.16 g, 30 mmol) in ethylalcohol (50 mL) was allowed to stir overnight at room temperature, then the mixture was concentrated in vacuo and the purification of the curde product using silica gel chromatography, eluting with 80% acetoacetate in *N*-hexane afforded the compound **3** (1.58 g, 9.49 mmol, 95.03%) [10].

Compound **3** (664 mg, 4 mmol) was dissovled in anhydrous THF (20 mL), then triphenylphosphine (1.10 g, 4.20 mmol) and carbon tetrabromide (1.39 g, 4.20 mmol) were added. The reaction mixture was stirred at room temperature for 3 h. The mixture was then filtered to remove the precipitates and the filtrate was concentrated to an oil. The crude product was purified using flash silica chromatography (eluting with 80% acetoacetate in *N*-hexane) to afford the compound **4** (477 mg, 2.09 mmol, 52.34%) [11].

A mixture of 5-nitro-1*H*-indole (200 mg, 1.23 mmol) or its derivatives and dry DMF (5 mL) was cooled to 0 °C and NaH (60 mg, 1.23 mmol) was added. After the addition, the reaction mixture was stirred at r.t. for 0.5–1 h, then compound **4** (282 mg, 1.23 mmol) was added, and the reaction mixture was allowed to stirred for an additional 2 h. After a sequence of extraction, wash, and concentration, the crude product compound **5** (222 mg, 0.72 mmol, 58.30%) was obtained and could be used in next step without further purifications [12].

Compound **5** (222 mg, 0.72 mmol) was dissolved in MeOH (10 mL), and 5%Pd/C (10 mg) was added, then H_2 was ventilated. After 0.5 h the reaction completed. Pd/C was removed by filtration, the filtrate was condensed in vacuo and chromatographic purification of the curde product over silica gel, eluting with 50% acetoacetate in *N*-hexane afforded the compound **6** (194 mg, 0.69 mmol, 96.5%).

A mixture of 4-chloro-3-(trifluoromethyl)aniline or its derivatives and CDI in dry CH_2Cl_2 (5 mL) was stirred for 1 h at room temperature, then compound **6** (194 mg, 0.69 mmol) or its derivatives was added. The mixture was allowed to stir for an additional 1 h to afford white precipitates. The solid was collected by filtration under vacuum, washed with water for three times and dried under vaccum to give compound **7a**–**7m** and **7p**–**7r** [13], but **7m** and **7n** was synthesis by a condensation reaction between benzoic acid and compound **6** promoted by EDCI. The yields of all compounds were between 65.7% and 82.4%. The general synthetic route is depicted in Scheme 1.

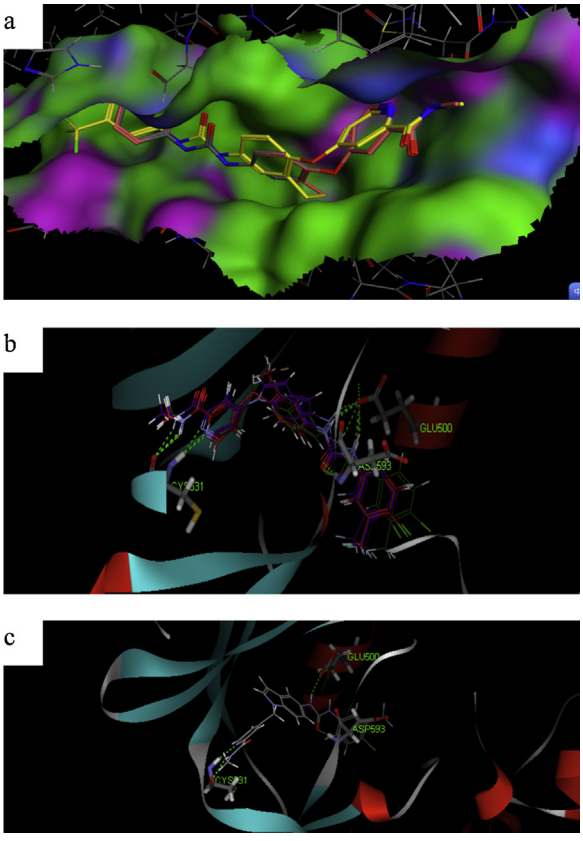

Fig. 2. (a) Predicted binding model (PDB: 1UWH) of Sorafenib (gray) and its derivatives with a methyl (brown) or ethyl (yellow) substitution center phenyl ring. (b) Overlap binding model of our hit compound (green) with compound A (purple), Sorafenib (blue) and its derivatives with a methyl (red) or ethyl (brown) substitution center phenyl ring. (c) The binding model of compound **7b**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

7a: 273 mg, 78.6%, mp 209 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.77 (d, 1H), 5.57 (s, 2H,), 6.50 (d, 1H, J = 2.94 Hz), 7.09 (d, 1H, J = 8.97 Hz), 7.27 (m, 2H, J = 3.00 Hz, J = 8.97 Hz), 7.51 (m, 1H), 7.58 (m, 2H), 7.73 (d, 2H,), 8.12 (s, 1H), 8.53 (dd, 1H, J = 6.15 Hz, J = 2.85 Hz), 8.60 (s, 1H), 8.73 (dd, 1H, J = 6.15 Hz, J = 2.85 Hz), 9.06 (s, 1H); ESI (m/z): 501.6 [M+H]. Anal. Calcd. for $C_{24}H_{19}ClF_3N_5O_2$: C, 57.43; H, 3.82; N, 13.95. Found: C, 57.67; H, 3.94; N: 13.79.

7b: 267 mg, 75.9%, mp 248 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.78 (d, 1H), 5.53 (s, 2H), 7.13 (d, 1H, J = 9.32 Hz), 7.25 (m, 1H), 7.37 (m, 1H, J = 9.32 Hz), 7.51 (m, 1H), 7.59 (m, 1H), 7.63 (m, 2H), 7.76 (m, 2H, J = 2.22 Hz, J = 5.28 Hz), 8.12 (d, 1H, J = 2.22 Hz), 8.56 (m, 1H, J = 5.28 Hz), 8.72 (m, 2H), 9.10 (s, 1H); ESI (m/z): 520.1 [M+H]. Anal. Calcd. for $C_{24}H_{18}ClF_4N_5O_2$: C, 55.45; H, 3.49; N, 14.62. Found: C, 55. 54; H, 4.02; N: 14.63.

B-Raf kinase assay: Kinase activity was measured as the percentage of ATP consumed following the kinase reaction using luciferase-luciferin-coupled chemiluminescence system. Reactions were conducted in a 384-well plate (PerkinElmer). B-Raf kinase reactions were initiated by adding test compounds (1 μL/well) and B-Raf kinase (4 μL/well, Signalchem) to the 384-well plate. The assay plate was centrifuged with 1000 rpm for 1 min to mix them and then pre-incubated at 30 °C for 30 min. ATP-Unactive MEK1 mixture (5 μL/well, obtained by mixing an equal volume of ATP and inactive MEK1, Sigma and Signalchem, respectively) was added to the assay plate. The assay plate was centrifuged with 1000 rpm for 1 min and incubated at 30 °C for 1 h. ADP-Glo reagent was added to each well and the assay plate was incubated at 27 °C for 30 min. Finally, the luminescence signal was