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Short communication

Determination of CH330331, a novel 4-anilinoquinazoline inhibitor of epidermal growth factor receptor tyrosine kinase, in human Caco-2 monolayers by high performance liquid chromatography with ultraviolet detection: Application to a trans-epithelial transport study

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Abstract

4-Anilinoquinazolines (e.g. Iressa and Glivec) are a class of epidermal growth factor receptor tyrosine kinase (EGFR-TK) inhibitors widely used to treat non-small cell lung cancer and other tumors. However, low clinical response rate, resistance, and host toxicity of currently available EGFR-TK inhibitors prompt the development of second generation of TK inhibitors with improved efficacy, selectivity, and less resistance. CH330331 is a recently synthesized novel 4-anilinoquinazoline analog with confirmed anticancer activity in vitro and in vivo. To predict its oral pharmacokinetic behavior and transport nature in the intestine before entering clinical trials, we have developed and validated a high performance liquid chromatographic (HPLC) method for the determination of CH330331 in Caco-2 (a human colon cancer cell line) monolayers. The developed HPLC method was sensitive and reliable, with acceptable accuracy (90–110% of nominal values) and precision (intra- and inter-assay R.S.D. < 10%). The total running time was within 10 min, with acceptable separation of the target analytes. The lower limit of quantitation (LLOQ) value for CH330331 was 200 ng/ml when an aliquot of 100 μl sample was injected onto the HPLC. The validated HPLC method was applied to characterize the epithelial transport of CH330331 in Caco-2 monolayers. The transport of CH330331 across the Caco-2 monolayers from the apical to basolateral side was 8- to 10-fold higher than that from the basolateral to apical side. Co-incubation of sodium azide or MK-571, but not verapamil, significantly inhibited the apical to basolateral transport of CH330331. These findings provide initial evidence that the intestinal absorption of CH330331 is mediated by an active mechanism. Further studies are required to explore the interaction of CH330331 with ATP-binding cassette transporters and the possible influence on its pharmacokinetics and pharmacodynamics.

Keywords: HPLC; Validation; CH330331; Caco-2 cells

Abbreviations: AP, apical; BCRP, breast cancer resistance protein; ANOVA, one-way analysis of variance; BL, basolateral; CV, coefficient of variation; EGFR, epidermal growth factor receptor; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulphoxide; HBSS, Hanks' balanced salt solution; HEPES, *N*-[2-hydroxyethyl]piperazine-*N*′-[4-butanesulfonic acid]; LLOQ, lower limit of quantitation; LOD, limit of detection; MK-571, 3-[[[3-[2-(7-chloro-2-quinolinyl)-(E)-ethenyl]phenyl][[3-(dimethylamino)-3-oxopropyl]thio]methyl]thio]propionic acid; MRP, multidrug resistance associated protein; MTT, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide; P_{app}, permeability coefficient; P-gP, P-glycoprotein; QC, quality control; TK, tyrosine kinase; TEER, transepithelial electrical resistance

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1. Introduction

Multiple components of mitogenic signaling pathways in normal and neoplastic cells have been identified, including the large family of protein kinases, which serve as critical components of signal transduction pathways. These protein kinases play a critical role in diverse biological processes, such as control of cell growth, metabolism, differentiation, and apoptosis [1]. The signal transduction of human epidermal growth factor receptor (EGFR) tyrosine kinases (TKs) that is frequently expressed in epithelial tumors is closely associated with tumor growth, angiogenesis, invasion, and metastasis [2–4]. The EGFR was the first receptor to be proposed as a target for cancer therapy [5], and recent insight into the role of receptor TK function in cancer cells culminated in the design of highly selective TK inhibitors. After two decades of intensive research, a series of antineoplastic agents have been developed and synthesized which preferentially inhibit EGFR tyrosine kinase [6,7]. To date, there are several anti-EGFR agents available for chemotherapy in the clinic [4]. These compounds have been widely used as oral agents to treat multifold tumors, including breast cancer, stomach cancer, ovary cancer, small cell lung cancer, and non-small cell lung cancer [4]. 4-Anilinoquinazolines, including gefitinib (Iressa), imatinib melysate (Glivec), and erlotinib (Tarceva) (Fig. 1), are a class of orally available synthetic small molecules designed to bind to the intracellular kinase domain of TKs [8–12]. These compounds are competitive inhibitors at the ATP binding site [13]. Treatment of appropriately selected patients with these drugs can alter the natural history of their disease and improve survival with a response rate of 5–10% in non-small cell lung cancers with activating mutations within the EGFR kinase domain [7]. However, despite the marked antitumor effect in animal studies, clinical response to these compounds is still poor for a high proportion of the cancer patients after failure of at least one prior chemotherapy regimen [7]. Furthermore, the cancer cell may develop resistance to these TK inhibitors due to acquired mutations in the *EGFR* gene [14,15]. All currently available TK inhibitors have some minor to moderate host toxicities [7]. Therefore, there is a need to develop second generation of novel anti-EGFR agents and TK inhibitors with advantages of lesser resistance and higher efficacy compared to first generation compounds. In a hope of identifying new TK inhibitors with improved specificity, selectivity and clinical efficacy, CH330331, an analog of 4-anilinoquinazoline, has been recently synthesized by the Sun Yat-sen University Cancer Center (Guangzhou, China). Its chemical structure is shown in Fig. 1.

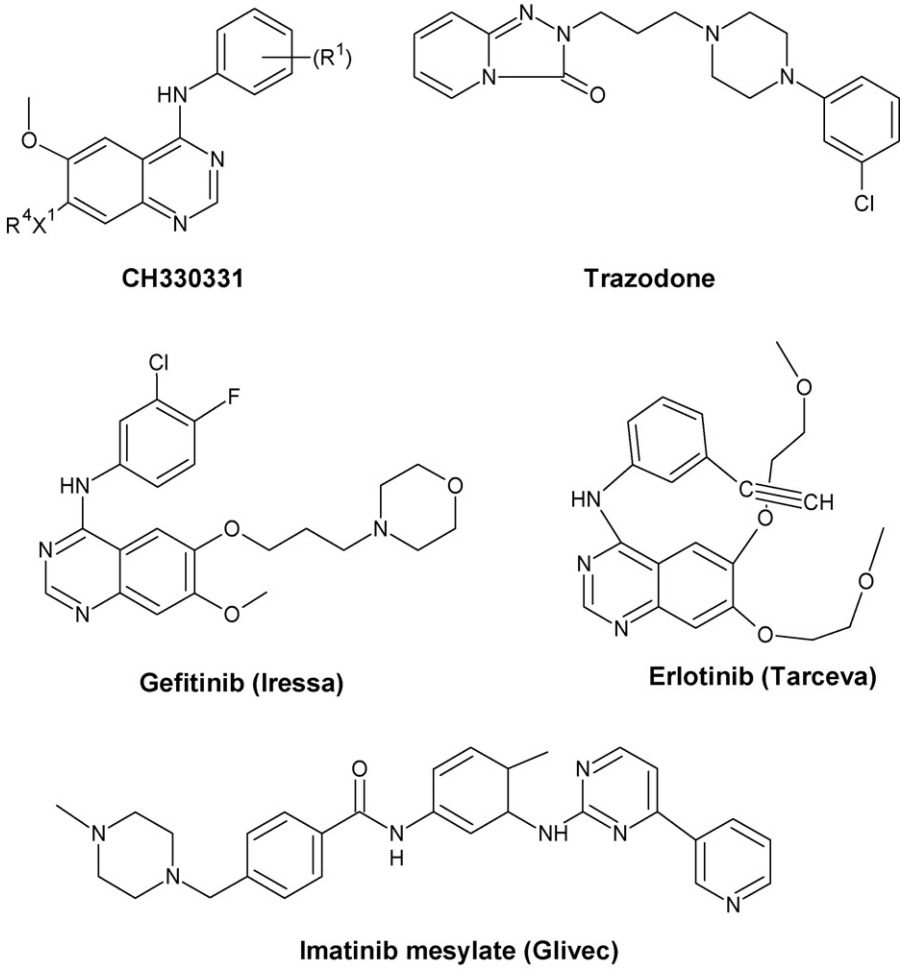

Fig. 1. Chemical structures of CH330331, trazodone (used as an internal standard), gefitinib (Iressa), Erlotinib (Tarceva) and imatinib mesylate (Glivec).