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Synthesis and biological evaluation of fluoro-substituted 3,4-dihydroquinazoline derivatives for cytotoxic and analgesic effects

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ABSTRACT

As a bioisosteric strategy to overcome the poor metabolic stability of lead compound KYS05090S, a series of new fluoro-substituted 3,4-dihydroquinazoline derivatives was prepared and evaluated for T-type calcium channel ($Ca_v3.2$) block, cytotoxic effects and liver microsomal stability. Among them, compound **8h** (KCP10068F) containing 4-fluorobenzyl amide and 4-cyclohexylphenyl ring potently blocked $Ca_v3.2$ currents (>90% inhibition) at 10 μM concentration and exhibited cytotoxic effect (IC_{50} = 5.9 μM) in A549 non-small cell lung cancer cells that was comparable to KYS05090S. Furthermore, **8h** showed approximately a 2-fold increase in liver metabolic stability in rat and human species compared to KYS05090S. Based on these overall results, **8h** (KCP10068F) may therefore represent a good backup compound for KYS05090S for further biological investigations as novel cytotoxic agent. In addition, compound **8g** (KCP10067F) was found to partially protect from inflammatory pain via a blockade of $Ca_v3.2$ channels.

1. Introduction

Calcium (Ca^{2+}) as a second messenger plays a vital role in cellular physiology and biochemistry, such as proliferation, differentiation, growth, cell death and apoptosis.[1–4] Thus, alterations in calcium signaling can cause defects in cell growth and are associated with certain types of cancer.[5–7] A number of research groups have suggested a potential role for voltage-activated Ca^{2+} channels, in particular T-type (Ca_v3), in the regulation of tumor growth and progression.[8–14] This T-type Ca^{2+} channel family (Ca_v3) contains three members: $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$.[15] A growing number of reports show that T-type Ca^{2+} channels are abnormally overexpressed in many types of human cancers compared to normal cells.[16,17] Therefore, T-type Ca^{2+} channel blockers can be regarded as new tools in cancer therapies.[18,19]

We have already reported that 3,4-dihydroquinazoline derivatives exhibited both strong T-type calcium blocking and anti-cancer effects.[20–25] An optimization study resulted in the lead compound KYS05090S for non-clinical studies.[26] We explored the *in vitro* metabolic stability of KYS05090S in mouse, rat, dog, and human liver microsomes using a single point metabolic assay.[27,28] Briefly, KYS05090S was incubated at a concentration of 1.0 μM with 1.0 mg/mL protein at 37 °C for 30 min. It showed lower metabolic stability with less than 30% remaining in liver microsomes of three species except dog (Table 1). *In vivo* pharmacokinetic (PK) studies of KYS05090S led to very low oral bioavailability in dog (*F*, 1.27%) and monkey (*F*, 2.01%) animals together with the formation of primary metabolites as shown in Fig. 1 (unpublished results).

To overcome this issue, herein, we decided to insert a fluorine atom as a bioisostere of hydrogen atom into various regions of 3,4-dihydroquinazoline scaffold hoping that the small fluorine atom will not impair the binding of new synthetic 3,4-dihydroquinazoline derivatives to the target T-type Ca^{2+} channel. The rapid

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Table 1
In vitro liver microsomal stability of KYS05090.[a]

Compound	% remaining after 30 min incubation			
	Human	Dog	Rat	Mouse
KYS05090S	16.6	>100	24.2	27.6

[a] The metabolic stability was expressed as percent remaining compound after 30 min incubation in liver microsomal enzyme.

oxidative metabolism by the liver enzymes, in particular the P450 cytochromes, is often found to limit bioavailability. A frequently employed strategy to circumvent this problem is to block the reactive site by the introduction of a fluorine atom.[29] In fact, this bioisosteric strategy is one of the most commonly used methods for developing new compounds with improved pharmacodynamics and/or pharmacokinetic properties and thus has led to many successful drugs.[29–31]

2. Results and discussions

2.1. Chemistry

A series of fluoro-substituted 3,4-dihydroquinazoline derivatives (**8**) except **8f** in Table 2 was prepared using a Heck reaction (for **8d** and **8j**)[32] and the procedure described previously by our group.[20,33] New compound **8f** (KCP10066F) was easily synthesized according to the procedure as shown in Scheme 1: Methyl 2-nitrocinnamate **1** was reduced with Zn (powder)/NH_4Cl into 2-aminocinnamate **2,** which was coupled with isocyanate (*in situ* prepared from 4-fluorobiphenyl-4-carboxylic acid **3** via Curtius rearrangement) to provide a urea **4**. The dehydration of **4** with $Ph_3P{\cdot}Br_2$ and Et_3N provided a carbodiimido **5**, which was subsequently coupled with piperazine compound **6** afforded 3,4-dihydroquinazoline ester **7**. The treatment of **7** with 5-fluorobenzylamine and 1,5,7-triazabicyclo[4.4.0] dec-5-ene (TBD) as a catalyst afforded the final compound **8f** (KCP10066F) under solvent-free condition.

2.2. Biological evaluation

All compounds were tested for their abilities to block transiently expressed human $Ca_v3.2$ ($hCa_v3.2$) currents by using whole-cell patch clamp recordings in HEK cells.[34] Most of compounds except **8a** and **8f** potently blocked $Ca_v3.2$ currents (>90% inhibition) at 10 μM concentration (Fig. 2A). Compound **8a** (KCP10048F) with a relatively short 4-isopropylphenyl ring at R^4 position was almost inactive, which was consistent with the reported result of SAR studies on the 3.4-dihydroquinazoline scaffold.[35] In contrast, compound **8d** (KCP10060F) and **8g** (KCP10067F) completely blocked $Ca_v3.2$ currents and their IC_{50} values determined from the fit to the dose-response curve were 5.6 and 5.1 μM, respectively, when cells were held at a hyperpolarized potential of −110 mV [Fig. 2C and D]. While the dose-response curve for compound **8d** could be fitted with a Hill coefficient of 0.8, that for compound **8g** required a Hill coefficient of 0.14. This suggests that block by **8g** required the interaction of more than one drug molecule with the channel and negative cooperativity. With respect to their channel selectivity, the two compounds showed poor $Ca_v3.2$ (T-type)/$Ca_v2.2$ (N-type) and (T-type)/$Ca_v1.2$ (L-type) calcium channel selectivity (Fig. 2B), suggesting that they are broad spectrum inhibitors of calcium channel activity.

Current–voltage relationship experiments for both compounds were acquired for $Ca_v3.2$ channels transiently transfected into HEK-293 cells by stepping to a series of test potentials ranging from −60 mV to +60 mV in 10 mV steps (lasting for 100 ms). Fig. 3A and B display averaged IV-traces from cells before and after the application of 5 μM **8d** (KCP10060F) and **8g** (KCP10067F), respectively. Each compound caused a prominent reduction of peak current density compared to the control across a range of test potentials. However, neither compound **8d** (KCP10060F) nor **8g** (KCP10067F) caused a shift in the half-activation potential.

A two-step voltage-clamp protocol was applied for determining the effects of the compounds on steady-state inactivation of $Ca_v3.2$. This involved an inactivating pre-pulse period that varied from

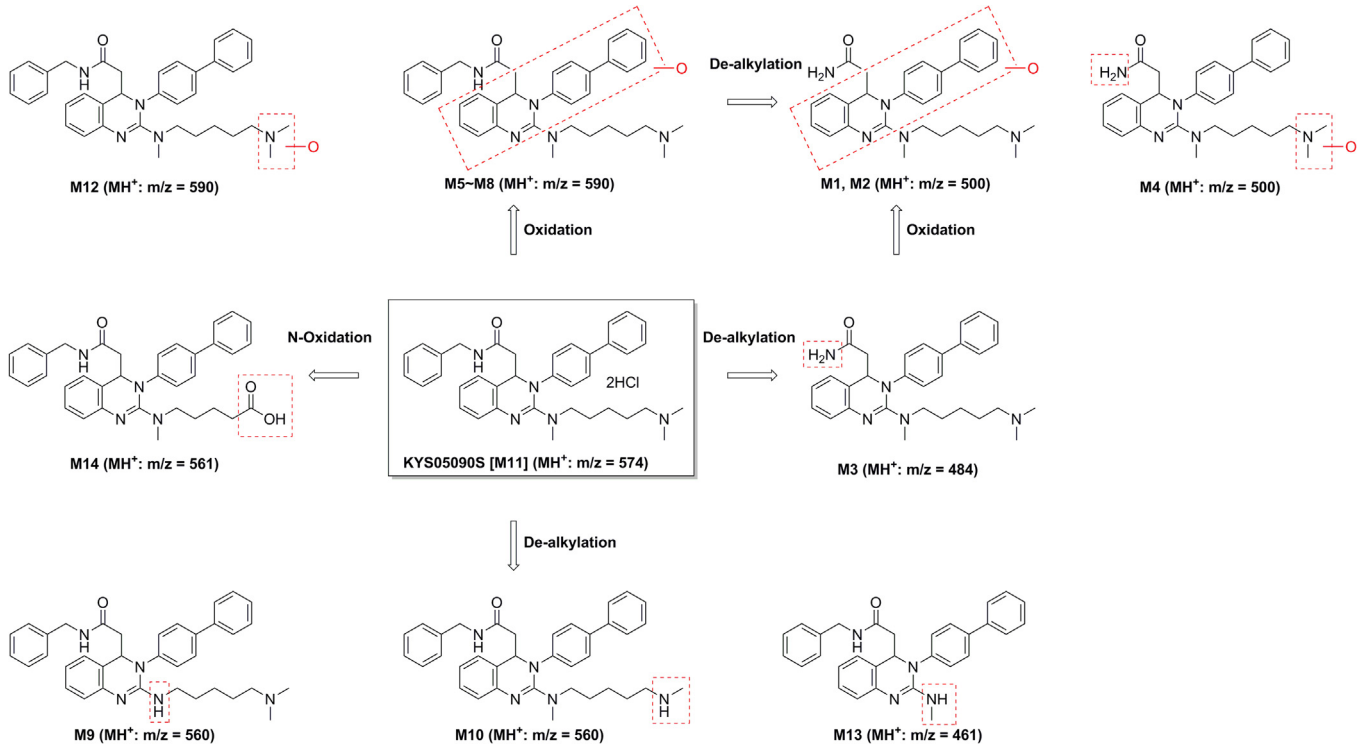

Fig. 1. The proposed metabolic pathways and identified metabolites of KYS05090S in human, monkey, dog, rat and mouse liver microsomes.