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In vivo evaluation of oral anti-tumoral effect of 3,4-dihydroquinazoline derivative on solid tumor

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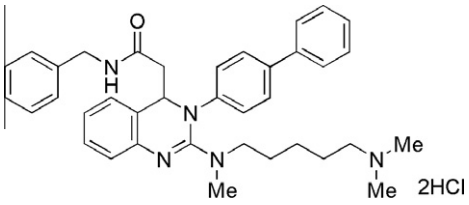
ABSTRACT

An extension of our previously reported 3,4-dihydroquinazoline derivative is investigated. Oral anti-tumoral activity of 3,4-dihydroquinazoline derivative (**KYS05090**) as potent and selective T-type calcium channel blocker was in vivo evaluated against A549 xenograft in BALB/$c^{nu/nu}$ nude mice. The rate of tumor volume increment in mouse model with **KYS05090**-treated group was remarkably slower than that of control group. With respect to tumor weight, it exhibited 60% and 67% tumor growth inhibition through oral administration of 1 and 5 mg/kg of bodyweight, respectively, compared to control and was more potent than paclitaxel (53%). In addition, **KYS05090** (10 and 50 mg/kg, po) was found to have a marked analgesic effect in acetic acid-induced writhing test, whereas it did not show any effect on hot plate test.

For several decades, medical oncologists have treated most of their patients with intravenous (iv) anticancer drugs, and consequently hospital services and clinical activities have been organized on the basis of this type of administration.[1] However, in the last six years the therapeutic scenario has been characterized by a steady increase in the availability of oral anticancer drugs with more than twenty oral anti-neoplastic drugs currently approved for use in United States and Europe.[2,3] Moreover, one-quarter of all anticancer agents under development are oral drugs.[4] The use of an oral anticancer therapy affects many relevant aspects of the clinical practice.

Calcium ion channels play a role in regulating calcium signaling during cell proliferation.[5] Among them, T-type calcium channel is particularly the predominant influx mechanism in most solid cancers by which extracellular calcium enters.[6] It was also found that the T-type calcium channel blockers mibefradil and pimozide have anti-proliferative effects on the mitogenic cell lines.[7] Recently, we reported that our 3,4-dihydroquinazoline derivative **1** (**KYS05090**: Fig. 1) as a selective T-type calcium channel blocker exhibited 49% tumor-weight inhibition compared with vehicle alone against A549 xenograft in BALB/$c^{nu/nu}$ nude mice through intravenous administration of 2 mg/kg of body weight (Table 1).[8]

Based on both the recent shift from iv to oral drug administration in oncology and the lower oral acute toxicity (LD_{50} = 693 mg/kg) and the higher oral bioavailability (F = 98%) of **KYS05090**,[8] we decided to evaluate the oral anti-tumoral effect of **KYS05090** on solid tumor using A549 xenograft in BALB/$c^{nu/nu}$ nude mice in comparison with paclitaxel as positive control.[9–11] A549 cells (5×10^6 cells/mL) were injected subcutaneously (s.c.) into the left flank region of BALB/$c^{nu/nu}$ nude mice and the tumors were allowed to grow. When tumor nodules were clearly visible (ca. 3 mm^3) after 8 days, **KYS05090** with two doses, 1 and 5 mg/kg in co-solvent (Ethanol: Cremophor EL: Distilled Water = 1:1:18) were orally (po) administered five times per week for consecutive 39 days and paclitaxel with 10 mg/kg dose in co-solvent was administered through intraperitoneal (ip) injection twice per week during the



KYS05090 (1)

Figure 1. Structure of **KYS05090**.

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Table 1
The pharmacological profiles of **KYS05090**

Calcium channel blocking (IC_{50})		Growth inhibition of cancer cells (GI_{50})	Tumor weight inhibition[a]	LD_{50}[b]		F[c]
T-type (α_{1G})	N-type (α_{1B})	A 549 (lung)	A549 xenograft	po	iv	
0.041 μM	4.9 μM	0.17 μM	49%	693 mg/kg	40 mg/kg	98%

[a] Percentage of tumor-weight inhibition versus control at 2 mg/kg dose (once/day).
[b] Acute toxicity post single administration on ICR mice.
[c] *F* denotes bioavailability.

same period. Tumor lengths and widths were measured weekly using a caliper and tumor volume was calculated as 1/2 × length × width × height until animal sacrifice. The results are documented in Figure 2 and Table 2. Figure 2a displays the effects of **KYS05090** on tumor growth in nude mice presented as tumor volume over time. There was statistically significant dose-dependent decrease in tumor volume with increasing administered dose. The rates of tumor volume increment in mouse model with both **KYS05090**-treated groups were remarkably slower than that of control group (vehicle only). Specifically, **KYS05090** at 1 mg/kg dose showed the strong potency equal to paclitaxel as positive control and in particular **KYS05090** at 5 mg/kg dose exhibited ca. threefold efficacy than paclitaxel at 39th day as shown in Figure 2a. At the end of experiment, all mice were weighed and sacrificed, and their tumors were excised. Tumors were weighed and the mean tumor weight was calculated. The tumor growth inhibition rates (TGIR) were calculated as follows: Tumor growth inhibition rates (TGIR,%) = 100 × (*C* − *T*)/*C*, where *T* is the average tumor weight of the treated and *C* the average tumor weight of the control (vehicle only). The results presented in Figure 2b and Table 2 showed that **KYS05090** exhibited better efficacy against A549 xenograft in BALB/$c^{nu/nu}$ nude mice than paclitaxel at both dosages: 60% and 67% inhibition at 1 and 5 mg/kg dose of **KYS05090**, respectively, compared to 53% inhibition of paclitaxel. With respect to body weight loss related with its chronic toxicity, there was no statistical difference between average total body weights in mice treated with **KSY05090**, paclitaxel (positive control) and vehicle (control). On the other hand, a little of body weight loss in mice treated with 5 mg/kg dose of **KSY05090** was observed during 19th–22nd day but the body weight was normally recovered after then as shown in Figure 2c. The mortality rate in group treated with **KYS05090** was zero during the experimental period, which supports the lower toxicity or the safety of **KYS05090**. This revealed that **KYS05090** may have a tumor suppressor function in human lung cancer cells and could be a promising treatment in oral anticancer therapy.

On the other hand, recent studies have indicated that T-type calcium channels are present throughout the pain pathway and may contribute to both peripheral and central sensitization.[12,13] Additionally, some observations suggest that pharmacological blockade of T-type channels may attenuate pain without significant effects.[14] Based on these studies, we decided to evaluate the peripheral and central antinociceptive effects of **KYS05090** because it is a potent T-type calcium channel blocker. The acetic acid-induced writhing test and hot plate test is generally used for the evaluation of peripheral and central antinociceptive activities, respectively.[15,16] It has also been reported that acetic acid-induced pain is related to peripheral nociceptive neurons[17] and heat induced pain is sensed by upper central nervous system.[18] For the evaluation of its peripheral antinociceptive effects, therefore, **KYS05090** with two doses (10 and 50 mg/kg) was orally administered and after 1 h, mice were injected ip with a 0.7% (v/v) acetic acid solution (10 mL/kg of body weight) as an irritant stimulus. After a 5-min period, the number of writhes was recorded for 20 min. In the case of its central antinociceptive effect, **KYS05090** with the same doses was orally administered and after 1 h, mice were kept on the heated surface of the plate (55 ± 0.5 °C) and the time of latency was measured for each group at 0 and 30 min.

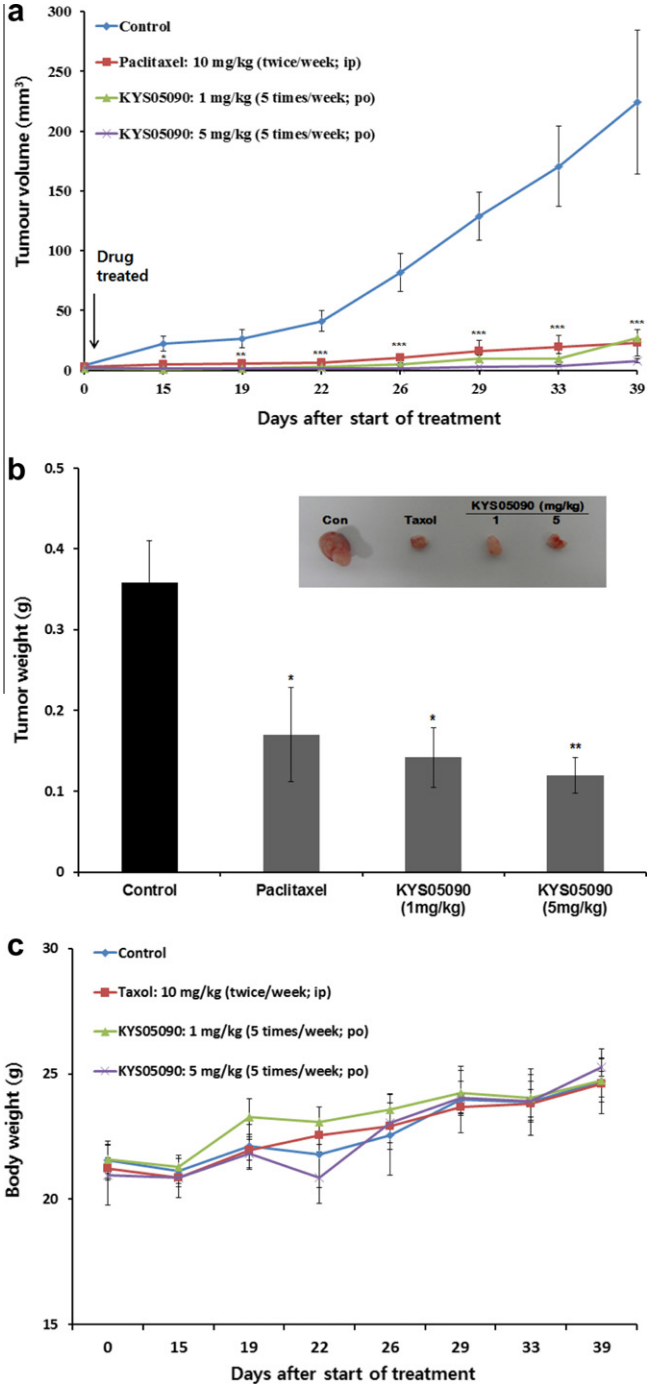


Figure 2. Oral anti-tumoral effect of **KYS05090** in mouse xenografts models. A549 cells (5×10^6) were injected subcutaneously into the left flank of nude mice. Mice were separated into four groups ($n = 5$ per each group). After 8 days when tumor nodules were clearly visible, paclitaxel and **KYS05090** were administered for 39 days. Control animals and the animals treated with paclitaxel or **KYS05090** were euthanized after 39 days and the tumors were excised and measured. Data points represent a means ± SE; (a) tumor volumes in mice of each group; (b) tumor weights in mice of each group measured on the last day of the experiment and the photographs of representative tumors: (c) body weights in mice of each group. $^{*}P$ <0.05, $^{**}P$ <0.01, and $^{***}P$ <0.001 compared with control group (Dunett's test).

Table 2
Oral anti-tumor efficacy of compound **KYS05090** against A549 xenograft in nude mice[a]

Compound	Dose (mg/kg)	AR[b]	No. of animals (*n*)	Tumor volume[c] (mm^3)	Tumor weight[c] (g)
Control[d]	—	—	5	224.40 ± 60.34	0.358 ± 0.052
KYS05090[d]	1	po	5	27.05 ± 7.03	0.142 ± 0.037 (60)[e]
	5	po	5	7.90 ± 1.92	0.120 ± 0.022 (67)[e]
Paclitaxel[d]	10	ip	5	22.90 ± 11.02	0.170 ± 0.058 (53)[e]

[a] During 39 days after administration.
[b] Administration route.
[c] Data are expressed as mean ± S.E.
[d] Co-solvent (Ethanol:Cremophor EL:DW = 1:1:18).
[e] Tumor growth inhibition rates (%) = 100 × (*C* − *T*)/*C*.

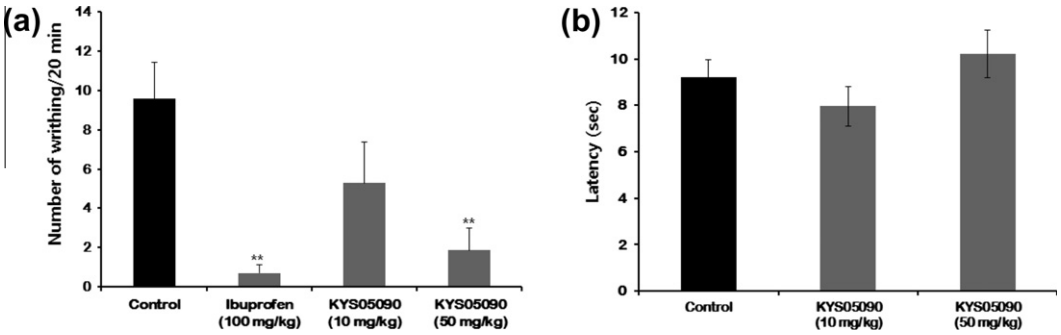
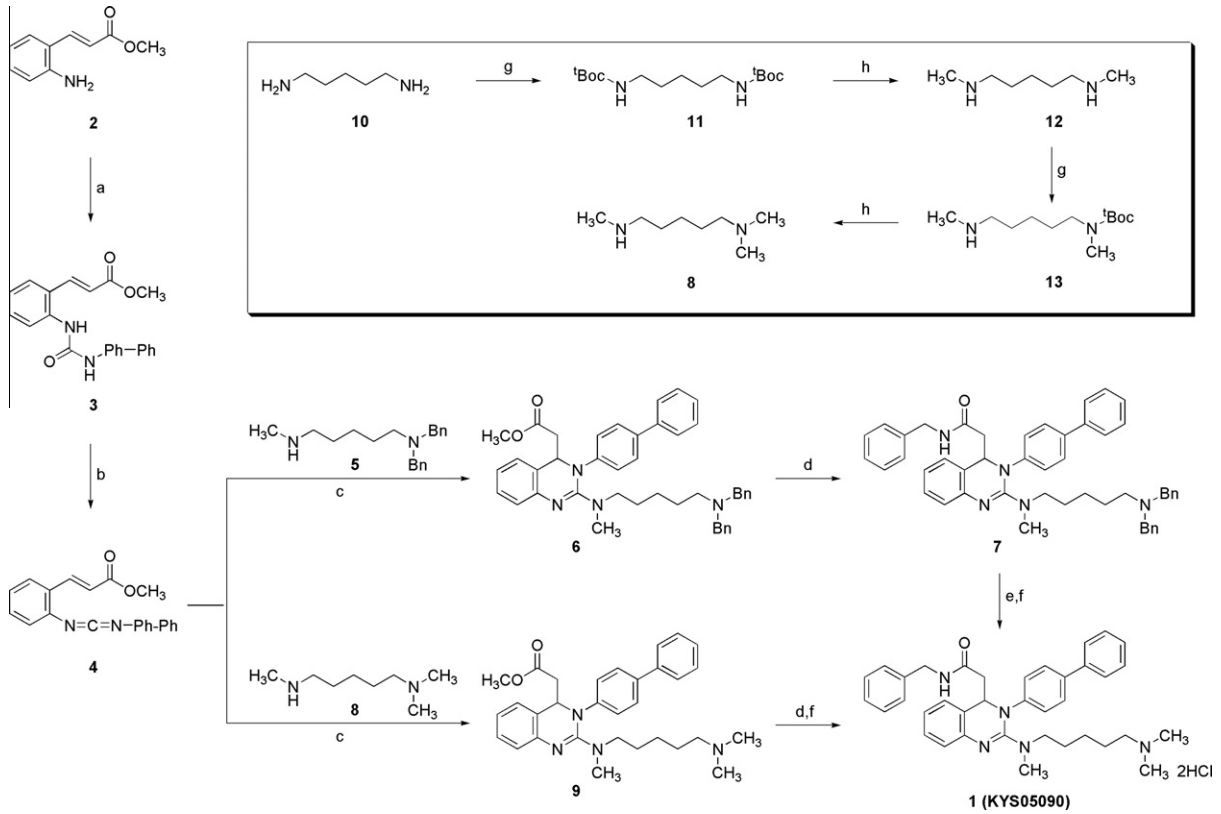


Figure 3. Effects of **KYS05090** in the acetic acid-induced writhing test in mice (*n* = 7 per each group) and hot plate test in mice (*n* = 10 per each group). (a) **KYS05090** was administered orally 1 h before injecting ip acetic acid (0.7% v/v, 10 mL/kg). (b) **KYS05090** was administered orally 1 h before recording the hot plate latencies. The data show the means ± S.D. $^{**}P$ <0.01 compared with the control group (Dunett's test).



Scheme 1. Reagents and conditions: (a) biphenyl-4-carboxylic acid, DPPA, Et_3N, toluene, rt to 100 °C, 72%; (b) PPh_3Br_2, Et_3N, CH_2Cl_2, 0 °C, 77%; (c) toluene, rt, 85% for **6** and 77% for **9**; (d) $PhCH_2NH_2$, TBD, 40 °C, ca. 89% for each reaction; (e) HCHO, H_2, 10% Pd/C, CH_3OH, 15 days (for 1.5 g of **7**), rt, 70%; (f) 2 N HCl, EtOAc, rt, >99%; (g) $(^tBoc)_2O$, HCl, MeOH, 0 °C to rt, 71% for **11** and 73% for **13**; (h) $LiAlH_4$, dry THF, 0 °C to reflux, 82% for **12** and 64% for **8**.

The effect of **KYS05090** on acetic acid-induced writhing behavior in mice is shown in Figure 3a: It dose-dependently and significantly reduced the number of writhes induced by acetic acid (0.7%, v/v, 10 mL/kg) in mice compared to control (vehicle) but had a weaker effect than ibuprofen, a positive control. In the case of hot-plate test, **KYS05090** did not produce dose-independently a reduction of the response to the hot-plate test compared to control as shown in Figure 3b. These overall results implicate that **KYS05090** as T-type calcium channel blocker has a peripheral antinociceptive effect, not central antinociceptive effect.

In the course of scale-up synthesis of **KYS05090** for its preclinical trial, we met the unexpected synthetic problem as following: The previously reported step (e) of Scheme 1, which is a one-pot debenzylation and dimethylation of compound **7**, took more than 2 weeks for the complete reaction of only 1.5 g-scale. This reaction phenomenon may be due to a plausible catalyst poisoning of 3,4-dihydroquinazoline moiety on the palladium catalyst as like the deactivation role of quinoline on the Lindlar catalyst.[19] In order to avoid this longer and tedious step (e), therefore, we decided to prepare directly *N*,*N*-dimethyl-*N*′-methylpentane-1,5-diamine (**8**) from pentane-1,5-diamine (**10**) prior to the coupling reaction with carbodiimide **4** as shown in Scheme 1. Diprotection of diamine **10** with di-*tert*-butyl dicarbonate using known procedure[20] afforded compound **11** in 71% yield. The reduction of both Boc groups with lithium aluminum hydride gave compound **12** in 82% yield.[21] The repeated mono-protection and subsequent reduction of compound **12** afforded the target nucleophile **8** in 47% two-step yield. The coupling reaction of **8** with carbodiimide **4** and the subsequent reactions were successfully carried out to provide **KYS05090** (**1**) according to the previously reported procedure as shown in Scheme 1.[8]

In conclusion, as a continuous part of our research for anti-tumoral agents for oral cancer therapy, in vivo efficacy of **KYS05090** as selective T-type calcium channel blocker was evaluated against A549 xenograft in BALB/$c^{nu/nu}$ nude mice. **KYS05090** exhibited not only potency in both patch-clamp and cellular assays, but also more potent oral anti-tumoral activity than paclitaxel in this xenograft mice model, which is consistent with good oral bioavailability. These overall results indicate that **KYS05090** combined with a peripheral antinociceptive effect potentially possess promising features as drug candidates for oral anticancer therapy. In addition, an efficient scale-up procedure for large production of **KYS05090** has been finally optimized via the direct preparation of side-chain present at C-2 position of 3,4-dihydroquinazoline ring. By applying this new scheme, ca. 100 g of **KYS05090** as a laboratory scale was prepared for further preclinical trial.

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References and notes

1. Banna, G. L.; Collova, E.; Gebbia, V.; Lipari, H.; Giuffrida, P.; Cavallaro, S.; Condorelli, R.; Buscarino, C.; Tralongo, P.; Ferrau, F. *Cancer Treat. Rev.* **2010**, *36*, 595.
2. Ruddy, K.; Mayer, E.; Partridge, A. *CA Cancer J. Clin.* **2009**, *59*, 56.
3. Weingart, S. N.; Brown, E.; Bach, P. B., et al *J. Natl. Compr. Canc. Netw.* **2008**, *6*, S1.
4. Kuppens, I. E.; Breedveld, P.; Beijnen, J. H.; Schellens, J. H. *Cancer Invest.* **2005**, *23*, 443.
5. Schreiber, R. *J. Membr. Biol.* **2005**, *205*, 129.
6. Gray, L. S.; Perez-Reyes, E.; Gomora, J. C.; Haverstick, D. M.; Shattock, M.; McLatchie, L.; Harper, J.; Brooks, G.; Heady, T.; Macdonald, T. L. *Cell Calcium* **2004**, *36*, 489.
7. Bertolesi, G. E.; Shi, C.; Elbaum, L.; Jollimore, C.; Rozenberg, G.; Barnes, S.; Kelly, M. E. *Mol. Pharmacol.* **2002**, *62*, 210.
8. Jung, S. Y.; Lee, S. H.; Kang, H. H.; Park, H. A.; Chang, S. K.; Kim, J.; Choo, D. J.; Oh, C. R.; Kim, Y. D.; Seo, J. H.; Lee, K.-T.; Lee, J. Y. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6633.
9. Zhong, G.; Zhang, S.; Li, Y.; Liu, X.; Gao, R.; Miao, Q.; Zhen, Y. *Cancer Lett.* **2010**, *295*, 124.
10. Lee, H. S.; Ryu, J. S.; Jeon, Y. J.; Hong, Y. S.; Lee, J. J.; Hong, S. K.; Kim, T. Y. *Cancer Chemother. Pharmacol.* **2007**, *60*, 197.
11. Labonté, P.; Kadhim, S.; Bowlin, T.; Mounir, S. *Hepatol. Res.* **2000**, *18*, 72.
12. Todorovic, S. M.; Jevtovic-Todorovic, V. *CNS Neurol. Disord. Drug Targets* **2006**, *5*, 639.
13. Perez-Reyes, E. *Physiol. Rev.* **2003**, *83*, 117.
14. Todorovic, S. M.; Jevtovic-Todorovic, V. *Br. J. Pharmacol.* **2011**, *163*, 484.
15. Bentley, G. A.; Newton, S. H.; Starr, J. *Br. J. Pharmacol.* **1981**, *73*, 325.
16. D'amour, F. E.; Smith, D. *J. Pharmacol. Exp. Ther.* **1941**, *72*, 74.
17. Julius, D.; Basbaum, A. I. *Nature* **2001**, *413*, 203.
18. Basbaum, A. I.; Bautista, D. M.; Scherrer, G.; Julius, D. *Cell* **2009**, *139*, 267.
19. Garcia-Mota, M.; Gomez-Diaz, J.; Novell-Leruth, G.; Vargas-Fuentes, C.; Bellarosa, L.; Bridier, B.; Perez-Ramirez, J.; Lopez, N. *Theor. Chem. Acc.* **2010**, *128*, 663.
20. Lee, D. W.; Ha, H.-J.; Lee, W. K. *Synth. Commun.* **2007**, *37*, 737.
21. Phuan, P.-W.; Kozlowski, M. C. *J. Org. Chem.* **2002**, *67*, 6339.