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Antitumor activity of 3,4-dihydroquinazoline dihydrochloride in A549 xenograft nude mice

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ABSTRACT

In the previous article we have reported that 3,4-dihydroquinazoline **1** is a potent and selective T-type calcium channel blocker that exhibited strong anti-cancer activity in vitro. Compound **1**·2HCl was further in vivo evaluated against A549 xenograft in BALB/c nude mice, which exhibited 49% tumor-weight inhibition through intravenous administration of 2 mg/kg of body weight and was more potent than doxorubicin. Moreover, compound **1**·2HCl has an oral bioavailability of 98% with LD_{50} values of 693 mg/kg (po route) and 40.0 mg/kg (iv route) of body weight. In addition, its efficient scale-up synthetic method was developed.

Calcium is essential for living organisms, particularly in cell physiology, where the movement of Ca^{2+} into and out of the cytoplasm functions as a signal for many cellular processes including cell cycle progression.[1] Among various Ca^{2+} channels, T-type (low voltage activated) Ca^{2+} channels have been known to play an important role in controlling cell proliferation and differentiation in many tissues.[2] In line with this molecular biology, the anti-cancer effects of a T-type Ca^{2+} channel antagonists (or blockers) on tumor cells in vivo have been reported by many researcher groups.[3] Our group also have reported the identification of 3,4-dihydroquinazoline compound **1**, which exhibits both selective/potent T-type calcium channel blocking effect and strong anti-cancer effect on cancer cell lines comparable to doxorubicin in vitro.[4] As a continuous work, the acute toxicity study, A549 xenograft study and pharmacokinetic study for compound **1** were performed. The results of these studies are reported herein.

Before in vivo study, we had a problem with the previously reported synthetic method for compound **1** which required multistep reactions, high expensive reagents and unstable intermediates. Therefore, a more efficient synthetic procedure was desired to furnish the quantities of compound **1** required for further in vivo biological evaluation study. We have modified the scale-up synthetic procedure for compound **1** as shown in Scheme 1. In the previous methods, iminophosphorane **3** was used as an intermediate for key carbodiimide **5**. However, this intermediate is too unstable to be completely isolated and store for long time. Furthermore, 4-biphenylyl isocyanate as a reagent for the reaction with compound **3** is highly expensive and also purification step via column chromatography has to be avoided for scale-up preparation. As a solution to these problems, we have developed an efficient two-step process for carbodiimide **5**: Curtius rearrangement using cheap reagent biphenyl-4-carboxylic acid/diphenyl phosphorazidate (DPPA)/Et_3N for urea **4** and subsequent dehydration using PPh_3Br_2 and Et_3N for carbodiimide **5** as shown in Scheme 1 (path c and d).[5] As a second modified procedure, the reaction of ester compound **7** with benzylamine and 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) as a catalyst under solvent-free condition afforded directly amide **9** in higher yield than the original two-step procedure (hydrolysis and amide coupling reaction) as shown in Scheme 1 (path f).[6] Finally, salt formation to enhance aqueous solubility of compound **1** was carried out using 2 N HCl in EtOAc to afford compound **1** dihydrochloride salt, which became highly water soluble. The cytotoxic effect of both free and salt forms on various cancer cell lines was then evaluated using MTT assay for comparison.[7] Both forms of compound **1** showed approximately similar cytotoxic activities against tested cancer cells as shown in Table 1, which means that the salt formation has no effect on cytotoxicity of compound **1** against cancer cells. This modified procedure seems to be more efficient and economical than the earlier reported method because of high overall yield (26%) and

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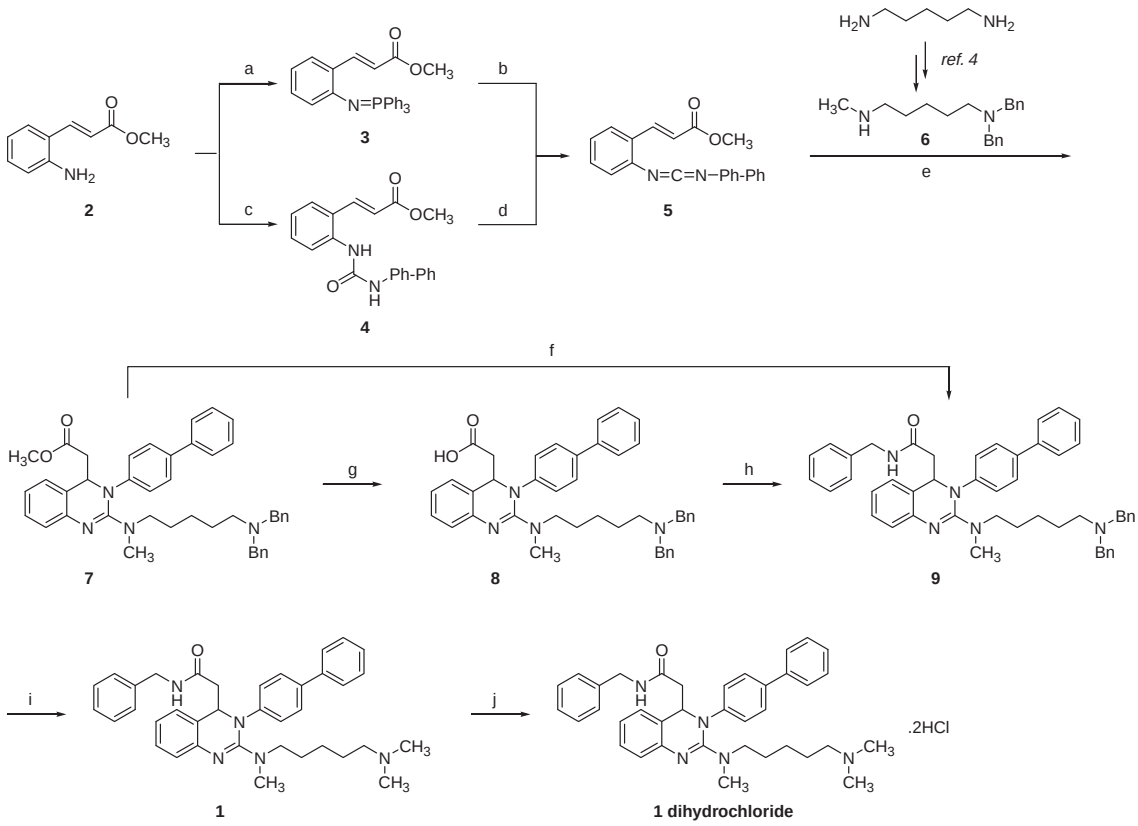

Scheme 1. Reagents and conditions: (a) PPh_3, C_2Cl_6, Et_3N, toluene, reflux, 60%; (b) 4-biphenylyl isocyanate, toluene, rt, 80%; (c) biphenyl-4-carboxylic acid, DPPA, Et_3N, toluene, rt to 100 °C, 72%; (d) PPh_3Br_2, Et_3N, CH_2Cl_2, 0 °C, 77%; (e) toluene, rt, >99%; (f) $PhCH_2NH_2$, TBD, 40 °C, 89%; (g) LiOH, THF–H_2O (1:1), 70 °C, >99%; (h) $PhCH_2NH_2$, HOBt, EDC, THF–CH_2Cl_2 (1:1), 0 °C to rt, >79%; (i) HCHO, H_2, 10% Pd/C, CH_3OH, 4 days, rt, 70%; (j) 2 N HCl, EtOAc, rt, >99%.

Table 1
In vitro cytotoxic activity of 3,4-dihydroquinazolines **1** on cancer cell lines

Form of compound	Cancer cell lines (IC_{50} in μM)						
	A549 (lung)	HeLa (cervix)	LNCAP (prostate)	SKOV (ovary)	A172 (brain)	MDA-MB-231 (breast)	SKBR-3 (breast)
Free base	5.17	4.04	4.70	5.00	4.83	2.65	2.17
2HCl salt	5.00	3.11	5.19	5.22	4.61	1.64	1.83

fewer stages (seven steps). Therefore, we have prepared a large quantity of compound **1**·2HCl using this modified procedure for its following in vivo screening.

Compound **1**·2HCl was profiled for its acute toxicity to ICR male mice using two kinds of administration route as shown in Table 2.[8] In the case of oral administration, all the mice given a single oral injection of **1**·2HCl at the dose of 250 mg/kg of body weight showed no clinical sign and body weight loss and the mortality rate was zero. At 500 mg/kg dose, however, clinical signs such as diarrhea and soiled perineal region were observed on some of tested mice on the injected day and disappeared on all of alive mice after 3rd day post injection. The mortality rate was 2/5

Table 2
Acute toxicity of compound **1**·2HCl post single administration on ICR mice[a]

Compound	Dose (mg/kg)	AR[b]	No. of animals[c]	Sex	Clinical signs	Mortality	LD_{50}
1·2HCl	250	po	5	Male	Normal	0	693 mg/kg
	500	po	5	Male	Diarrhea; soiled perineal region	2	
	1000	po	5	Male	Diarrhea; soiled perineal region	4	
	26.6	iv	5	Male	Normal	0	40.0 mg/kg
	34.6	iv	5	Male	Panting; inanimation; loss of locomotor activity; erosion	1	
	45.0	iv	5	Male	Panting; inanimation; loss of locomotor activity; erosion	4	
Control[d]	—	po/iv	5/5	Male	Normal	0	—

[a] 14 days post single administration.
[b] Administration route.
[c] Number of mice tested in the group.
[d] 10 mL/kg of saline, vehicle for compound **1**·2HCl salt.

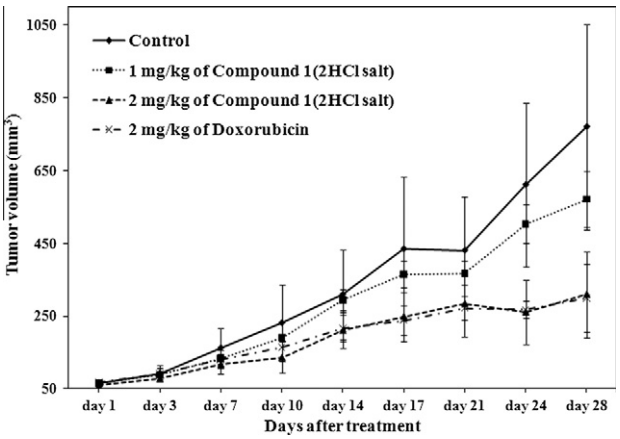

Figure 1. Effects of compound **1**·2HCl on tumor growth in BALB/c nude mice injected with 5 million human lung tumor A549 cells. The results are presented as tumor volume over time.

(40%). At 1000 mg/kg dose, the mortality rate was 4/5 (80%) and the same clinical signs were observed too. In addition, a little of body weight loss was observed compared with the vehicle control (p <0.05). Based on these data, oral LD_{50} value for compound **1**·2HCl was decided to be 693 mg/kg, which means that compound 1·2HCl is relatively less toxic compared to other cancer chemotherapeutic agents. In the case of intravenous administration, all the mice given a single intravenous injection of **1**·2HCl at the dose of 26.6 mg/kg of body weight showed no clinical sign and body weight loss and the mortality rate was zero. At the dose of 34.6 mg/kg, the mortality rate was 20% (1/5) and various clinical signs such as panting, inanimation, loss of locomotor activity and erosion were observed post injection and disappeared slowly on alive mice. At high dose of 45.0 mg/kg, the same clinical signs were observed and four of the treated mice were dead within 10 min post injection. In particular, a little of body weight loss was observed on the alive mouse compared with control, which may be related with the toxicity of compound **1**. Anyway, intravenous LD_{50} value for compound **1**·2HCl was decided to be 40.0 mg/kg.

Based on these in vitro anti-cancer data and acute toxicity data, we further evaluated the in vivo antitumor activity of compound **1**·2HCl against A549 xenograft in BALB/c nude mice model in comparison with doxorubicin as positive control and saline was used as blank control.[9,10] Compound **1**·2HCl with two doses (1 and 2 mg/kg) and control with 5 mL/kg dose were administered once daily through intravenous administration route for consecutive 28 days and doxorubicin with 2 mg/kg was administered once per 2 days through intravenous administration route for consecutive 4 weeks. The results are documented in Figure 1 and Table 3. Figure 1 displays the effects of compound **1**·2HCl on tumor growth in nude mice presented as tumor volume over time. There was statistically significant dose-dependent decrease in tumor volume with increasing compound **1**·2HCl dose. In particular, compound **1**·2HCl at 2 mg/kg dose showed the strong potency equal to doxorubicin. With respect to tumor weight, the results presented in Table 3 showed that compound **1**·2HCl through intravenous administration of 1 mg/kg exhibited good efficacy against A549 xenograft in BALB/c nude mice by 29% comparable to a doxorubicin (30%) at dosage 2 mg/kg. At 2 mg/kg dose, compound **1**·2HCl showed the 1.6-fold potency (49%) than doxorubicin compared with vehicle alone. With respect to body weight related with toxicity, there was no statistical difference between average total body weights in mice treated with compound **1**·2HCl and control, whereas significant body weight loss in mice was continuously observed since 8th day after treating with doxorubicin (data not shown). The present study implies that compound **1**·2HCl could be effective in the treatment of human lung cancer.

Table 4
Pharmacokinetic parameters for compound **1**·2HCl in rat after iv administration

Parameter[a]	Dose	
	5 mg/kg	10 mg/kg
AUC (ng h/mL)	127.95 ± 42.70	279.09 ± 98.22
C_{max} (ng/mL)	459.57 ± 113.65	1026.37 ± 475.85
$t_{1/2}$ (min)	522.96 ± 105.84	1192.29 ± 257.78
CL (mL/min/kg)	600.06 ± 306.88	411.89 ± 152.49
V_d (L/kg)	0.33 ± 0.17	0.61 ± 0.21
MRT (min)	305.62 ± 66.89	431.17 ± 68.32
F (%)	98.32 ± 31.02	70.09 ± 28.18

[a] AUC is area-under-the-curve; C_{max} is maximum concentration in plasma; $t_{1/2}$ is half-life in plasma; CL is plasma clearance; V_d is volume of distribution; MRT is mean residence time; F denotes bioavailability.

Based on these biological results, pharmacokinetic parameters of compound **1**·2HCl were obtained from in sprague dawley rats (n = 4) using two dosages (5 and 10 mg/kg) and summarized in Table 4. Pharmacokinetic parameters generally showed the dose-dependent pattern. First of all, compound **1**·2HCl is orally well absorbed at two dosages. The 98% oral bioavailability (F%) at low dose (5 mg/kg) is particularly gratifying. The decreased oral bioavailability at high dose (10 mg/kg) is thought to result from the saturation at the absorption site. This pharmacokinetic profile shows the possibility that compound **1**·2HCl would be developed as oral anti-cancer agent.

In conclusion, compound **1**·2HCl has relatively low toxicity, in vivo good efficacy, good water solubility and excellent oral bioavailability. All these results revealed that compound **1**·2HCl may have a tumor suppressor function in human lung cancer cells and could be a promising treatment in anti-cancer therapy. An efficient and economical synthesis of large quantities of compound **1**·2HCl has been developed.

Table 3
Antitumor efficacy of compound **1**·2HCl against A549 xenograft in nude mice[a]

Compound	Dose (mg/kg)	AR[b]	No. of animals	Sex	Tumor weight[c] (g)	Tumor weight-inhibition[d] (%)
Control[e]	—	iv	4	Male	0.537 ± 0.172	—
1·2HCl	1	iv	4	Male	0.381 ± 0.055	29
1·2HCl	2	iv	4	Male	0.273 ± 0.078[f]	49
Doxorubicin[g]	2	iv	4	Male	0.375 ± 0.046	30

[a] During 29 days post xenograft.
[b] Administration route.
[c] Data are expressed as mean ± S.E.
[d] Percentage of tumor-weight inhibition versus control.
[e] 5 mL/kg saline, vehicle for compound **1**.
[f] p <0.05 versus the vehicle-treated control group.
[g] 10 mL/kg of doxorubicin hydrochloride in saline solution.

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