Bioorganic & Medicinal Chemistry Letters 18 (2008) 3899–3901

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

T-type Ca^{2+} channel blockers suppress the growth of human cancer cells

Jae Ho Heo [a], Han Na Seo [a], Yun Jeong Choe [a], Sujin Kim [a], Chun Rim Oh [b], Young Deuk Kim [b], Hyewhon Rhim [c], Dong Joon Choo [a], Jungahn Kim [a], Jae Yeol Lee [a,*]

[a] Research Institute for Basic Sciences and Department of Chemistry, College of Sciences, Kyung Hee University, 1 Hoegi-Dong, Dongdaemun-Gu, Seoul 130-701, Republic of Korea
[b] DongWoo Syntech Co., Ltd, Bondae-Ri Geumwang-Eup, Eumsung-Goon, Choongcheungbook-Do 369-901, Republic of Korea
[c] Life Sciences Division, Korea Institute of Science & Technology, PO Box 131, Cheongryang, Seoul 130-650, Republic of Korea

ARTICLE INFO

Article history:
Received 20 May 2008
Revised 6 June 2008
Accepted 11 June 2008
Available online 14 June 2008

Keywords:
T-type calcium channel blocker
3,4-Dihydroquinazoline
Doxorubicin
Anti-cancer

ABSTRACT

In order to further clarify the role of T-type Ca^{2+} channels in cell proliferation, we have measured the growth inhibition of human cancer cells by using our potent T-type Ca^{2+} channel blockers. As a result, **KYS05090**, a most potent T-type Ca^{2+} channel blocker, was found to be as potent as doxorubicin against some human cancer cells without acute toxicity. Therefore, this letter provides the biological results that T-type calcium channel is important in regulating the important cellular phenotype transition leading to cell proliferation, and thus novel T-type Ca^{2+} channel blocker presents new prospects for cancer treatment.

Calcium plays a critical role as an intracellular signal and controls many different cell processes,[1] of which intracellular calcium (Ca^{2+}) regulates proliferation, differentiation, growth, cell death, and apoptosis.[2] Thus, alterations in Ca^{2+} signaling can be the cause of defects in cell growth and are associated with some cancers. A number of research groups have suggested a potential role of T-type Ca^{2+} channels in controlling cell proliferation.[3–8] If a proliferation is a principal characteristic of cancer cells, therefore, a modulator of T-type Ca^{2+} channels in cancer cells responsible for proliferation is of potential clinical significance. For example, the known inhibitors of T-type Ca^{2+} channels, for example, both mibefradil and pimozide, have been demonstrated to be effective in decreasing cell proliferation in normal cells as well as breast cancer cells (Fig. 1).[9–15] In addition, it is recently reported that inhibition of T-type channels reduces cell proliferation via a p53-dependent $p21^{CIP1}$ pathway in certain esophageal carcinomas.[16] In light of these findings, we have recently demonstrated that through the assay of eight compounds showing a broad range of channel blocking effects (34–91% inhibition at 10 μM concentration), there is a close connection between channel blocking effect and growth inhibition of human cancer cells.[17]

As a continuous study, we have set out to select and screen 13 compounds showing the good inhibitory activity (its IC_{50} is less than 1.0 μM) against calcium influx from our library of compounds as illustrated in Figure 2.[18–22] The general synthetic method of 3,4-dihydroquinazoline compounds is described in Scheme 1.[20] The channel blocking activity of selected compounds was evaluated against HEK293 cells which stably express both T-type calcium channel $Ca_v3.1$ with α_{1G} subunit and potassium channel Kir2.1.[23]

The selected compounds were again evaluated for their growth inhibition against five human cancer cell lines [human lung carcinoma (A549), human prostate cancer (DU 145), human colon cancer (HT-29), human malignant melanoma (SK-MEL-2), and human ovarian cancer (SK-OV-3)] using sulforhodamine B (SRB) assay.[24]

Table 1 provides the results of assays used to measure the inhibition of calcium influx and the growth inhibition of human cancer cells by the compounds as illustrated in Figure 1. The entry of compounds was arranged in order of the channel blocking activity for the easy data comparison.

Doxorubicin was used as a reference for anti-cancer activity and also tabulated with data for comparison. Before discussing the results, we have already confirmed that most compounds showed little cytotoxicity on HEX293 cell at 100 μM concentration except **KYS05080**, which showed the serious cytotoxicity at that concentration. From these data, a brief relationship profile emerged as follows: first, most compounds showed the linear correlation between cell growth inhibitory activity and T-type channel blocking effect except **KYS05055** and **KYS05056**, which showed no anticancer activity against three cell lines (A549, DU 145, and SK-OV-3) and the poor activity against another cell lines (HT-29 and SK-MEL-2) although they show the good channel blocking effect. Second, most compounds exhibited the similar growth inhibitory activities against five cancer cells except for compounds **KYS05055** and **KYS05056**. Among compounds, **KYS05090**, the most potent channel blocker, showed the best activity (their GI_{50} values are less than

* Corresponding author. Tel.: +82 2 961 0726; fax: +82 2 966 3701.
E-mail address: ljy@khu.ac.kr (J.Y. Lee).

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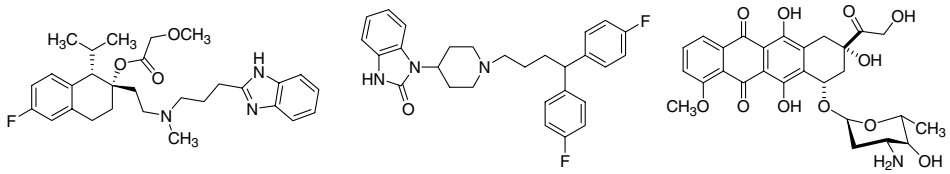
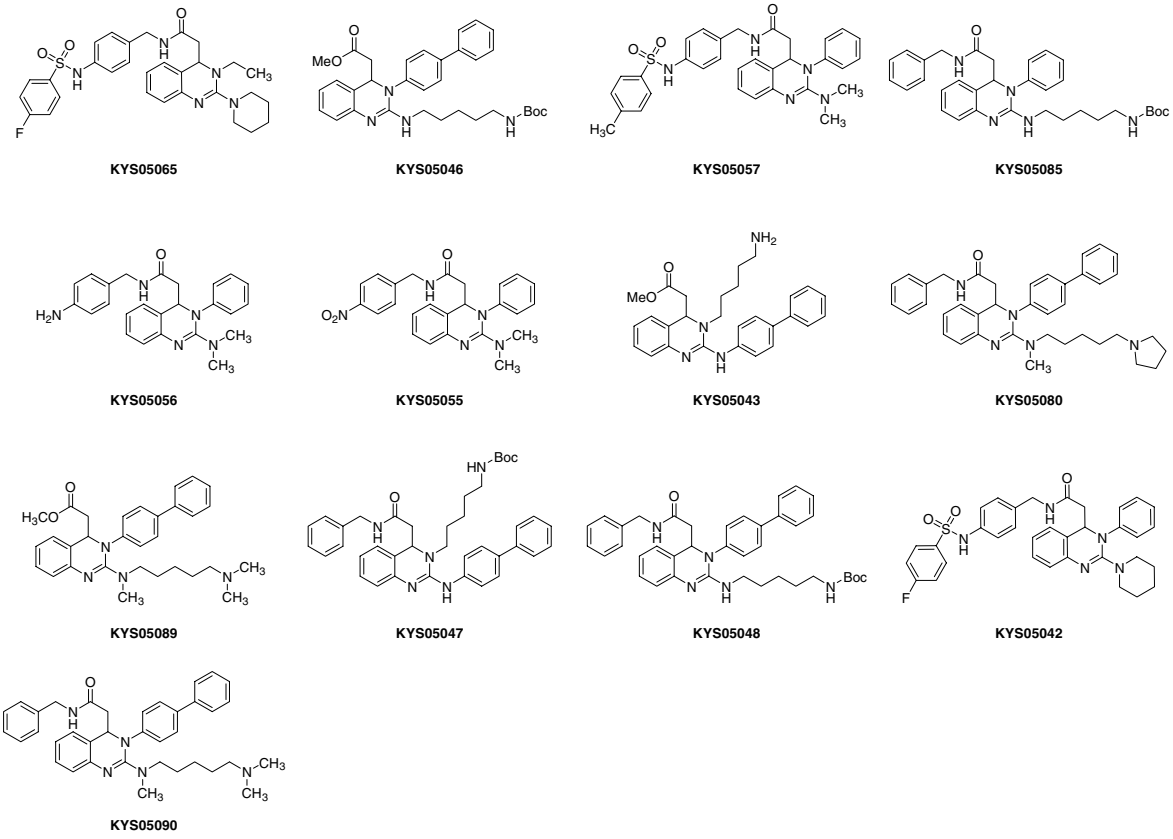
doi:10.1016/j.bmcl.2008.06.034



mibefradil pimozide doxorubicin

Figure 1. Structures of mibefradil, pimozide, and doxorubicin.



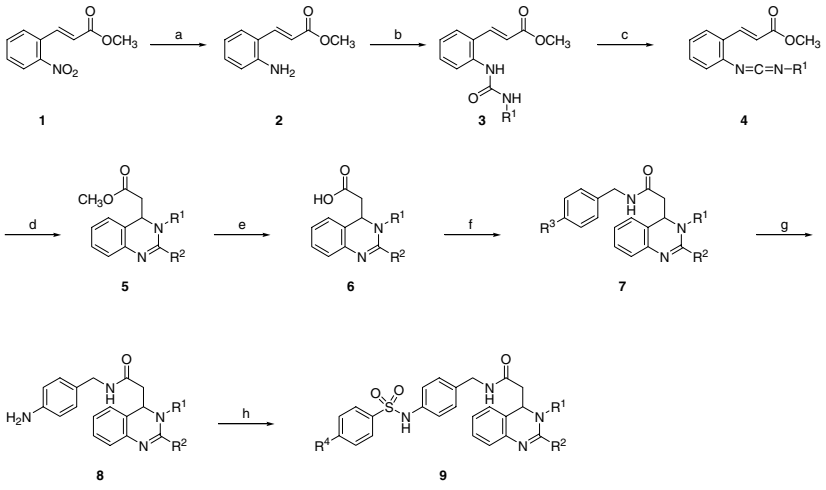
KYS05065 KYS05046 KYS05057 KYS05085

KYS05056 KYS05055 KYS05043 KYS05080

KYS05089 KYS05047 KYS05048 KYS05042

KYS05090

Figure 2. 3,4-Dihydroquinazoline derivatives studied in SRB assay.



Scheme 1. Reagents and conditions: (a) $SnCl_2{\cdot}2H_2O$, EtOAc, 70 °C; (b) R^1NCO, benzene, rt; (c) $Ph_3P{\cdot}Br_2$, Et_3N, CH_2Cl_2, 0 °C; (d) R^2H, THF, rt; (e) $LiOH{\cdot}H_2O$, THF–H_2O (1:1), 60 °C; (f) *p*-R^3-$BnNH_2$, HOBT, EDC, rt; (g) 10% Pd(C), MeOH, rt; (h) *p*-R^4-$PhSO_2Cl$, pyridine, 0 °C to rt.

Table 1
Biological data for selected compounds

Compound	T-type calcium channel blocking effect (IC_{50}: μM)[a,b]	Growth inhibition of cancer cell (GI_{50}: μM)[b]				
		A549[c]	DU 145[d]	HT-29[e]	SK-MEL-2[f]	SK-OV-3[g]
KYS05065	1.04 ± 0.25	24.46 ± 5.79	17.21 ± 1.39	13.94 ± 1.13	17.03 ± 1.56	26.14 ± 6.08
KYS05046	0.68 ± 0.18	3.83 ± 0.55	3.83 ± 1.08	2.08 ± 0.43	4.31 ± 2.56	4.61 ± 0.30
KYS05057	0.63 ± 0.04	14.27 ± 1.05	6.52 ± 1.00	3.15 ± 0.66	8.53 ± 1.65	15.61 ± 1.14
KYS05085	0.57 ± 0.05	7.74 ± 2.60	4.26 ± 0.56	3.18 ± 0.52	2.02 ± 0.40	7.18 ± 1.18
KYS05056	0.38 ± 0.15	>100	>100	28.28 ± 1.42	43.95 ± 7.68	>100
KYS05055	0.35 ± 0.07	>100	>100	22.31 ± 1.00	31.62 ± 3.05	>100
KYS05043	0.30 ± 0.09	2.90 ± 0.38	4.09 ± 0.71	1.08 ± 0.28	1.80 ± 0.48	3.99 ± 1.07
KYS05080	0.26 ± 0.01	0.87 ± 0.18	2.49 ± 0.53	0.61 ± 0.12	0.69 ± 0.15	2.76 ± 0.76
KYS05089	0.23 ± 0.03	1.77 ± 0.16	1.78 ± 0.17	0.52 ± 0.24	1.91 ± 0.11	1.95 ± 0.04
KYS05047	0.17 ± 0.03	1.87 ± 0.05	1.79 ± 0.04	1.70 ± 0.16	1.59 ± 0.27	2.01 ± 0.32
KYS05048	0.16 ± 0.02	1.83 ± 0.22	1.64 ± 0.09	1.67 ± 0.18	1.48 ± 0.31	1.61 ± 0.09
KYS05042	0.11 ± 0.06	1.93 ± 0.13	1.71 ± 0.16	1.71 ± 0.17	1.73 ± 0.28	1.95 ± 0.23
KYS05090	0.041 ± 0.001	0.17 ± 0.02	0.19 ± 0.02	0.04 ± 0.01	0.48 ± 0.13	0.66 ± 0.13
Doxorubicin	ND[h]	0.16 ± 0.01	0.06 ± 0.01	0.21 ± 0.04	0.11 ± 0.01	0.12 ± 0.02

[a] T-type calcium channel (α_{1G}) expressed on HEX293 cell.
[b] Value was determined from dose–response curve and obtained from three independent experiments.
[c] Human lung carcinoma (A549).
[d] Human prostate cancer (DU 145).
[e] Human colon cancer (HT-29).
[f] Human malignant melanoma (SK-MEL-2).
[g] Human ovarian cancer (SK-OV-3).
[h] ND, not determined.

1 μM) across all the cell lines in the series as shown in Table 1. This compound was found to be as potent as doxorubicin against human lung carcinoma (A549) and showed in particular 4-fold more potency against human colon cancer (HT-29) compared to doxorubicin. For the acute toxicity study, **KYS050590** as a corn oil mixture was orally single administered at dose of 1000 mg/kg to two CD-1(ICR) mice, and no mortality was observed during 5 days (the data not shown). Therefore, **KYS050590** did not appear to have significant toxicity. This result implies a possibility that **KYS050590** works by stopping cancer cells from multiplying, and thus it stops cancer from growing (cytostatic effect) rather than killing cancer cells (cytotoxic effect).

In conclusion, our potent T-type channel blockers were evaluated for the growth inhibition of human cancer cells and **KYS050590** possess GI_{50} similar to that of Doxorubicin. This result provided a strong evidence for the correlation between inhibition of calcium influx and anti-cancer activity. Therefore, this study presents new prospects for cancer treatment. Now, the evaluation of in vivo anti-tumor efficacy and chronic toxicity in CD-1(ICR) mice is in progress and its data will be announced in the future.

Acknowledgments

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2007-313-C00475) and DongWoo Syntech Co., Ltd. (20071065).

References and notes

1. Berridge, M. J.; Lipp, P.; Bootman, M. D. *Nat. Rev. Mol. Cell Biol.* **2000**, *1*, 11.
2. Berridge, M. J. *Neuron* **1998**, *21*, 13.
3. Lory, P.; Bidaud, I.; Chemin, J. *Cell Calcium* **2006**, *40*, 135.
4. Gray, L. S.; Macdonaldm, T. L. *Cell Calcium* **2006**, *40*, 115.
5. McCalmont, W. F.; Heady, T. N.; Patterson, J. R.; Lindenmuth, M. A.; Haverstick, D. M.; Gray, L. S.; Macdonald, T. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3691.
6. McCalmont, W. F.; Patterson, J. R.; Lindenmuth, M. A.; Heady, T. N.; Haverstick, D. M.; Gray, L. S.; Macdonald, T. L. *Bioorg. Med. Chem.* **2005**, *13*, 3821.
7. Chen, H.-T.; Chang, W.-C.; Chen, J.-S.; Lu, Y.-C.; Hsu, S.-S.; Wang, J.-L.; Cheng, H.-H.; Cheng, J.-S.; Jiann, B.-P.; Chiang, A.-J.; Huang, J.-K.; Jan, C.-R. *Life Sci.* **2005**, *76*, 2091.
8. Haverstick, D. M.; Heady, T. N.; Macdonald, T. L.; Gray, L. S. *Cancer Res.* **2000**, *60*, 1002.
9. Schmitt, R.; Clozel, J. P.; Iberg, N.; Buhler, F. R. *Arterioscler. Thromb. Vasc. Biol.* **1995**, *15*, 1161.
10. Rodman, D. M.; Reese, K.; Harral, J.; Fouty, B.; Wu, S.; West, J.; Hoedt-Miller, M.; Tada, Y.; Li, K. X.; Cool, C.; Fagan, K.; Cribbs, L. *Circ. Res.* **2005**, *96*, 864.
11. Nilius, B.; Prenen, J.; Kamouchi, M.; Viana, F.; Voets, T.; Droogmans, G. *Br. J. Pharmacol.* **1997**, *121*, 547.
12. Lijnen, P.; Fagard, R.; Petrov, V. *J. Cardiovasc. Pharmacol.* **1999**, *33*, 595.
13. Wondergem, R.; Gong, W.; Monen, S. H.; Dooley, S. N.; Gonce, J. L.; Conner, T. D.; Houser, M.; Ecay, T. W.; Ferslew, K. E. *J. Physiol.* **2001**, *532*, 661.
14. Panner, A.; Cribbs, L. L.; Zainelli, G. M.; Origitano, T. C.; Singh, S.; Wurster, R. D. *Cell Calcium* **2005**, *37*, 105.
15. Strobl, J. S.; Melkoumian, Z.; Peterson, V. A.; Hylton, H. *Breast Cancer Res. Treat.* **1998**, *51*, 83.
16. Lu, F.; Chen, H.; Zhou, C.; Liu, S.; Guo, M.; Chen, P.; Zhuang, H.; Xie, D.; Wu, S. *Cell Calcium* **2008**, *43*, 49.
17. Lee, J. Y.; Park, S. J.; Park, S. J.; Lee, M. J.; Lee, M. J.; Rhim, H.; Seo, S. H.; Kim, K.-S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5014.
18. Lee, Y. S.; Lee, B. H.; Park, S. J.; Kang, S. B.; Rhim, H.; Park, J.-Y.; Lee, J.-H.; Jeong, S.-W.; Lee, J. Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3379.
19. Rhim, H.; Lee, Y. S.; Park, S. J.; Chung, B. Y.; Lee, J. Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 283.
20. Park, S. J.; Park, S. J.; Lee, M. J.; Rhim, H.; Kim, Y.; Lee, J.-H.; Chung, B. Y.; Lee, J. Y. *Bioorg. Med. Chem.* **2006**, *14*, 3502.
21. Choi, J. Y.; Seo, H. N.; Lee, M. J.; Park, S. J.; Park, S. J.; Jeon, J. Y.; Kang, J. H.; Pae, A. N.; Rhim, H.; Lee, J. Y. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 471.
22. Seo, H. N.; Choi, J. Y.; Choe, Y. J.; Kim, Y.; Rhim, H.; Lee, S. H.; Kim, J.; Joo, D. J.; Lee, J. Y. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5740.
23. Kim, T.; Choi, J.; Kim, S.; Kwon, O.; Nah, S. Y.; Han, Y. S.; Rhim, H. *Biochem. Biophys. Res. Commun.* **2004**, *324*, 401. For the recordings of α_{1G} T-type Ca^{2+} currents, the standard whole-cell patch–clamp method was utilized. Briefly, borosilicate glass electrodes with a resistance of 3–4 MΩ were pulled and filled with the internal solution containing (in mM): 130 KCl, 11 EGTA, 5 Mg-ATP, and 10 HEPES (pH 7.4). The external solution contained (in mM): 140 NaCl, 2 $CaCl_2$, 10 HEPES, and 10 glucose (pH 7.4). α_{1G} T-type Ca^{2+} currents were evoked every 15 s by a 50 ms depolarizing voltage step from −100 to −30 mV. The molar concentrations of test compounds required to produce 50% inhibition of peak currents (IC_{50}) were determined from fitting raw data into dose–response curves. The current recordings were obtained using an EPC-9 amplifier and Pulse/Pulsefit software program (HEKA, Germany).
24. Skehan, P.; Streng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107. All cell lines were grown in RPMI 1640 (Gibco BRL) supplemented with 10% (V/V) heat inactivated Fetal Bovine Serum (FBS), and maintained at 37 °C in a humidified atmosphere with 5% CO_2. The cells were seeded into 96-well plate. Various concentrations of samples were added to each well in triplicate, and then incubated at 37 °C with 5% CO_2 for 72 h such that time cells are in the exponential phase of growth at the time of drug addition. After incubation, the 100 μL of formalin solution was gently added to the wells. Microplates were left for 30 min at room temperature, washed 5 times with tap water. The 100 μL of 0.4% SRB solution was added to each well and left at room temperature for 30 min. SRB was removed and the plates were washed 5 times with 1% acetic acid before air drying. Bound SRB was solubilized with 100 μL of 10 mM unbuffered Tris-base solution (Sigma) and plates were left on a plate shaker for at least 10 min. The optical density was measured using a microplate reader (Versamax, Molecular Devices) with a 520 nm wavelength and the growth inhibition concentration was expressed as a GI_{50}.