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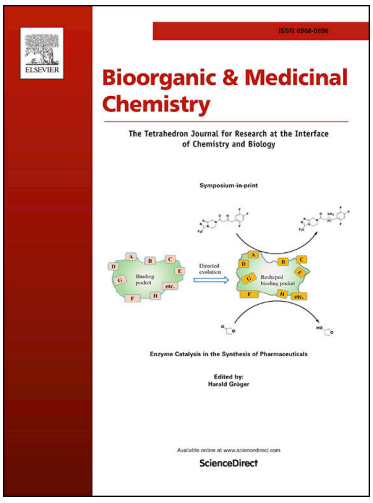


# Synthesis and Cytotoxic Effects of 2-Thio-3,4-dihydroquinazoline Derivatives as Novel T-Type Calcium Channel Blockers

Yunchan Nam, Ki Deok Ryu, Changyoung Jang, Yoon Hyoung Moon, Misong Kim, Dohyeong Ko, Kyung-Sook Chung, Maria A. Gandini, Kyung-Tae Lee, Gerald W. Zamponi, Jae Yeol Lee

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# Synthesis and Cytotoxic Effects of 2-Thio-3,4-dihydroquinazoline Derivatives as Novel T-Type Calcium Channel Blockers

Yunchan Nam [a,1], Ki Deok Ryu [a,1], Changyoung Jang [a], Yoon Hyoung Moon [a], Misong Kim [a], Dohyeong Ko [a], Kyung-Sook Chung [b], Maria A. Gandini [c], Kyung-Tae Lee [b*], Gerald W. Zamponi [c*], Jae Yeol Lee [a,d*]

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**Abstract:** In our previous work, a series of 2-amino-3,4-dihydroquinazoline derivatives using an electron acceptor group was reported to be potent T-type calcium channel blockers and exhibit strong cytotoxic effects against various cancerous cell lines. To investigate the role of the guanidine moiety in the 2-amino-3,4-dihydroquinazoline scaffold as a pharmacophore for dual biological activity, a new series of 2-thio-3,4-dihydroquniazoline derivatives using an electron donor group at the C2-position was synthesized and evaluated for T-type calcium channel blocking activity and cytotoxic effects against two human cancerous cell lines (lung cancer A549 and colon cancer HCT-116). Among them, compound **6g** showed potent inhibition of $Ca_v3.2$ currents (83% inhibition) at 10 μM concentrations. The compound also exhibited $IC_{50}$ values of 5.0 and 6.4 μM against A549 and HCT-116 cell lines, respectively, which are comparable to the parental lead compound **KYS05090**. These results indicate that the isothiourea moiety similar to the guanidine moiety of 2-amino-3,4-dihydroquinazoline derivatives may be an essential pharmacophore for the desired biological activities. Therefore, our preliminary work can provide the opportunity to expand a chemical repertoire to improve affinity and selectivity for T-type calcium channels.

**Key Words:** T-type calcium channel; 2-thio-3,4-dihydroquinazoline; current inhibition; cytotoxicity

## 1. Introduction

The calcium ($Ca^{2+}$) channel family is divided into three phylogenetic subfamilies: $Ca_v1$ (L-type), $Ca_v2$ (P/Q, N, and R-types) and $Ca_v3$ (T-type).[1] Both, $Ca_v1$ and $Ca_v2$ are activated by strong membrane depolarizations whereas $Ca_v3$ is low voltage activated. All calcium channels participate in the activation of intracellular signaling pathways that control many cell processes such as proliferation, differentiation, mitogenesis, metastasis, and apoptosis.[2-7] On the other hand, aberrant calcium channel activity has been linked to a number of pathological conditions, and consequently calcium channels are important drug targets.[8] This also includes the family of T-type calcium channels (i.e., $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$ which are expressed widely and differentially throughout nerve, muscle and heart, with both gain and loss of function mutations in $Ca_v3$ encoding genes being linked to a range of disorders, including epilepsy, movement disorders, and autism.[1,9] As a result, there have been considerable efforts in the development of new T-type calcium channel inhibitors with the goal of identifying inhibitors that preferentially block T-type over non T-type channels (and even blockers with selectivity within the $Ca_v3$ family) to minimize the potential for adverse effects.[10] Indeed, a number of recent studies have identified various pharmacophores acting on T-type calcium channels selectively, whereas others have yielded compounds that target both $Ca_v2$ and $Ca_v3$ calcium channels.[11-15]

Anomalous regulation of T-type calcium channels has been linked to many cancer processes although T-type $Ca^{2+}$ channel expression levels depend on cancer type, stage, and T-type $Ca^{2+}$ channel isoform.[16-19] It is possible that survival of cancer cells requires increased basal $Ca^{2+}$ influx and remodeled $Ca^{2+}$ signaling pathways for enhancing proliferation, promoting invasiveness and conferring chemotherapeutic resistance.[20-21] During the past few decades a number of research groups have highlighted T-type $Ca^{2+}$ channels as promising cancer biomarkers and anti-neoplastic targets.[22-25]

T-type $Ca^{2+}$ channel blockers have been developed with the intent of arresting cancer cell proliferation or inducing cancer cell death since the expression of T-type $Ca^{2+}$ channels in human

retinoblastoma Y79 cells was first reported in 2002.[26] The pharmacological effects of T-type $Ca^{2+}$ channel blockers on cancer cells are supported by the fact that they reduced proliferation due to effects on the p53-dependent p21 upregulation pathway[27] halting the $G_1$-S progression. Moreover, the T-type blocker mediated decrease in cell survival was shown to involve caspase-dependent apoptosis[28,29] or autophagy deregulation[30] through p38-MAPK or nutrient stress pathways. The role of T-type channels in cancer cell survival is complex due to the intricate nature of the underlying signaling pathways.[31] It was demonstrated that T-type calcium channel blockers such as mibefradil and pimozide recruit different cytotoxic pathways to the mitogenic cell lines in which pimozide decreases mRNA levels of Bcl-2 whereas mibefradil activates caspases.[32] Along these lines, our 3.4-dihydroquinazoline derivative, **KYS05090**, showed a cytotoxic effect through the induction of autophagy and apoptosis via ROS generation based on the inhibition of glucose uptake.[33]

In our previous studies, most structures of 3,4-dihydroquinazoline derivatives consisting of various groups at the C2-, N3-, C4-positions showed both cytotoxic effects against various cancer cell lines that paralleled T-type $Ca^{2+}$ channel blocking effects.[33-38] The best lead compound in our hands, **KYS05090** with an *N',N'*-dimethyl-*N*-methylpentane-1,5-diamino group at the C2-position, a biphenyl group at the N3-position, and an *N*-benzylacetamide group at the C4-position was evaluated and synthesized through an optimal design.[39] However, it was limited in commercial chemical utility and a further modification of the functional group at the C2-position was needed. The limitation had to be overcome because functional variations into the C2-position should be important for improving the selectivity of T-type $Ca^{2+}$ channels among all $Ca^{2+}$ channels on the basis of our previous work reporting selective $Ca^{2+}$ channel activity according to only C2-position differences between **KYS05044** and **KYS05089** (**Figure 1**).[39] Therefore, we needed to switch the synthetic pathway to obtain a surrogate structure with an electron donor group similar to the guanidine moiety. Among a variety of donor groups, a thiourea group containing a sulfur atom was chosen as a synthetic intermediate due to the fact that not only can it easily form the final structure through simple $S_N2$

reaction, but also the sulfur version of the final compounds from a thiourea does not affect the original electronic interaction with targets that has been shown in **KYS05090**.

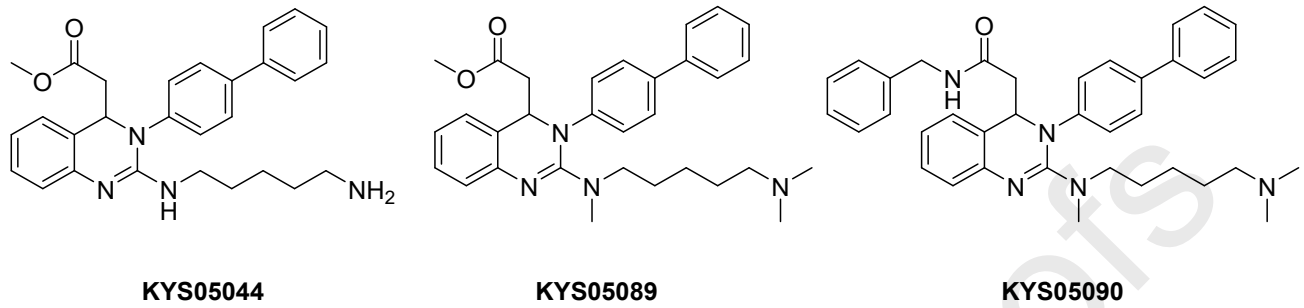



**Figure 1**. Structures of **KYS05044**, **KYS05089** and **KYS05090**.

## 2. Results and discussion

### *2.1. Chemistry*

The requested core precursor, 3,4-dihydroquinazoline-2-thione, as a surrogate of a guanidine moiety is an attractive heterocyclic structure, which may be applicable to biological and pharmaceutical activities.[40] In this study, we concentrated on an efficient synthetic approach of the 2-thio-3,4-dihydroquinazoline scaffold and the biological properties to evaluate the scaffold differentiation by first comparing T-type channel blocking activity of 2-amino-3,4-dihydroquinazoline, **KYS05090** containing a guanidine moiety and chemical variations at C2-position. A series of 2-thio-3,4-dihydroquinazoline derivatives as a guanidine-surrogate of **KYS05090** was synthesized as described below (**Scheme 1**). 2-Nitrocinnamic acid **1** was coupled with benzylamine or 4-fluorobenzylamine via EDC [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide] amide coupling reaction.[41] Compound **2** was reduced with Zn (powder)/$NH_4Cl$ into compound **3**. In the synthesis of compound **4**, chemoselective aza-Michael addition in which nitrogen acts as a nucleophile, not sulfur, was required (**Figure 2**).

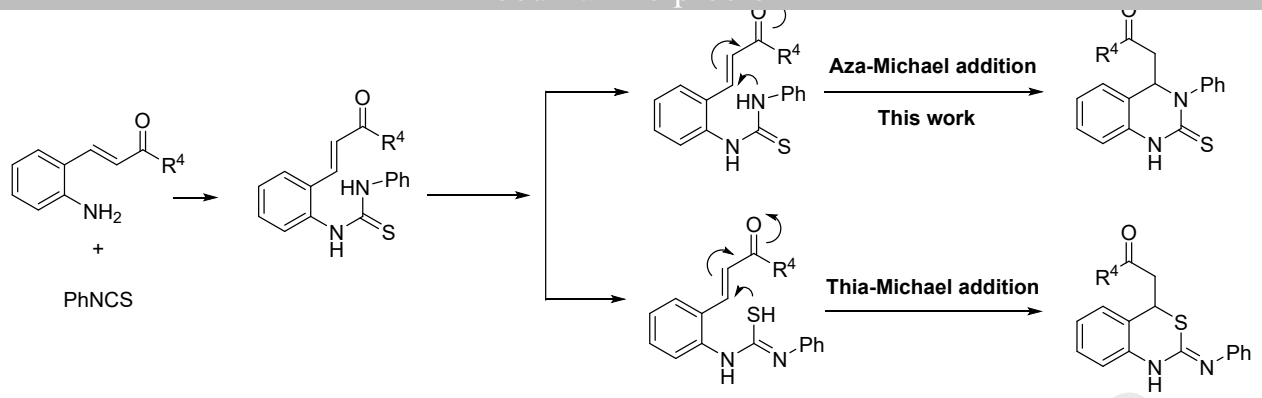
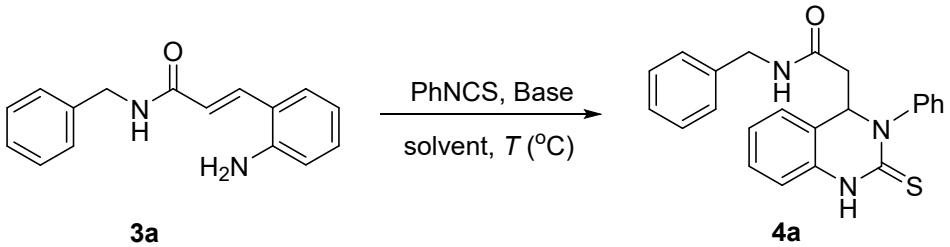



**Figure 2.** The chemoselective aza- and thia-Michael reactions.

So, we screened several solvents and bases to find optimal chemoselective aza-Michael addition conditions (**Table 1**). Our first attempt without any base did not provide the desired product **4a** (entry 1 and 2 in **Table 1**). Moreover, the reaction proceeded very slowly. However, addition of a base in DMSO solvent improved the yield to around 70% (entry 3 and 4 in **Table 1**). Additionally, the reaction time can be reduced also with a high yield (91%) when the reaction temperature was increased up to 80 $^{o}$C (entry 5 and 6 in **Table 1**). As a result, compound **4**, 3.4-dihydroquinazoline-2-thione, was smoothly synthesized via selective aza-Michael addition in our optimal condition ($K_2CO_3$ base, 80 $^{o}$C, 2 h). Subsequently, the 2-thio-3,4-dihydroquinazoline derivatives were synthesized via two successive nucleophilic substitution reactions (step **d** and **e** in **Scheme 1**).[42] Isothiocyanates with a biphenyl moiety, which are not commercially available, were prepared via a two-step reaction as shown below in **Scheme 1**: 4-Bromoaniline was coupled with phenyl or 4-fluorophenylboronic acid via Suzuki coupling into compound **8**.[43] The aniline moiety of **8** was reacted with carbondisulfide ($CS_2$) to afford the desired product **9** through desulfuration of an unstable dithiocarbamate intermediate.[44]

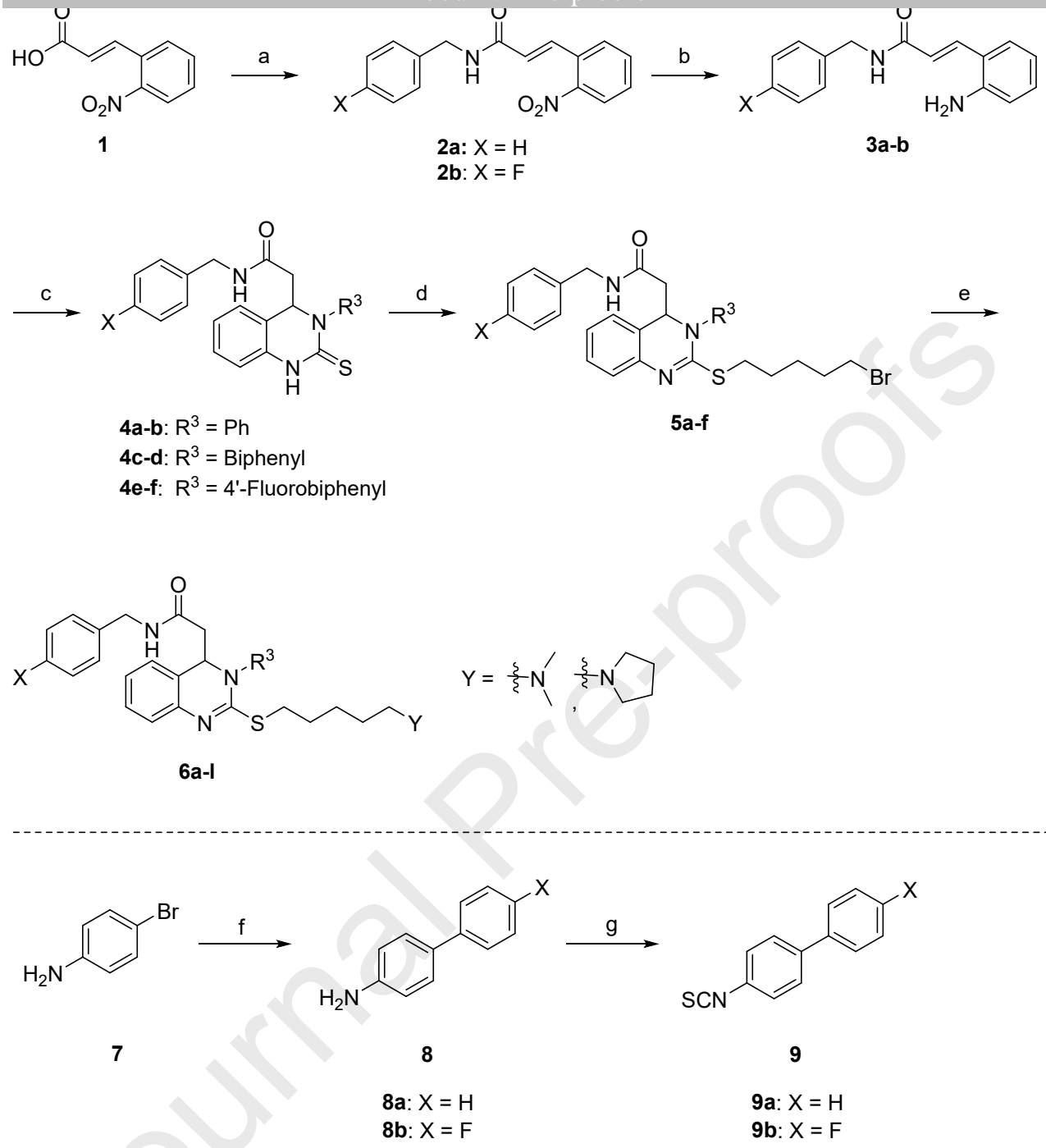
**Table 1.** Optimization of reaction conditions for the synthesis of compound **4** [a]




**3a** **4a**

| Entry | solvent | *T* (°C) | base [b] | time (h) | yield of **4a** (%) [c] |
|---|---|---|---|---|---|
| 1 | Water | 80 | - | 12 | 0 |
| 2 | DMSO | r.t. | - | 12 | 0 |
| 3 | DMSO | r.t. | DIPEA | 12 | 69 |
| 4 | DMSO | r.t. | $K_2CO_3$ | 12 | 73 |
| 5 | DMSO | 40 | $K_2CO_3$ | 15 | 81 |
| 6 | DMSO | 80 | $K_2CO_3$ | 2 | 91 |

[a] The reaction was conducted with **3a** (0.10 g, 0.40 mmol), phenylisothiocyante (0.052 mL ,0.44 mmol) and base (0.12 mmol) in solvent (5 mL); [b] DIPEA is diisopropylethylamine (Hunig's base); [c] Isolated yield.

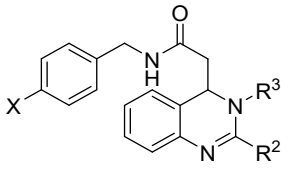


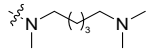
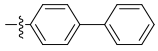
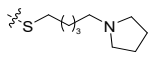
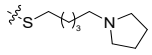
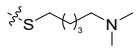
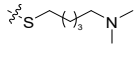
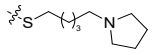
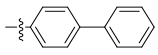
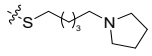
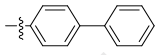
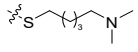
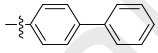
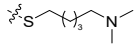
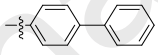
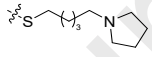
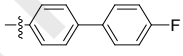
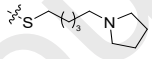
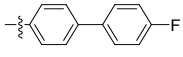
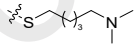
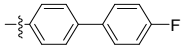
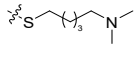
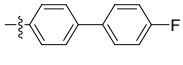
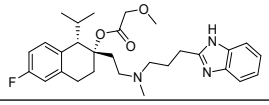




**Scheme 1.** Synthesis of 2-thio-3,4-dihydroquinazoline derivatives (**6**) and reagent **9**. Reagents and conditions: (a) benzylamine or 4-fluorobenzylamine, EDC HCl, *N*-hydroxybenzotriazole (HOBt), DCM/THF (1:1, *v/v*), rt, 71-78%; (b) Zn, $NH_4Cl$, MeOH, reflux, 95- 97%; (c) phenyl isothiocyanate or compound **9**, $K_2CO_3$, DMSO, 80 °C, 80-91%; (d) 1,5-dibromopentane, $K_2CO_3$, DMF, 60 °C, 60-74%; (e) dimethylamine or pyrrolidine, $K_2CO_3$, DMF, 0 °C to rt, 22-89%; (f) phenylboronic acid, $Pd(PPh_3)_4$, $K_2CO_3$, EtOH/$H_2O$/toluene (3:3:10,*v/v/v*.), 100 °C, 76-80%; (g) i) $CS_2$, 1,4-diazabicyclo[2.2.2]octane (DABCO), acetone, rt, ii) di-*tert*-butyl dicarbonate, DCM, 0 °C to rt, 70-73%.

**Table 2.** Biological activity of 2-thio-3,4-dihydroquinazoline derivatives as sulfur-analogues of **KYS05090**



| Entry | $R^2$ | $R^3$ | X | T-type Calcium channel inhibition: % inhibition (at 10 μM) (Estimated $IC_{50}$ in μM) [a] | | | Cytotoxicity: $IC_{50}$ (μM) [b] | |
|---|---|---|---|---|---|---|---|---|
| | | | | $Ca_v3.1$ ($\alpha_{1G}$) [c] | $Ca_v3.2$ ($\alpha_{1H}$) [d] | $Ca_v3.3$ ($\alpha_{1I}$) [e] | A549 [f] | HCT-116 [g] |
| **KYS05090** |  |  | H | 98.0±1.6 (0.26) | 89.3±2.1 (3.6) | ND [h] | 6.0 | 5.6 |
| **6a** |  |  | H | 60.4±19.8 (77.01) | 61.6±10.5 (7.71) | 78.3±6.0 (6.19) | 98.1 | 31.3 |
| **6b** |  |  | F | 60.1±13.1 (13.50) | 40.0±15.3 (57.43) | 22.1±13.5 (663.90) | 89.9 | 27.1 |
| **6c** |  |  | H | 46.5±13.2 (18.51) | 51.8±12.2 (13.18) | 76.0±3.2 (3.20) | 138.2 | 38.8 |
| **6d** |  |  | F | 26.7±15.4 (554.90) | 7.0±7.0 (832.30) | inactive | 36.8 | 38.8 |
| **6e** |  |  | H | 93.9±1.7 (0.66) | 73.1±19.3 (6.90) | 92.7±2.3 (0.81) | 14.9 | 5.5 |
| **6f** |  |  | F | 51.5±1.3 (14.97) | 69.0±23.7 (276.10) | 55.9±21.4 (13.63) | 19.8 | 6.2 |
| **6g** |  |  | F | 31.0±7.4 (27.38) | 83.1±15.4 (3.14) | 69.3±7.02 (6.10) | 5.0 | 6.4 |
| **6h** |  |  | H | 76.2±17.3 (7.77) | 83.9±8.6 (2.40) | 85.6±4.5 (1.76) | 18.1 | 9.6 |
| **6i** |  |  | H | 83.9±7.0 (2.18) | 97.5±1.1 (0.26) | 68.2±6.6 (5.10) | 17.8 | 12.5 |
| **6j** |  |  | F | 44.5±2.1 (12.55) | 76.6±9.8 (4.88) | 35.1±5.3 (19.78) | 12.6 | 7.7 |
| **6k** |  |  | H | 97.6±0.8 (0.25) | 75.6±12.7 (4.18) | 66.7±27.7 (9.06) | 17.6 | 9.2 |
| **6l** |  |  | F | 96.2±1.0 (0.40) | 84.8±11.5 (2.76) | 92.0±4.4 (0.96) | 11.3 | 9.3 |
| **Mibefradil** |  | | | 95.9 ± 1.7 (1.34) [i] | ND | ND | 24.9 | ND |

[a] Estimated $IC_{50}$ values were obtained by measuring the percentage of inhibition of $Ca_v3$ currents by 10 μM compound, using patch clamp electrophysiology (n≥3) , and then calculating the $IC_{50}$ by solving the Hill equation $Ir=1/(1+[C]/IC_{50})$ where Ir is the fraction of current remaining after application of the compounds, and [C] is the concentration of the compound (10 μM) ; [b] MTT assay; [c, d, e] T-type $Ca^{2+}$ channels ($\alpha_{1G}$ , $\alpha_{1H}$ and $\alpha_{1I}$ expressed in tsA-201 cells; [f] Human non-small cell lung cancer cells; [g] Human colon cancer cells; [h] Not determined; [i] Reported data by our group.[39]

*2.2. Biological evaluation*

The synthetic compounds were broadly divided into two classes to compare a structural differentiation of the biological activities even though fluorine was added to $R^3$ and $R^4$ as bioisosteres. One consisted of a combination of variable $R^2$ and fixed $R^3$ similar to **KYS05090** as $R^2$ (5-dimethylaminopentyl), $R^2$-alternative (5-pyrrolidinopentyl) and $R^3$ (biphenyl). The other was a combination of variable $R^2$ and $R^3$-alternatives as $R^2$ (5-dimethylaminopentyl), $R^2$-alternative (5-pyrrolidinopentyl) and $R^3$-alternative (phenyl).

T-type $Ca^{2+}$ channel blocking abilities of all compounds were determined at 10 μM concentrations using a patch-clamp assay involving heterologously expressed T-type $Ca^{2+}$ channels. Human embryonic kidney tsA-201 cells were cultured and transfected using the calcium phosphate method as described previously.[45] Cells were plated on glass coverslips and transfected with either 3 μg of human $Ca_v3.1$, $Ca_v3.2$ or $Ca_v3.3$ cDNA. Plasmid encoding enhanced fluorescent protein (pEGFP) (0.5μg) was included into the transfection mix as a transfection marker. Electrophysiological recordings were performed according to the whole cell configuration of the patch-clamp technique 72 h after transfection at room temperature. The external recording solution contained (in mM): 20 $BaCl_2$, 1 $MgCl_2$, 40 Tetraethylammonium Chloride (TEACl), 65 CsCl, 10 HEPES, and 10 glucose, pH 7.4. Borosilicate glass pipettes were filled with internal solution containing (in mM): 140 CsCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 5 EGTA, 10 HEPES, 2 Na-Adenosinetriphosphate (ATP), and 0.3 Mg-Guanosinetriphosphate (GTP), pH 7.3. Compounds were prepared daily in external solution and were applied locally to cells. Currents were evoked by depolarization from a holding potential of −110 mV to a test potential of −20 mV. The interpulse interval was 20 s, and the test pulse was 100 ms long. The effects of the compounds were assessed by normalizing the current amplitude in the presence of the compound to that observed before adding the drug. Independent experiments were performed using the vehicle DMSO (0.1%) and no inhibition was observed.

The overall *in vitro* T-type $Ca^{2+}$ channel blocking activities and anti-neoplastic effects of new synthetic compounds are summarized in **Table 2** and **Figure 3**. Data obtained with **KYS05090**

and mibefradil are also tabulated in **Table 2** as a standard as well as a positive control for the activities. As shown in **Figure 3**, some compounds similarly inhibited all three T-type channel isoforms, while some exhibited a preference for $Ca_v3.2$ (**6j** for example). We focused our analysis predominantly on $Ca_v3.1$ ($\alpha_{1G}$) and $Ca_v3.2$ ($\alpha_{1H}$) since the correlation between $Ca_v3.3$ overexpression and some tumors is relatively lower compared with $Ca_v3.1$ and $Ca_v3.2$ channels.[46] A detailed examination of $Ca_v3.1$ and $Ca_v3.2$ reveals that similar to our previous SAR (structure-activity relationship) results,[32] the modification of the N3-position in 2-thio-3,4-dihydroquinazoline core structure corresponded to the results of 2-amino-3,4-dihydroquinazoline so that most compounds with the biphenyl group at $R^3$ position gave more effective inhibitory activities on both $Ca_v3.1$ and $Ca_v3.2$ transfected cells rather than those of other derivatives that contain the phenyl group. Moreover, **6e** (93.9±1.7% inhibition), **6k** (97.6±0.8% inhibition), and **6l** (96.2±1.0% inhibition) were similar or more potent in comparison to mibefradil (95.9±1.7% inhibition) and **KYS05090** (98.0±1.6% inhibition). For $Ca_v3.2$ ($\alpha_{1H}$) blocking activity, **6i** (97.5±1.1% inhibition) exhibited a greater degree of inhibition than **KYS05090**. These results are consistent with our previous SAR studies[36,39] even though there was a substitution to a sulfur atom at the C2-position of 3,4-dihydroquinazoline. However, it was an unexpected result that **6a** and **6b** (60.4±19.8 and 60.1±13.1% against $Ca_v3.1$, respectively) provided more active inhibition of $Ca_v3.1$ ($\alpha_{1G}$) than those of biphenyl derivatives **6f**, **6g**, and **6j** (51.5±1.3, 31.0±7.4, and 44.5±2.1% against $Ca_v3.1$, respectively), and **6d** mediated exceptionally low T-type $Ca^{2+}$ channel inhibition (26.7±15.4 and 7.0±7.0% against $Ca_v3.1$ and $Ca_v3.2$, respectively).

Some T-type $Ca^{2+}$ channel blockers including small organic molecules and peptide toxins either block the pore or inhibit gating allosterically.[1] T-type channel activity can also be regulated via second messengers or post-translational modifications that regulate cell surface density.[47-48] Although a direct action of our compounds on the channel are the most likely scenario, we cannot rule out the possibility that some of the compounds might affect channel trafficking or regulation.

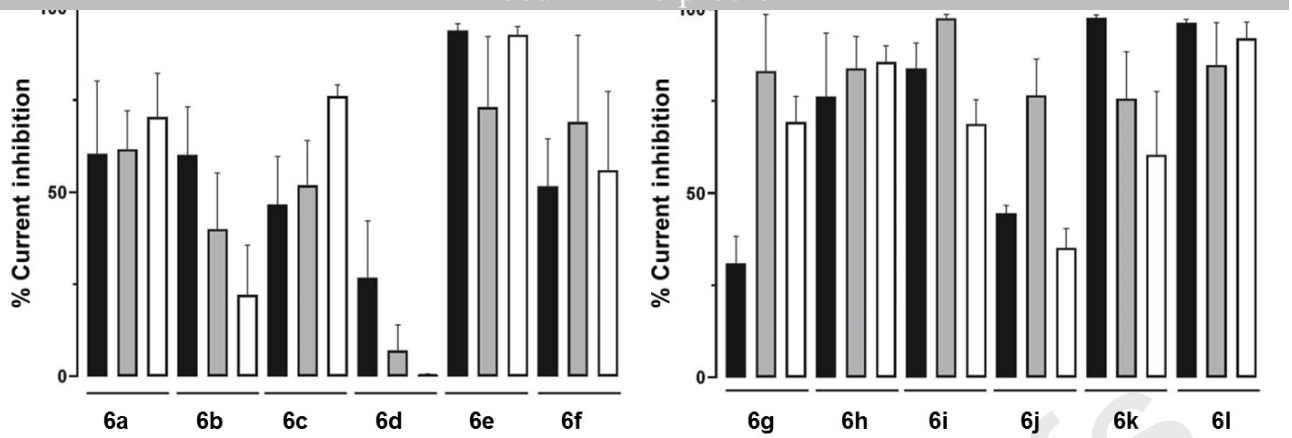



**Figure 3.** Inhibition of human $Ca_v3.1$ (black bars), human $Ca_v3.2$ (grey bars) and human $Ca_v3.3$ (white bars) calcium channels. Compounds were applied at a test concentration of 10 μM, and the percentage of current inhibition determined (n = 3-7).

All prepared 2-thio-3,4-dihydroquinazoline derivatives were evaluated for cytotoxic activity against A549 (human non-small cell lung cancer cells) and HCT-116 (human colon cancer cells) cell lines using an MTT assay.[49] There was also a steep SAR with the $R^3$ group: The compounds (**6a-d**) having phenyl group at $R^3$ position exhibited poorer anti-proliferative activities than compounds (**6e-6l**) having biphenyl group at the same position. The $IC_{50}$ values of compounds containing a biphenyl group at $R^3$ position against both A549 and HCT-116 cells were at least 3-fold more active than compounds containing a phenyl group at $R^3$ position. On the other hand, all of these compounds exhibited greater cytotoxic effects than mibefradil ($IC_{50}$ = 24.9 μM) against A549 cell lines. Among the synthetic derivatives, **6g** was most active against both A549 and HCT-116 cell lines ($IC_{50}$ = 5.0 and 6.5 μM respectively). In addition, it had a better anti-proliferative effect than **KYS05090** against A549 cell lines. In the case of cytotoxicity against HCT-116 cell lines, compounds containing a biphenyl group at $R^3$ position showed better or similar activities ($IC_{50}$ = 5.5 into 9.6 μM) compared with **KYS05090** ($IC_{50}$ = 5.6 μM) except **6i** ($IC_{50}$ = 12.5 μM). For some compounds, T-type $Ca^{2+}$ channel blocking activity was low, yet they mediated potent cytotoxicity. It is possible that these compounds might affect cancer cells by acting on another target, or by altering T-type channel

mediated processes that do not involve a reduction in calcium entry. Therefore, further biochemical or biological studies will be needed to explain the SAR correlation obtained in 10 μM concentrations.

## 3. Conclusions

In summary, a series of 2-thio-3,4-dihydroquinazoline was synthesized and biologically evaluated to identify their T-type $Ca^{2+}$ channel ($Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$) blocking effects and cytotoxic effects against A549 (human non-small cell lung cancer cells) and HCT-116 (human colon cancer cells). These findings add to a large body of recent literatures that reported a range of different pharmacophores and their SAR against heterologously expressed T-type calcium channels,[10] although SAR comparison between our compound series and these previous works is difficult given the wide range of chemical structures of T-type channel blockers. Within our compound series, a synthetic optimization of aza-Michael addition reaction via addition of a base led successfully to the synthesis of 2-thio-3,4-dihydroquinazoline derivatives in good yield (80-91% yield at the aza-Michael addition step). The biological data showed that most compounds having the biphenyl group at $R^3$ position of 2-thio-3,4-dihydroquinazoline derivatives were more effective than compounds containing the phenyl group at the same position with regard to both T-type calcium channel blockade and cytotoxicity, which is consistent with our previous research of SAR studies. Therefore, these results indicate that the guanidine moiety of **KYS05090** would be not a necessary functional group for both T-type calcium channel block and cytotoxicity against cancer cell lines, and thus provide the opportunity to expand the available repertoire of chemical diversities for T-type calcium channel block. In addition, **6g** exhibited better ($IC_{50}$ = 5.0 μM against A549 cell) or similar ($IC_{50}$ = 6.2 μM against HCT-116 cell) cytotoxic effect in comparison with the reference compound **KYS05090**. Thus, **6g** could be a backup compound of **KYS05090** for further biological investigations as an anti-neoplastic agent.

## 4. Experimental section

### *4.1. Chemistry*

#### *4.1.1. General*

All solvents and commercially available reagents were used without additional purification. Reactions were monitored by analytical thin layer chromatography (TLC, Merck, silica gel 60 $F_{254}$) with UV light (254 nm). Visualization was performed by incubation with *p*-methoxyanisaldehyde (PMA) solution and heating with a heat gun. Flash column chromatography was performed using a silica gel (Merck, 230-400 mesh). NMR spectra ($^{1}H$ and $^{13}C$) were recorded on Bruker Avance 400 MHz spectrometer. High-resolution mass spectra were recorded with JEOL JMS-700 MStation mass spectrometer (ionization mode: FAB+). HPLC for chemical purities of final compounds **6a-6l** was carried out on Waters 1525, equipped with Waters PDA 2998 (280 nm).

#### *4.1.2. General preparation of compound **8*** [43]

To a solution of 4-bromoaniline (**7**, 2.00 g, 11.60 mmol) and phenylboronic acid (1.56 g, 12.78 mmol) in a mixed solution of water, ethanol and toluene (30 mL, $H_2O$:EtOH:toluene=3:3:10, *v/v*) were added $Pd(PPh_3)_4$ (0.13 g, 0.12 mmol) and $K_2CO_3$ (16.00 g, 116.00 mmol) neatly. The reaction mixture was purged with Ar gas. The reaction mixture was stirred at 100 $^{o}$C for 12 h in Ar atmosphere and concentrated under reduced pressure to give the crude product. The crude product was extracted with dichloromethane (50 mL) and water (40 mL), dried ($MgSO_4$) and concentrated *in vacuo.* The residue was purified by flash column chromatography ($CH_2Cl_2$:hexane = 2:5) to provide desired product **8**.

*[1,1'-biphenyl]-4-amine* (**8a**, 1.75 g, 80%): $^{1}H$ NMR (400 MHz, DMSO-$d_6$) δ 7.53 (2H, d, $J$ = 7.6 Hz), 7.37 (4H, t, $J$ = 7.6 Hz), 7.21 (1H, t, $J$ = 7.2 Hz), 6.65 (2H, d, $J$ = 8.4 Hz), 5.23 (2H, s).

*4'-fluoro-[1,1'-biphenyl]-4-amine* (**8b**, 1. 65 g, 76%): $^{1}H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.56 – 7.53 (2H, m), 7.32 (2H, d, $J$ = 8.4 Hz), 7.18 (2H, t, $J$ = 5.0 Hz), 6.63 (2H, d, $J$ = 8.4 Hz), 5.21 (2H, s).

*4.1.3. General preparation of Compound* ***9*** [41]

To a solution of **8a** or **8b** (10.38 mmol) and DABCO (3.49 g, 31.15 mmol) in acetone (10 mL) was dropwise added $CS_2$ (20 mL). The reaction mixture was stirred for 1 h at room temperature and then filtered. The filter cake was dissolved in dichloromethane (30 mL). To this solution, were added di-*tert*-butyl dicarbonate (2.24 g, 10.28 mmol) and DMAP (4-dimethylaminopyridine ) (0.02 g, 0.21 mmol) and stirred for 1 h at room temperature. The reaction mixture was extracted with dichloromethane (45 mL) and water (30 mL), dried with $MgSO_4$ and evaporated under reduced pressure. Flash column chromatography (DCM:hexane = 1:5) gave the desired product **9**.

*4-isothiocyanato-1,1'-biphenyl* (**9a,** 1.78 g, 70%): $^1$H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.48 (4H, m), 7.38 (2H, t, *J* = 7.4 Hz), 7.32 – 7.30 (1H, m), 7.28 – 7.19 (2H, m).

*4-fluoro-4'-isothiocyanato-1,1'-biphenyl* (**9b**, 1.74 g, 73%): $^1$H NMR (400 MHz, DMSO-$d_6$) 7.75 – 7.73 (4H, ,m), 7.52 (2H, d, *J* = 8.4 Hz), 7.31 (2H, t, *J* = 9.0 Hz).

*4.1.4. General preparation of compound* ***2*** [41]

A solution of 2-nitrocinnamic acid (**1**, 2.00 g, 10.35 mmol) in THF (15 mL) and DCM (15 mL) was treated with EDC·HCl (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) salt (4.96 g, 25.88 mmol), HOBt (*N*-hydroxy-benzotriazole) (4.20 g, 31.05 mmol) and benzylamine or 4-fluorobenzylamine (31.05 mmol) neatly. The reaction mixture was stirred for 30 min under ice bath, further stirred at room temperature overnight and concentrated under reduced pressure. The reaction mixture was dissolved with a mixed solvent of $CH_2Cl_2$ (100 mL) and water (20 mL), extracted with $CH_2Cl_2$, dried ($MgSO_4$) and concentrated under reduced pressure. The concentrated residue was recrystallized with co-solvent of $CH_2Cl_2$, methanol and ethyl ether to give the desired product **2**.

*(E)-N-benzyl-3-(2-nitrophenyl)acrylamide* (**2a**, 2.28 g, 78%): $^1$H NMR (400 MHz, $CDCl_3$) *δ* 8.79 (1H, t, *J* = 5.4 Hz), 8.06 (1H, d, *J* = 8.0 Hz), 7.79 – 7.73 (3H, m), 7.65 – 7.63 (1H, m), 7.37 – 7.25 (1H, m), 6.70 (1H, d, *J* = 15.6 Hz) 4.42 (2H, d, *J* = 6.0 Hz).

*(E)-N-(4-fluorobenzyl)-3-(2-nitrophenyl)acrylamiae* (**2b**, 2.21 g, 71%): $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.78 (1H, s), 8.06 (1H, d, *J* = 8.0 Hz), 7.76 (3H, m), 7.65 (1H, m), 7.35 (2H, m), 7.18 (2H, t, *J* = 9.2 Hz), 6.68 (1H, d, *J* = 15.6 Hz), 4.40 (2H, d, *J* = 5.6 Hz).

*4.1.5. General preparation of compound **3***

Zinc powder 2 g was stirred with 2% HCl aqueous solution (4 mL) for 30 min. The mixture was then filtered and washed with water (10 mL) and diethyl ether (10 mL). The filter cake (activated zinc powder) was dryed *in vacuo*. To a solution of **2** (3.54 mmol) in methanol (20 mL) were added purified zinc (1.57 g, 28.3 mmol) and ammonium chloride (0.42g, 7.79 mmol) at room temperature. The reaction mixture was stirred at reflux for 16 h. The resulting mixture was cooled to room temperature and filtered through Celite 545. The filtrate was concentrated *in vacuo* and dissolved with $CH_2Cl_2$. The organic solution was washed with sat. $NaHCO_3$ solution, dried ($MgSO_4$) and evaporated *in vacuo* to provide the desired product **3**.

*(E)-3-(2-aminophenyl)-N-benzylacrylamide* (**3a**, 0.91 g, 95%): $^1$H NMR (400 MHz, $CDCl_3$) *δ* 7.74 (1H, d, *J* = 15.6 Hz), 7.29 – 7.19 (6H, m), 7.09 – 7.05 (1H, m), 6.66 (1H, t, *J* = 7.4 Hz), 6.61 (1H, d, 8.0 Hz), 6.25 (1H, d, *J* = 15.2 Hz), 5.22 (1H, br s), 4.49 (2H, d, *J* = 5.6 Hz), 2.79 (2H, br s).

*(E)-3-(2-aminophenyl)-N-(4-fluorobenzyl)acrylamide* (**3b**, 1.0 g, 97%) $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.51 (1H, t, *J* = 5.2 Hz), 7.65 (1H, d, *J* = 15.6 Hz), 7.31 (3H, m), 7.16 (2H, t, *J* = 8.8 Hz), 7.04 (1H, t, *J* = 7.2 Hz), 6.68 (1H, d, *J* = 7.6 Hz), 6.55 (1H, t, *J* = 7.6 Hz), 6.45 (1H, d, *J* = 15.6 Hz), 5.39 (2H, s), 4.38 (2H, d, *J* = 6.0 Hz).

*4.1.6. General preparation of compound **4***

To a solution of compound **3** (0.65 mmol) and phenylisothiocyanate (0.72 mmol) in DMSO (10 mL) was added $K_2CO_3$ (0.03 g,0.20 mmol). The reaction mixture was stirred for 2 h at 80 °C and poured into a mixed solution of EtOAc (45 mL) and water (60 mL). The extracted combinded organic

layer was dried with $MgSO_4$ and evaporated under reduced pressure. The crude product was then purified by flash column chromatography using the gradient eluent system (EA: Hex = 1:5 to 1:1) for **4a** and **4b** or purified by recrystalization with THF and hexane for **4c – 4f**.

*N-benzyl-2-(3-phenyl-2-thioxo-1,2,3,4-tetrahydroquinazolin-4-yl)acetamide* (**4a**, 0.18 g, 72%): $^1$H NMR (400 MHz, DMSO) $\delta$ 11.03 (1H, s), 8.32 (1H, t, $J$ = 5.6 Hz), 7.46 – 7.28 (5H, m) 7.27 – 7.20 (4H, m), 7.14 (1H, d, $J$ = 7.6 Hz), 7.05 – 6.97 (4H, m), 5.16 (1H, dd, $J$ = 10 Hz and 4 Hz), 4.22 (1H, dd, $J$ = 15 Hz and 6 Hz), 4.05 (1H, dd, $J$ = 14.6 Hz and 6.2 Hz), 2.77 (1H, dd, $J$ = 13.6 Hz and 4 Hz), 2.63 (1H, dd, $J$ = 14 Hz and 10 Hz ).

*N-benzyl-2-(3-(4-fluorophenyl)-2-thioxo-1,2,3,4-tetrahydroquinazolin-4-yl)acetamide* (**4b,** 0.16 g ,61%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.41 (1H, s), 7.38 – 7.28 (5H, m), 7.22 – 7.14 (1H, m), 7.10 - 7.03 (1H, m), 6.93 – 6.81 (6H, m), 5.76 (1H, br s), 5.22 (1H, dd, $J$ = 9.6 Hz and 4.4 Hz ), 6.25 (1H, d, $J$ = 15.2 Hz), 5.22 (1H, br s), 5.15 (1H, dd, $J$ = 9.6 Hz and 3.6 Hz), 4.16 (1H, dd, $J$ = 14.8 Hz and 6.0 Hz), 4.06 – 3.97 (1H, m), 2.82 (1H, dd, $J$ = 13.6 Hz and 4.0 Hz), 2.59 – 2.56 (1H, m).

*2-(3-([1,1'-biphenyl]-4-yl)-2-thioxo-1,2,3,4-tetrahydroquinazolin-4-yl)-N-benzylacetamide* (**4c**, 0.21 g, 71%): $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.07 (1H, s), 8.35 (1H, s), 7.73 – 7.71 (4H, m), 7.50 – 7.49 (4H, m), 7.42 – 7.40 (1H, m), 7.31 (1H, t, $J$ = 8.0 Hz), 7.25 – 7.20 (4H, m), 7.16 – 7.02 (4H, m), 5.21 (1H, dd, $J$ = 9.4 Hz and 3.6 Hz), 4.22 (1H, dd, $J$ = 14.9 Hz and 5.9 Hz), 4.09 – 4.04 (1H, m), 2.82 – 2.79 (1H, m), 2.68 – 2.66 (1H, m).

*2-(3-([1,1'-Biphenyl]-4-yl)-2-thioxo-1,2,3,4-tetrahydroquinazolin-4-yl)-N-(4-fluorobenzyl)acetamide* (**4d**, 0.22 g, 69%): $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.08 (1H, s), 8.36 (1H, t, $J$ = 5.4 Hz ), 7.73 – 7.71 (4H, m), 7.52 – 7.51 (4H, m), 7.50 – 7.49 (1H, m), 7.42 – 7.38 (1H, m), 7.33 – 7.29 (1H, m) 7.15

– 7.13 (1H, m), 7.10 – 6.99 (6H, m), 5.20 (1H, dd, *J* = 9.4 Hz and 3.6 Hz), 4.22 – 4.17 (1H, m), 4.08 – 4.01 (1H, m), 2.82 – 2.78 (1H, m), 2.67 – 2.61 (1H, m).

*N-benzyl-2-(3-(4'-fluoro-[1,1'-biphenyl]-4-yl)-2-thioxo-1,2,3,4-tetrahydroquinazolin-4-yl)acetamide* (**4e**, 0.19 g , 62%): $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.04 (1H, s), 8.34 (1H, s), 7.80 – 7.75 (4H, m), 7.71 – 7.74 (3H, m), 7.50 – 7.39 (3H, m), 7.25 – 7.19 (1H, m), 7.10 – 7.01 (6H, m), 5.20 (1H, dd, *J* = 9.4 Hz and 3.6 Hz), 4.18 – 4.16 (1H, m), 4.09 – 4.04 (1H, m), 2.80 – 2.74 (1H, m), 2.65 – 2.59 (1H, m).

*2-(3-(4'-fluoro-[1,1'-biphenyl]-4-yl)-2-thioxo-1,2,3,4-tetrahydroquinazolin-4-yl)-N-(4-fluorobenzyl)acetamide* (**4f**, 0.20 g, 63%): $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.07 (1H, s), 8.35 (1H, s), 7.85 – 7.76 (4H, m), 7.65 – 7.62 (2H, m), 7.50 – 7.39 (3H, m), 7.25 – 7.05 (7H, m), 5.19 (1H, dd, *J* = 9.4 Hz and 3.6 Hz), 4.20 – 4.16 (1H, m), 4.10 – 4.06 (1H, m), 2.79 – 2.70 (1H, m), 2.64 – 2.60 (1H, m).

*4.1.7. General preparation of compound* ***5***

To a solution of 1,5-dibromopentane (0.07 mL, 0.52 mmol) and $K_2CO_3$ (0.07 g, 0.52 mmol) in DMF (6 mL) was added dropwise **4** (0.26 mmol). The reaction mixture was stirred at room temperature for 7 h. The reaction mixture was then poured into a mixed solution of ethyl ether (30 mL) and water (15 mL). The exracted organic layer was dried with $MgSO_4$, evaporated *in vacuo* and purified by flash column chromatography (EA: hexane = 1:3) to give the desired product **5**.

*N-benzyl-2-(2-((5-bromopentyl)thio)-3-phenyl-3,4-dihydroquinazolin-4-yl)acetamide* (**5a**, 0.2 g, 70%): $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$ 8.35 (1H, t, *J* = 5.6 Hz), 7.43 – 7.32 (5H, m), 7.28 – 7.21 (4H, m), 7.09 – 7.07 (3H, m), 7.01 (1H , t, *J* = 7.2 Hz), 6.96 (1H, d, *J* = 7.2 Hz), 5.08 (1H, dd, *J* = 5.2 Hz and 5.6 Hz), 4.26 – 4.10 (2H, m), 3.53 (2H , t, *J* = 6.6 Hz), 3.15 – 3.08 (1H, m), 2.99 – 2.92 (1H, m), 2.71 – 2.66 (1H, m), 2.57 – 2.51 (1H, m), 1.86 – 1.79 (2H, m), 1.64 – 1.58 (2H, m), 1.48 – 1.43 (2H, m).

*2-(2-[(5-bromopentyl)thio]-3-phenyl-3,4-dihydroquinazolin-4-yl)-N-(4-fluorobenzyl)acetamide* (**5b,** 0.22 g, 64%): $^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$ 8.21 (1H, t, $J$ = 5.6 Hz), 7.48 – 7.40 (4H, m), 7.32 – 7.21 (4H, m), 7.11 – 7.13 (3H, m), 7.09 (1H , t, $J$ = 7.2 Hz), 6.87 (1H, d, $J$ = 7.2 Hz), 5.11 (1H, dd, $J$ = 5.2 Hz and 5.6 Hz), 4.22 – 4.15 (2H, m), 3.46 (2H , t, $J$ = 6.6 Hz), 3.22 – 3.20 (1H, m), 2.94 – 2.90 (1H, m), 2.72 – 2.53 (2H, m), 1.83 – 1.75 (2H, m), 1.59 – 1.54 (2H, m), 1.46 – 1.44 (2H, m).

*2-(3-([1,1'-biphenyl]-4-yl)-2-((5-bromopentyl)thio)-3,4-dihydroquinazolin-4-yl)-N-benzylacetamide* (**5c**, 0.24 g, 67%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.52 – 7.49 (4H, m), 7.40 – 7.56 (2H, m), 7.31 – 7.26 (3H, m), 7.22 – 7.16 (5H, m), 7.02 (2H, d, $J$ = 2.0 Hz), 7.00 – 6.95 (2H, m), 5.71 (1H, s), 5.15 (1H, dd, $J$ = 5.6 Hz and 6 Hz), 4.27 – 4.18 (2H, m), 3.34 – 3.30 (2H, m), 3.24 – 3.22 (1H, m), 3.04 – 3.02 (1H, m), 2.69 – 2.65 (1H, m), 2.49 – 2.46 (1H, m), 1.83 – 1.78 (2H, m), 1.63 – 1.58 (2H, m), 1.50 – 1.44 (2H, m).

*2-(3-([1,1'-biphenyl]-4-yl)-2-((5-bromopentyl)thio)-3,4-dihydroquinazolin-4-yl)-N-(4-fluorobenzyl)acetamide* (**5d**, 0.22 g, 69%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.62 – 7.60 (4H, m), 7.50 – 7.46 (2H, m), 7.40 – 7. 38 (3H, m), 7.33 – 7. 30 (2H, m), 7.09 – 7.03 (4H, m), 6.98 – 6.94 (2H, m), 5.62 (1H, s), 5.23 (1H, t, $J$ = 7.0 Hz), 4.34 – 4.27 (2H, m), 3.43 (2H, t, $J$ = 6.8 Hz), 3.30 – 3.26 (1 H, m), 3.12 – 3.05 (1H, m), 2.79 – 2.75 (1H, m), 2.63 – 2.60 (1H, m), 1.94 – 1.89 (2H, m), 1.74 – 1.69 (3H, m), 1.62 – 1.55 (3H, m).

*N-benzyl-2-(2-[(5-bromopentyl)thio]-3-(4'-fluoro-[1,1'-biphenyl]-4-yl)-3,4-dihydroquinazolin-4-yl)acetamide* (**5e**, 0.18 g, 60%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.58 – 7.53 (4H, m), 7.39 (2H, d, $J$ = 8.0 Hz), 7.32 – 7.18 (5H, m), 7.26 – 7.11 (4H, m), 7.07 –7.06 (2H, m), 5.76 (1H, s), 5.23 (1H, t, $J$ = 8.0 Hz), 4.34 – 4.31 (1H, m), 3.44 – 3.41(1H, m), 3.27 – 3.24 (1H, m), 3.05 – 3.01 (1H, m), 2.80 – 2.75 (1H, m), 2.62 – 2.56 (1H, m), 1.93 – 1.88 (2H, m), 1.73 – 1.68 (2H, m), 1.60 – 1.54 (2H, m).

*2-(2-[(5-bromopentyl)thio]-3-(4'-fluoro-[1,1'-biphenyl]-4-yl)-3,4-dihydroquinazolin-4-yl)-N-(4-fluorobenzyl)acetamide* (**5f**, 0.20 g, 74%), $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.58 – 7.53 (4H, m), 7.38 (2H, d, $J$ = 8.4 Hz), 7.32 – 7.24 (2H, m), 7.16 (2H, t, $J$ = 8.8 Hz), 7.09 – 7.02 (4H, m), 6.96 (1H, t, $J$ = 8.4 Hz), 5.67 (1H, s), 5.21(1H, t, $J$ = 7.6 Hz), 4.34 – 4.27 (2H, m), 3.43 (2H, t, $J$ = 6.8 Hz), 3.27 – 3.25 (1H, m), 3.06 – 3.03 (1H, m), 2.75 – 2.73 (1H, m), 2.62 – 2.58 (1H, m), 1.94 – 1.88 (2H, m), 1.74 – 1.70 (2H, m), 1.60 – 1.55 (3H, m).

*4.1.8. General preparation of compound* ***6***

To a solution of **5** (0.19 mmol), $K_2CO_3$ (0.05 g, 0.38 mmol) in DMF (5 mL) was added dropwise dimethylamine (ca. 2 M in THF solution, 0.95 mL) or pyrrolidine ( 0.03 mL,0.38 mmol) at 0 $^o$C. The reaction mixture was then warmed to room temperature and stirred for 3 h. The reaction mixture was treated with EtOAc (20 mL) and water (30 mL). The extracted organic layer was dried with $MgSO_4$ and concentrated under reduced pressure to provide a crude product, which was purified by flash column chromatography (DCM:MeOH:$NH_4OH$= 100:3:1 into 100:9:1) to afford the desired product **6**.

*N-benzyl-2-(3-phenyl-2-[(5-(pyrrolidin-1-yl)pentyl)]thio)-3,4-dihydroquinazolin-4-yl)acetamide* (**6a,** 0.05 g, 50%): $^1$H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.1 (10H, m), 7.00 – 6.93 (4H, m), 5.74 (1H, s), 5.09 (1H, t, $J$ = 8 Hz), 4.27 (1H, dd, $J$ = 14.6 Hz and 5.6 Hz), 4.18 (1H, dd, $J$ = 14.8 and 5.2 Hz), 3.12 – 3.05 (1H, m), 2.96 – 2.89 (1H, m), 2.66 (1H, dd, $J$ = 12.8 Hz and 5.6 Hz), 2.50 (1H, s), 2.46 (4H, s), 2.38 (2H, t, $J$ = 7.6 Hz), 1.71 (4H, s), 1.58 – 1.57 (2H, m), 1.47 (2H, t. $J$ = 7.2 Hz), 1.35 (2H, t, $J$ = 7.2 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 169.1, 157.3, 142.6, 141.7, 137.7, 129.2, 128.6, 128.5, 127.8, 127.4, 127.4, 127.2, 125.6, 125.0, 124.5, 123.2, 60.3, 56.3, 54.1, 43.6, 41.9, 31.3, 29.1, 28.2, 26.8, 23.3; Purity: 96%; HRMS (FAB+): m/z estimated for $C_{32}H_{39}N_4OS$ $[M+H]^+$ 527.2839, found: 527.2845.

*N-(4-fluorobenzyl)-2-(3-phenyl-2-[(5-(pyrrolidin-1-yl)pentyl]thio)-3,4-dihydroquinazolin-4-yl)acetamide* (**6b**, 0.02 g, 22 %): $^1$H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.16 (5H, m), 7.07 (1H, d, *J* = 7.6 Hz), 7.00 – 6.93 (4H, m), 6.82 (2H, t, *J* = 8.4 Hz), 6.62 (1H, s), 5.11 (1H, dd, *J* = 8.4 Hz and 6.0 Hz), 4.25 (1H, dd, *J* = 14.8 Hz and 6.4 Hz), 4.12 (1H, dd, *J* = 14.8 Hz and 5.6 Hz), 3.03 – 2.92 (6H, m), 2.85 – 2.80 (2H, m), 2.71 (1H, dd, *J* = 13.6 Hz and 5.6 Hz), 2.55 (1H, dd, *J* = 13.6 Hz and 8.4 Hz), 1.91 (4H, s), 1.75 (2H, t, *J* = 3.6 Hz), 1.62 (2H, t, *J* = 7.2 Hz), 1.48 – 1.30 (2H, m); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 169.1, 157.1, 142.4, 141.7, 134.0, 129.5, 129.4, 129.2, 128.5, 127.3, 125.7, 125.2, 124.5, 123.1, 115.3, 115.1, 60.2, 55.4, 53.6, 42.6, 41.7, 30.6, 28.7, 25.6, 25.5, 23.3; Purity: 93%; HRMS (FAB+): m/z estimated for $C_{32}H_{38}FN_4OS$ $[M+H]^+$ 545.2745, found: 545.2750.

*N-benzyl-2-(2-[(5-(dimethylamino)pentyl]thio)-3-phenyl-3,4-dihydroquinazolin-4-yl)acetamide* (**6c**, 0.05 g, 49%): $^1$H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.11 (10H, m), 7.00 (2H, d, *J* = 6.0 Hz), 6.97 – 6.94 (2H, m), 5.68 (1H, s), 5.10 (1H, dd, *J* = 8.2 Hz and 6.4 Hz), 4.28 (1H, dd, *J* = 15.2 Hz and 5.6 Hz), 4.19 (1H, dd, *J* = 14.8 Hz and 5.2 Hz), 3.10 – 3.05 (1H, m), 2.96 – 2.93 (1H, m), 2.67 (1H, dd, *J* = 13.2 Hz and 6.0 Hz), 2.48 (1H, dd, *J* = 13.2 Hz and 8.4 Hz), 2.22 (2H, t, *J* = 7.6 Hz), 2.16 (6H, s), 1.61 – 1.57 (2H, m), 1.45 – 1.40 (2H, m), 1.35 – 1.30 (2H, m); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 169.0, 157.2, 142.6, 141.7, 137.6, 129.2, 128.6, 127.8, 127.4, 127.3, 127.2, 125.5, 125.0, 124.5, 123.2, 60.3, 59.3, 45.0, 43.7, 42.0, 31.1, 29.7, 29.1, 26.6, 26.4; Purity: 95%; HRMS (FAB+): m/z estimated for $C_{30}H_{37}N_4OS$ $[M+H]^+$ 501.2683, found: 501.2686.

*N-(4-fluorobenzyl)-2-(2-[(5-(dimethylamino)pentyl]thio)-3-phenyl-3,4-dihydroquinazolin-4-yl) acetamide* (**6d**, 0.03 g, 32 %): $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.36 (1H, t, *J* = 6.0 Hz), 7.43 – 7.33 (5H, m), 7.26 (1H, t, *J* = 7.2), 7.14 – 7.06 (5H, m), 7.00 (1H, t, *J* = 7.2 Hz), 6.94 (1H, d, *J* = 6.8 Hz), 5.07 (1H, dd, *J* = 8.4 Hz and 5.6 Hz), 4.22 (1H, *J* = 15.2 Hz and 6.0 Hz), 4.11 (1H, dd, *J* = 15.2 Hz and 5.6 Hz), 3.13 – 3.10 (1H, m), 2.96 – 2.93 (1H, m), 2.68 (1H, dd, *J* = 13.6 Hz and 5.2 Hz), 2.56 – 2.52 (1H, m), 2.21 (2H, t, *J* = 7.2 Hz), 2.13 (6H, s), 1.61 – 1355 (2H, m), 1.43 – 1.37 (2H, m), 1.35 –

1.24 (2H, m); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 169.0, 157.2, 142.6, 141.7, 129.5, 129.4, 129.2, 128.6, 127.3, 127.2, 125.5, 124.9, 124.5, 123.3, 115.5, 115.2, 60.3, 59.4, 45.2, 43.0, 42.0, 31.2, 29.1, 26.9, 26.5; Purity: 96%; HRMS (FAB+): m/z estimated for $C_{30}H_{36}FN_4OS$ $[M+H]^+$ 519.2588, found:519.2594.

*N-benzyl-2-(3-([1,1'-biphenyl]-4-yl)-2-([5-(pyrrolidin-1-yl)pentyl]thio)-3,4-dihydroquinazolin-4-yl) acetamide* (**6e**, 0.03 g, 25%): $^1$H NMR (400 MHz, $CDCl_3$) δ 7.50 (4H, t, *J* = 7.6 Hz), 7.36 (2H, t, *J* = 7.6 Hz), 7.30 – 7.02 (8H, m), 7.01 – 6.98 (2H, m), 6.95 (2H, s), 5.77 (1H, s), 5.13 (1H, t, *J* = 8.0 Hz), 4.29 (1H, dd, *J* = 14.8 Hz and 6.0 Hz), 4.20 (1H, dd, *J* = 14.8 Hz and 5.2 Hz) 3.13 – 2.97 (1H, m), 2.95 – 2.4 (1H, m), 2.68 (2H, dd, *J* = 13.4 Hz and 6.0 Hz), 2.51 – 2.48 (1H, m), 2.44 (4H, s), 2.37 (2H, t, *J* = 7.6 Hz), 1.69 (4H, s), 1.65 – 1.59 (2H, m), 1.49 – 1.45 (2H, m), 1.38 – 1.32 (2H, m); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 169.1, 157.2, 141.8, 141.7, 140.1, 139.9, 137.7, 128.9, 128.6, 128.5, 127.8, 127.5, 127.4, 127.1, 125.5, 125.1, 124.6, 123.3, 60.4, 56.3, 54.1, 43.7, 41.9, 31.3, 29.1, 28.3, 26.9, 23.4; Purity: 93%; HRMS (FAB+): m/z estimated for $C_{38}H_{43}N_4OS$ $[M+H]^+$ 603.3152, found: 603.3158.

*N-(4-fluorobenzyl)-2-(3-([1,1'-biphenyl]-4-yl)-2-([5-(pyrrolidin-1-yl)pentyl]thio)-3,4-dihydroquinazolin-4-yl)acetamide* (**6f**, 0.04 g, 30%): $^1$H NMR (400 MHz, $CDCl_3$) δ 7.50 – 7.48 (4H, m), 7.37 – 7.11 (7H, m), 7.98 – 6.85 (6H, m), 5.96 (1H, s), 5.13 (1H, s), 4.24 – 4315 (2H, m), 3.07 (1H, m), 3.00 (1H, m), 2.69 – 2.67 (1H, m), 2.53 – 2.45 (7H, m), 1.73 (4H, s), 1.62 – 1.53 (5H, m), 1.36 (2H, s); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 169.1, 157.1, 141.8, 141.7, 140.1, 139.9, 133.6, 129.5, 129.4, 128.8, 128.6, 127.8, 127.5, 127.3, 127.1, 125.5, 125.1, 124.6, 123.3, 115.4, 115.2, 60.4, 56.2, 54.1, 42.9, 41.9, 31.2, 29.7, 29.1, 27.9, 26.6, 23.3; Purity: 98%; HRMS (FAB+): m/z estimated for $C_{38}H_{42}FN_4OS$ $[M+H]^+$ 621.3058, found:621.3063.

*N-(4-fluorobenzyl)-2-(3-([1,1'-biphenyl]-4-yl)-2-([5-(dimethylamino)pentyl]thio)-3,4-dihydroquinazolin-4-yl)acetamide* (**6g**, 0.08 g, 75%): $^1$H NMR (400 MHz, $CDCl_3$) δ 7.60 (4H, t, *J* =

7.6 Hz), 7.47 (2H, t, *J* = 7.6 Hz), 7.39 (3H, d, *J* = 8.0 Hz), 7.31 – 7.21 (2H, m), 7.10 – 7.04 (4H, m), 6.95 (2H, t, *J* = 8.4 Hz), 5.96 (1H, s), 5.23 (1H, t, *J* = 7.6 Hz), 4.36 (1H, dd, *J* = 14.8 Hz and 5.6 Hz), 4.26 (1H, dd, *J* = 14.8 Hz and 5.2 Hz), 3.21 – 3.16 (1H, m), 3.13 – 3.08 (1H, m), 2.78 (1H, dd, *J* = 13.6 Hz and 5.6 Hz), 2.60 (1H, dd, *J* = 13.2 Hz and 8.4 Hz), 2.39 (2H, t, *J* = 6.0 Hz), 2.30 (6H, s), 1.74 – 1.70 (2H, m), 1.58 (2H, s), 1.48 – 1.43 (2H, m); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 169.2, 163.3, 160.8, 157.2, 141.8, 141.7, 140.1, 139.9, 133.6, 129.5, 129.4, 128.9, 128.6, 127.8, 127.6, 127.4, 127.1, 125.5, 125.1, 124.6, 123.3, 115.4, 115.2, 60.4, 59.5, 45.4, 42.9, 41.8, 31.3, 29.2, 27.1, 26.7; Purity: 98%; HRMS (FAB+): m/z estimated for $C_{36}H_{40}FN_4OS$ $[M+H]^+$ 595.2901, found: 595.2903.

*N-benzyl-2-(3-([1,1'-biphenyl]-4-yl)-2-([5-(dimethylamino)pentyl]thio)-3,4-dihydroquinazolin-4-yl) acetamide* (**6h**, 0.10 g, 89%): $^1$H NMR (400 MHz, $CDCl_3$) δ 7.60 (4H, t, *J* = 7.6 Hz), 7.47 (2H, t, *J* = 7.6 Hz), 7.41 – 7.38 (3H, m), 7.31 – 722 (5H, m), 7.11 (2H, d, *J* = 5.6 Hz), 7.06 – 7.05 (2H, m), 5.78 (1H, s), 5.24 (1H, dd, *J* = 8.0 Hz and 6.0 Hz), 4.40 (1H, dd, *J* = 14.4 Hz and 6.0 Hz), 4.31 (1H, dd, *J* = 14.8 Hz and 5.2 Hz), 3.21 – 3.19 (1H, m), 3.09 – 3.06 (1H, m), 2.79 (1H, dd, *J* = 13.2 Hz and 6.0 Hz), 2.59 (1H, dd, *J* = 13.2 Hz and 8.4 Hz), 2.34 (2H, t, *J* = 7.2 Hz), 2.27 (6H, s), 1.74 – 1.69 (2H, m), 1.55 – 1.52 (2H, m), 1.48 – 1.42 (2H, m); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 169.1, 157.1, 141.8, 141.7, 140.2, 139.9, 137.7, 128.9, 128.6, 128.5, 127.8, 127.5, 127.4, 127.3, 127.1, 125.5, 125.1, 124.6, 123.3, 60.4, 59.5, 45.3, 43.7, 42.0, 31.3, 29.2, 27.0, 26.7; Purity: 97%; HRMS (FAB+): m/z estimated for $C_{36}H_{41}N_4OS$ $[M+H]^+$ 577.2996, found: 577.3001.

*N-benzyl-2-(3-(4'-fluoro-[1,1'-biphenyl]-4-yl)-2-([5-(pyrrolidin-1-yl)pentyl]thio)-3,4-dihydroquinazolin-4-yl)acetamide* (**6i**, 0.06 g, 50%): $^1$H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.52 (4H, m), 7.39 (2H, d, *J* = 7.6 Hz), 7.31 – 7.21 (5H, m), 7.17 – 7.11 (4H, m), 7.06 (2H, d, *J* = 3.6 Hz), 5.98 (1H, s), 5.23 (1H, dd, *J* = 8.0 Hz and 7.6 Hz), 4.40 (1H, dd, *J* = 14.4 Hz and 6.0 Hz), 4.30 (1H, dd, *J* = 14.8 Hz and 5.2 Hz), 3.19 – 3.06 (2H, m), 2.82 – 2.74 (5H, m), 2.64 – 2.58 (3H, m), 1.87 (4H, s), 1.74 – 1.61 (4H, m), 1.48 – 1.44 (2H, m); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 169.2, 163.8, 161.3, 157.1,

141.9, 141.6, 138.8, 137.9, 136.2, 128.7, 128.6, 128.5, 127.8, 127.6, 127.5, 127.3, 125.6, 125.2, 124.6, 123.2, 115.8, 115.6, 60.4, 56.2, 54.1, 43.5, 41.9, 31.2, 29.7, 29.1, 27.9, 26.7, 23.3; Purity: 93%; HRMS (FAB+):m/z estimated for $C_{38}H_{42}FN_4OS$ $[M+H]^+$ 621.3058, found: 621.3063.

*N-(4-fluorobenzyl)-2-(3-(4'-fluoro-[1,1'-biphenyl]-4-yl)-2-([5-(pyrrolidin-1-yl)pentyl]thio)-3,4-dihydroquinazolin-4-yl)- acetamide* (**6j**, 0.03 g, 28%): $^1$H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.41 (4H, m), 7.26 (2H, d, *J* = 8.0 Hz), 7.19 – 7.17 (1H, m), 7.10 (1H, d, *J* = 8.0 Hz), 7.06 – 7.02 (2H, m), 6.99 – 6.90 (4H, m), 6.83 (2H, t, *J* = 7.2 Hz), 6.00 (1H, s), 5.10 (1H, t, *J* = 7.2 Hz), 4.13 (1H, dd, *J* = 14.4 Hz and 6.0 Hz), 4.15 (1H, dd, *J* = 14.8 Hz and 4.8 Hz), 3.14 – 3.07 (1H, m), 2.97 – 2.90 (1H, m), 2.65 (1H, dd, *J* = 13.6 Hz and 6.0 Hz), 2.47 (1H, dd, *J* = 13.6 Hz and 8.4 Hz), 2.38 (4H, s), 2.33 (2H, t, *J* = 7.6 Hz), 1.66 (4H, s), 1.61 – 1.56 (2H, m), 1.45 – 1.41 (2H, m), 1.36 – 1.31 (2H, m); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 169.1, 163.8, 163.3, 161.4, 160.9, 157.1, 141.9, 141.7, 138.9, 136.3, 136.2, 133.5, 133.5, 129.5, 129.4, 128.7, 128.6, 128.5, 127.6, 127.3, 125.5, 125.0, 124.6, 123.3, 115.8, 115.6, 115.4, 115.2, 60.4, 56.4, 54.2, 42.9, 41.9, 31.3, 29.7, 29.2, 28.4, 26.9, 23.4; Purity: 96%; HRMS (FAB+):m/z estimated for $C_{38}H_{41}F_2N_4OS$ $[M+H]^+$ 639.2964, found: 639.2969.

*N-benzyl-2-(2-([5-(dimethylamino)pentyl]thio)-3-(4'-fluoro-[1,1'-biphenyl]-4-yl)-3,4-dihydroquinazolin-4-yl)acetamide* (**6k**, 0.03 g, 25%) $^1$H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.52 (4H, m), 7.38 (2H, d, *J* = 8.4 Hz), 7.33 – 7.22 (5H, m), 7.17 – 7.11 (4H, m), 7.06 – 7.05 (2H, m), 5.81 (1H, s), 5.22 (1H, t, *J* = 8.0 Hz), 4.39 (1H, dd, *J* = 14.8 Hz and 5.6 Hz), 4.31 (1H, dd, *J* = 14.8 Hz and 5.2 Hz), 3.25 – 3.18 (1H, m), 3.11 – 3.04 (1H, m), 2.78 (1H, dd, *J* = 13.2 Hz and 6.0 Hz), 2.58 (1H, dd, *J* = 13.2 Hz and 8.4 Hz), 2.30 (2H, t, *J* = 7.2 Hz), 2.24 (6H, s), 1.73 – 1.69 (2H, m), 1.55 – 1.50 (2H, m), 1. 47 – 1.41 (2H, m); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 169.0, 157.0, 141.8, 141.6, 138.9, 137.6, 136.3, 128.7, 128.6, 128.5, 127.8, 127.7, 127.5, 127.4, 125.5, 125.1, 124.6, 123.3, 115.8, 115.6, 60.4, 59.5, 45.2, 43.8, 42.0, 31.3, 29.7, 29.2, 27.0, 26.6; Purity: 98%; HRMS (FAB+):m/z estimated for $C_{36}H_{40}FN_4OS$ $[M+H]^+$ 595.2901, found: 595.2907.

*N-(4-fluorobenzyl)-2-(2-([5-(dimethylamino)pentyl]thio)-3-(4'-fluoro-[1,1'-biphenyl]-4-yl)-3,4-dihydroquinazolin-4-yl)acetamide* (**6l**, 0.04 g, 38%) $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.42 (4H, m), 7.28 (2H, d, *J* = 8.4 Hz), 7.2 – 7.12 (2H, m), 7.05 (2H, t, *J* = 8.8 Hz), 7.00 – 6.92 (4H, m), 6.85 (2H, t, *J* = 8.8 Hz), 5.82 (1H, s), 5.11 (1H, t, *J* = 7.6 Hz), 4.26 (1H, dd, *J* = 15.2 Hz and 5.6 Hz), 4.17 (1H, dd, *J* = 14.8 Hz and 5.2 Hz), 3.15 – 3.08 (1H, m), 3.00 – 2.94 (1H, m), 2.66 (1H, dd, *J* = 13.2 Hz and 6.0 Hz ), 2.48 (1H, dd, *J* = 13.6 Hz and 8.0 Hz), 2.19 (2H, t, *J* = 7.2 Hz), 2.13 (6H, s), 1.63 – 1.58 (2H, m), 1.42 – 1.38 (2H, m), 1.36 – 1.31 (2H, m); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 169.1, 163.8, 163.3, 161.4, 160.9, 157.1, 141.8, 141.7, 138.9, 136.3, 136.2, 133.5, 133.4, 129.5, 129.5, 128.7, 128.7, 128.6, 127.7, 127.3, 125.4, 125.0, 124.6, 123.4, 115.8, 115.6, 115.5, 115.3, 60.4, 59.5, 45.3, 43.0, 41.9, 31.3, 29.7, 29.2, 27.1, 26.7; Purity: 98%; HRMS (FAB+):m/z estimated for $C_{36}H_{39}F_2N_4OS$ $[M+H]^+$ 613.2807, found: 613.2813.

*4.2. Biological evalutation*

*4.2.1. Cell Culture for MTT assay*

Human non-small cell lung cancer A549 cells and Human colon cancer HCT-116 cells were obtained from the Korean cell line bank (Seoul, Korea). Cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS), penicillin (100U/Ml) and streptomycin sulfate (100 µg/mL). Cells were cultured at 37 $^{o}$C in an atmosphere of 5% $CO_2$.

*4.2.2. MTT assay*

The cells (5x105/mL) were seeded in each well containing 100 µL of the RPMI medium supplemented with 10% FBS in a 96-well plate. Various concentrations of synthetic compound were added and incubated for 24 h. MTT (5 mg/mL stock solution) was added and the plates were incubated for an additional 4 h. The medium was discarded and the formazan blue, which was formed was measured at 540 nm by an automatic microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA).

*4.2.3. tsA-201 cell culture and cDNA transfection*

Human embryonic kidney tsA-201 cells were cultured and transfected using the calcium phosphate method as describe previously.[43] Cells were plated on glass coverslips and transfected with either 3μg of human $Ca_v3.1$, $Ca_v3.2$ or $Ca_v3.3$ cDNA. pEGFP (0.5μg) was included into the transfection mix as a transfection marker.

*4.2.4. Electrophysiology*

Electrophysiological recordings were performed according to the whole cell configuration of the patch-clamp technique 72 h after transfection at room temperature. The external recording solution contained (in mM): 20 $BaCl_2$, 1 $MgCl_2$, 40 TEACl, 65 CsCl, 10 HEPES, and 10 glucose, pH 7.4. Borosilicate glass pipettes were filled with internal solution containing (in mM): 140 CsCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 5 EGTA, 10 HEPES, 2 Na-Adenosine-Tri-Phosphate (ATP), and 0.3 Mg-GTP, pH 7.3. Drugs were prepared daily in external solution and were applied locally to cells. The effects of the compounds (10 μM) were assessed by normalizing the current amplitude in the presence of the compound to that observed before adding the drug. In dependent experiment was performed using the vehicle DMSO (0.1%) and no inhibition was observed. Currents were evoked by depolarization from a holding potential of −110 mV to a test potential of −20 mV. The interpulse interval was 20 s, and the test pulse was 100 ms long.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Acknowledgments**

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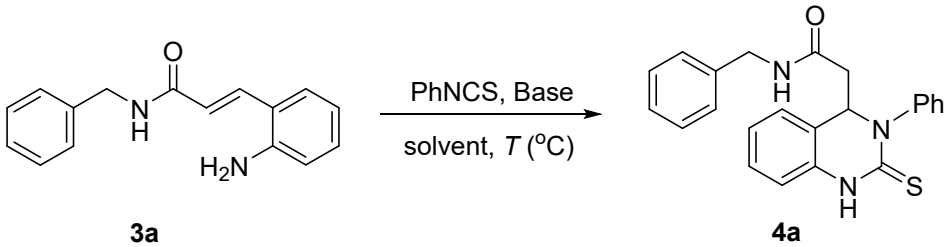
**Table 1.** Optimization of reaction conditions for the synthesis of compound **4** [a]

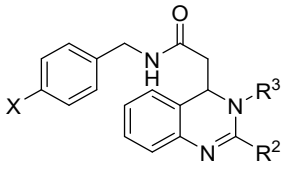


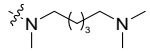
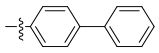
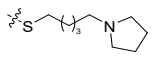
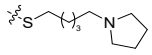
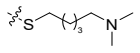
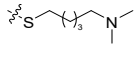
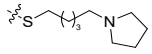
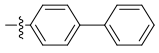
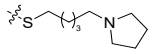
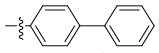
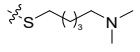
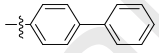
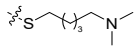
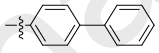
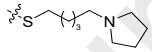
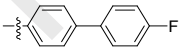
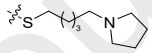
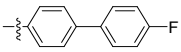
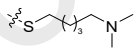
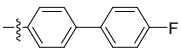
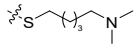
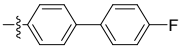
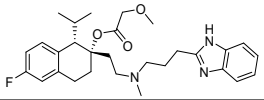

**3a** **4a**

| Entry | solvent | *T* (°C) | base [b] | time (h) | yield of **4a** (%) [c] |
|---|---|---|---|---|---|
| 1 | Water | 80 | - | 12 | 0 |
| 2 | DMSO | r.t. | - | 12 | 0 |
| 3 | DMSO | r.t. | DIPEA | 12 | 69 |
| 4 | DMSO | r.t. | $K_2CO_3$ | 12 | 73 |
| 5 | DMSO | 40 | $K_2CO_3$ | 15 | 81 |
| 6 | DMSO | 80 | $K_2CO_3$ | 2 | 91 |

[a] The reaction was conducted with **3a** (0.10 g, 0.40 mmol), phenylisothiocyante (0.052 mL ,0.44 mmol) and base (0.12 mmol) in solvent (5 mL); [b] DIPEA is diisopropylethylamine (Hunig's base); [c] Isolated yield.

**Table 2.** Biological activity of 2-thio-3,4-dihydroquinazoline derivatives as sulfur-analogues of **KYS05090**



| Entry | $R^2$ | $R^3$ | X | T-type Calcium channel inhibition: % inhibition (at 10 μM) (Estimated $IC_{50}$ in μM) [a] | | | Cytotoxicity: $IC_{50}$ (μM) [b] | |
|---|---|---|---|---|---|---|---|---|
| | | | | $Ca_v3.1$ ($\alpha_{1G}$) [c] | $Ca_v3.2$ ($\alpha_{1H}$) [d] | $Ca_v3.3$ ($\alpha_{1I}$) [e] | A549 [f] | HCT-116 [g] |
| **KYS05090** |  |  | H | 98.0±1.6 (0.26) | 89.3±2.1 (3.6) | ND [h] | 6.0 | 5.6 |
| **6a** |  |  | H | 60.4±19.8 (77.01) | 61.6±10.5 (7.71) | 78.3±6.0 (6.19) | 98.1 | 31.3 |
| **6b** |  |  | F | 60.1±13.1 (13.50) | 40.0±15.3 (57.43) | 22.1±13.5 (663.90) | 89.9 | 27.1 |
| **6c** |  |  | H | 46.5±13.2 (18.51) | 51.8±12.2 (13.18) | 76.0±3.2 (3.20) | 138.2 | 38.8 |
| **6d** |  |  | F | 26.7±15.4 (554.90) | 7.0±7.0 (832.30) | inactive | 36.8 | 38.8 |
| **6e** |  |  | H | 93.9±1.7 (0.66) | 73.1±19.3 (6.90) | 92.7±2.3 (0.81) | 14.9 | 5.5 |
| **6f** |  |  | F | 51.5±1.3 (14.97) | 69.0±23.7 (276.10) | 55.9±21.4 (13.63) | 19.8 | 6.2 |
| **6g** |  |  | F | 31.0±7.4 (27.38) | 83.1±15.4 (3.14) | 69.3±7.02 (6.10) | 5.0 | 6.4 |
| **6h** |  |  | H | 76.2±17.3 (7.77) | 83.9±8.6 (2.40) | 85.6±4.5 (1.76) | 18.1 | 9.6 |
| **6i** |  |  | H | 83.9±7.0 (2.18) | 97.5±1.1 (0.26) | 68.2±6.6 (5.10) | 17.8 | 12.5 |
| **6j** |  |  | F | 44.5±2.1 (12.55) | 76.6±9.8 (4.88) | 35.1±5.3 (19.78) | 12.6 | 7.7 |
| **6k** |  |  | H | 97.6±0.8 (0.25) | 75.6±12.7 (4.18) | 66.7±27.7 (9.06) | 17.6 | 9.2 |
| **6l** |  |  | F | 96.2±1.0 (0.40) | 84.8±11.5 (2.76) | 92.0±4.4 (0.96) | 11.3 | 9.3 |
| **Mibefradil** | |  | | 95.9 ± 1.7 (1.34) [i] | ND | ND | 24.9 | ND |

[a] Estimated $IC_{50}$ values were obtained by measuring the percentage of inhibition of $Ca_v3$ currents by 10 μM compound, using patch clamp electrophysiology (n≥3) , and then calculating the $IC_{50}$ by solving the Hill equation $Ir=1/(1+[C]/IC_{50})$ where Ir is the fraction of current remaining after application of the compounds, and [C] is the concentration of the compound (10 μM) ; [b] MTT assay; [c, d, e] T-type $Ca^{2+}$ channels ($\alpha_{1G}$ , $\alpha_{1H}$ and $\alpha_{1I}$ expressed in tsA-201 cells; [f] Human non-small cell lung cancer cells; [g] Human colon cancer cells; [h] Not determined; [i] Reported data by our group

***Graphical Abstract***

# Synthesis and Cytotoxic Effects of 2-Thio-3,4-dihydroquinazoline Derivatives as Novel T-Type Calcium Channel Blockers

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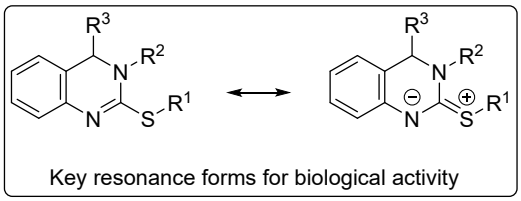


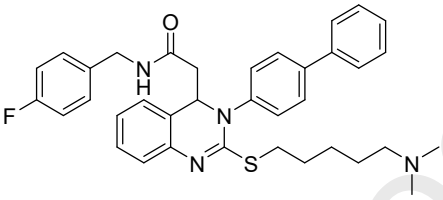


**6g**

T-type calcium channel blocking effect:
83% inhibition against Cav3.2 (@ 10 μM)

Cytotoxicity:
5.0 and 6.4 μM against A549 and HCT-116 cancer cell lines

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: