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Synthesis and SAR studies of a novel series of T-type calcium channel blockers

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Abstract—For the novel, potent, and selective T-type Ca^{2+} channel blockers, a series of sulfonamido-containing 3,4-dihydroquinazoline derivatives were prepared and evaluated for their blocking actions on T- and N-type Ca^{2+} channels. Among them, **9c** (**KYS05064**, $IC_{50} = 0.96 \pm 0.22$ μM) was found to be as potent as Mibefradil and also showed the highest selectivity for T-type Ca^{2+} channel with no effect on N-type Ca^{2+} channel.

1. Introduction

Voltage-gated calcium channels (VGCC) mediate the entry of calcium ions into excitable cells and are also involved in a variety of calcium-dependent processes, including muscle contraction, hormone or neurotransmitter release, gene expression, cell motility, cell division, and cell death. Voltage-gated calcium channels fall into two major functional classes: high voltage-activated (HVA) and low voltage-activated (LVA) channels.[1–4] Of them, T-type calcium channels are transient or low voltage-activated channels and found in cardiac myocyte membranes, the sinoatrial node, Purkinje cells of the heart, and the central nervous system.

T-type channels are strongly associated with the generation of rhythmical firing patterns in the mammalian CNS.[5–7] Furthermore, many reports have suggested that T-type channels are implicated in pathogenesis of epilepsy and neuropathic pain.[8–11] In spite of many of these researches, however, investigation of the role of T-type channels in physiological processes was limited by two factors: a lack of potent and selective T-type channel blockers and a lack of information about T-type channels at the molecular level. Until recently, three genes encoding T-type Ca^{2+} channel pore-forming subunits were identified and designated $Ca_v3.1$ (α_{1G}), $Ca_v3.2$ (α_{1H}), and $Ca_v3.3$ (α_{1I}).[12–15] However, only limited progress has been made to date in the quest to identify both potent and selective compounds, except Kurtoxin ($IC_{50} = 15$ nM) and Mibefradil ($IC_{50} = 1$ μM) for T-type channel blockade.[16–18]

Our discovery and initial optimization of the screening hit compound **KYS05001** (**7a**, $IC_{50} = 1.16 \pm 0.04$ μM) leading to potent compound, **KYS05041** (**9a**, $IC_{50} = 0.17 \pm 0.08$ μM), **KYS05042** (**9e**, $IC_{50} = 0.11 \pm 0.06$ μM), and **KYS05044** ($IC_{50} = 0.56 \pm 0.10$ μM) have recently been disclosed as shown in Figure 1.[19,20] While both **KYS05041** and **KYS05042** have potent blocking effects, they showed less selectivity for T-type channel over N-type channel. Although **KYS05044** has both acceptable potency and selectivity for T-type channel, in addition, it had a synthetic limitation due to the difficulty in separating the regioisomers in the course of synthesis as reported previously.[20] We thus set out to identify new lead compounds with higher potency and selectivity for T-type channel than **KYS05041/2** via the

Keywords: T-type calcium channel blockers; Selectivity; Potency; 3,4-Dihydroquinazoline; Sulfonamido group.
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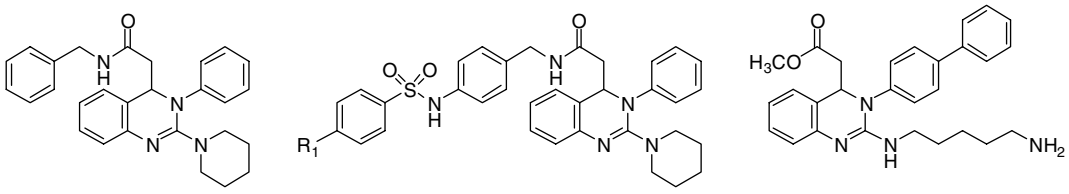


Figure 1. 3,4-Dihydroquinazoline derivatives as T-type Ca^{2+} channel blockers.

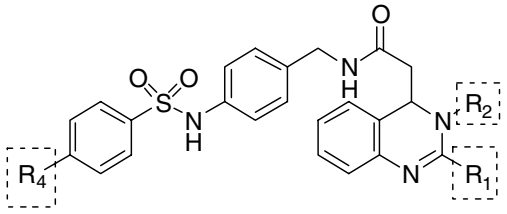


Figure 2. SAR study via the modifications of R_1, R_2, and R_4 substituents.

modification of R_1, R_2, and R_4 substituents of sulfonamido-containing 3,4-dihydroquinazoline as shown in Figure 2.

2. Results and discussion

2.1. Chemistry

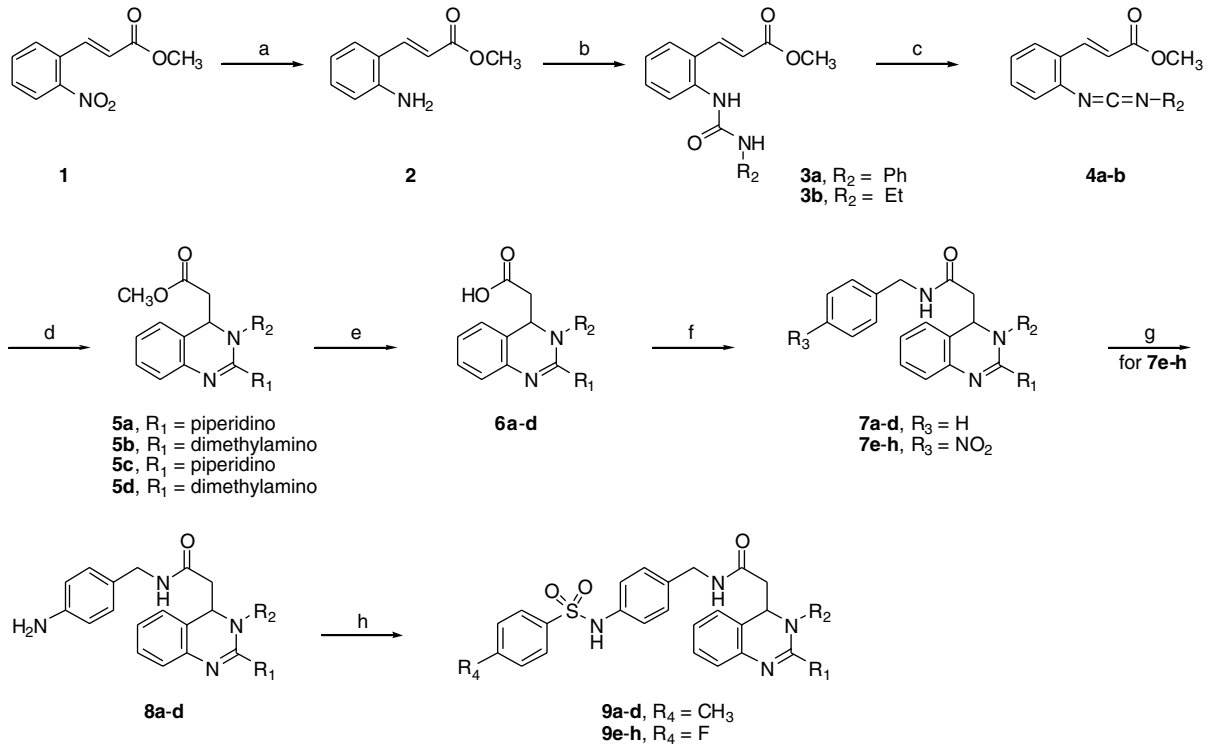
As shown in Scheme 1, a series of **KYS05041/2** analogues for SAR study were easily prepared by the same procedure as described previously by our group.[19,20] With respect to R_2 position, ethyl and phenyl groups were introduced, respectively, and the R_1 position was introduced by secondary amines such as dimethylamine and piperidine. Finally, the group of R_4 position was retained as methyl and fluoro substituents, respectively, as reported previously.[19]

Carbodiimides (**4a**,**b**), an intermediate of this reaction, were prepared in good yields by the reaction of ureas (**3a**,**b**) with $Ph_3P{\cdot}Br_2$ and Et_3N. The ureas (**3a**,**b**) were prepared in moderate to good yields (66–96%) from the reaction of the corresponding isocyanate with 2-aminocinnamate, which was quantitatively prepared through the reduction of methyl 2-nitrocinnamate (**1**) with $SnCl_2$ in ethyl acetate. The regioselective addition of secondary amines such as piperidine and dimethylamine into the carbodiimides (**4a**, **b**) followed by subsequent intramolecular conjugate addition to an α,β-unsaturated ester afforded the corresponding 3,4-dihydroquinazoline derivatives (**5a**–**d**) in 68–88% yields. The methyl ester group of compounds (**5a**–**d**) was hydrolyzed with LiOH in THF-H_2O at 70 °C to provide the free carboxylic compounds (**6a**–**d**) in quantitative yields, which were directly coupled with benzylamine by using EDC and HOBT to give the amides **7a**–**h** in 25–88% yields. Hydrogenation of the nitro group of some compounds (**7e**–**h**) using 10% Pd(C) in MeOH afforded *N*-(4-aminobenzylamido)-3,4-dihydroquinazolines (**8a**–**d**) in 71–97% yields, which were finally coupled with the respective phenylsulfonyl chloride in the presence of pyridine to provide the sulfonamido-3,4-dihydroquinazoline derivatives (**9a**–**h**) in 12–94% yields.

2.2. Channel blocking effects

The in vitro calcium channel blocking activities of all synthetic derivatives were determined in T-type channels stably expressed in *Xenopus* oocytes (α_{1H}) and HEK293 cells (α_{1G}). As preliminary assays, all synthetic compounds (100 μM) were evaluated for their inhibitory effects on α_{1H} T-type Ca^{2+} channels expressed in *Xenopus* oocytes by a two-electrode voltage clamp method.[21] The compounds were again re-evaluated for the blocking effects on α_{1G} T-type Ca^{2+} channels expressed in HEK293 cells at 10 μM concentration by whole-cell patch-clamp methods.[22] Next, these compounds were screened against α_{1B} N-type Ca^{2+} channels (high voltage-activated Ca^{2+} channels) stably expressed in HEK293 cell for the evaluation of ion channel selectivity. In vitro blocking effects of all compounds are summarized in Table 1, and the previously reported compounds (**7a**, **7e**, **8a**, **9a**, and **9e**) and Mibefradil were also tabulated with data for comparison.

Against the α_{1H} T-type Ca^{2+} channel (*Xenopus* oocyte), new synthetic compounds showed broad spectrum of inhibitory activities (% inhibition in >10 to 97.2 range). Of them, 2-piperidino-3-ethyl derivatives (**9c** and **9g**) containing sulfonamido groups showed improved inhibitory activities (92.0 and 97.2%, respectively) compared to both Mibefradil (86%) and the parent compounds: **9a** (84.7%) and **9e** (80.5%). Meanwhile, some compounds such as **7c**, **7d**, and **8d** showed lower blocking effects (>10%), which result cannot be properly explained at this stage. Next, all of compounds including the parent compounds (**9a** and **9e**) were re-evaluated in HEK293 cells (α_{1G}) at lower concentration (10 μM). Compared with the primary inhibitory activity, their profiles had a similar inhibitory trend to those against *Xenopus* oocyte (α_{1H}) with values of 34.4–92.3% inhibition range with the exception of some compounds (**7b**, **8b**, and **8c**), which had greater blocking effects (77.5–83.8%) than expected on the primary activity data. Among new synthetic derivatives, **9c**, the second potent compound against the α_{1H} T-type Ca^{2+} channel (*Xenopus* oocyte), was nearly equipotent (91.3 ± 0.6% inhibition) comparable to Mibefradil (95.9 ± 1.7% inhibition), **7a** (90.1 ± 2.3% inhibition), and **7e** (92.3 ± 1.3% inhibition) against the α_{1G} T-type Ca^{2+}



Scheme 1. Reagents and conditions: (a) $SnCl_2{\cdot}2H_2O$, EtOAc, 70 °C; (b) R_2NCO, benzene, rt; (c) $Ph_3P{\cdot}Br_2$, Et_3N, CH_2Cl_2, 0 °C; (d) R_1H, 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.

channel (HEK293 cell). With respect to the IC_{50} values, however, all of the compounds not only showed a broad range of potent efficacy (IC_{50} value: 0.35 ± 0.07 to 24.16 ± 3.48 μM range) but also were less potent than the parent compounds (**9a** and **9e**, $IC_{50} = 0.17 \pm 0.08$ and 0.11 ± 0.06 μM, respectively). A few of the compounds (**7b**, **7f**, **8b**, **8c**, **9b**, and **9f**) were more than 2-fold potent than Mibefradil as shown in Table 1. In particular, compound **9c** ($IC_{50} = 0.96 \pm 0.22$ μM) was found to be as potent as Mibefradil ($IC_{50} = 1.34 \pm 0.49$ μM).

With respect to channel selectivity (T/N-type channel), the replacement of R_1 and/or R_2 group with dimethylamino and/or ethyl groups, respectively, in general resulted in an improvement in selectivity as for most compounds, except for some compounds (**8d**, **9b**, and **9f**) as shown in Table 1. However, their selectivities were not acceptable (T/N: <10-fold) except for compounds **8b**, **8c**, **9c**, and **9h**. In addition, the parent and most potent compounds (**9a** and **9e**) also exhibited lower selectivity for T-type channels by only 6.4- and 3.3-fold, respectively. As for the selective compounds, compound **8b** showed more improved selectivity (32.2-fold) for T-type channel. In particular, compounds **9c** and **9h** exerted no effect on N-type channels and thus exhibited the specific selectivity. This result indicates that compounds **9c** and **9h** preferentially block T-type Ca^{2+} channel. Meanwhile, compound **9c** was equipotent with Mibefradil, while compound **9h** (**KYS05071**) was about 3-fold less potent than Mibefradil as for the blocking activity against T-type Ca^{2+} channel. In order to analyze cytotoxicities of all synthesized compounds in HEK293 cells, finally, MTT assay was conducted as follows:[23] the cultured HEK293 cells were treated with each compound at a concentration of 10 and 100 μM, respectively. At this time, the cells treated with a solvent, that is, 0.1% DMSO, were used as a negative control and the cells treated with H_2O_2 inducing cytotoxicity were used as a positive control. As a result, most compounds showing 50% or more inhibitory effect on HEK293 cells did not show any cytotoxicity at both concentrations but only compound **9f** showed cytotoxicity at a high concentration (100 μM). Therefore, these findings mean that compound **9c** would really be regarded as a new promising lead compound for selective T-type Ca^{2+} channel blocker with respect to the IC_{50} value and channel selectivity.

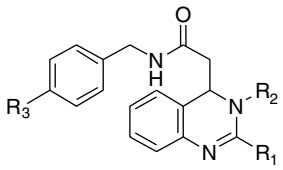
In conclusion, the replacement of phenyl group of R_2 position in our original series (**9a** and **9e**) of T-type Ca^{2+} channel blocker with ethyl group resulted in a new lead compound of potent and selective T-type Ca^{2+} channel blocker, **9c** (**KYS05064**), in comparison with Mibefradil and our original compounds (**9a** and **9e**). Encouraged by these findings, the pharmacokinetic profiles for **9c** (**KYS05064**) are in progress and will be announced in the future.

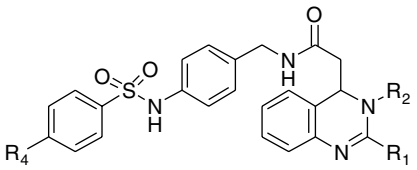
3. Experimental

3.1. Chemistry

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and have not been corrected. The IR spectra were performed on a Perkin-Elmer 16F-PC FT-IR in KBr pellets. ^1H NMR and

Table 1. In vitro calcium channel blocking effects of 3,4-dihydroquinazolines





Compounds (library code)	R_1	R_2	R_3	R_4	*Xenopus* oocyte (T-type: α_{1H}) % Inhibition (100 μM)	HEK293 cells (T-type: α_{1G}) % Inhibition[a] (10 μM)	HEK293 cells (T-type: α_{1G}) IC_{50}[b] (μM)	HEK293 cell (N-type: α_{1B}) % Inhibition[a] (10 μM)	Selectivity (T/N-type)
7a (KYS05001)	–N⬡	—⬡	H		77.0	90.1 ± 2.3	1.16 ± 0.04	28.1 ± 1.7	3.2
7b (KYS05073)	$-N(CH_3)_2$	—⬡	H		27.7	81.7 ± 0.5	0.49 ± 0.13	8.3 ± 0.9	9.8
7c (KYS05074)	–N⬡	$-CH_2CH_3$	H		>10	59.3 ± 1.9	4.58 ± 2.14	9.3 ± 0.5	6.3
7d (KYS05075)	$-N(CH_3)_2$	$-CH_2CH_3$	H		>10	34.4 ± 1.4	24.16 ± 3.48	27.6 ± 0.5	1.2
7e (KYS05034)	–N⬡	—⬡	NO_2		91.9	92.3 ± 1.3	2.34 ± 0.36	75.3 ± 2.4	1.2
7f (KYS05055)	$-N(CH_3)_2$	—⬡	NO_2		61.1	87.8 ± 2.2	0.35 ± 0.07	9.5 ± 2.6	9.2
7g (KYS05062)	–N⬡	$-CH_2CH_3$	NO_2		69.6	79.1 ± 0.8	0.87 ± 0.02	18.4 ± 3.1	4.3
7h (KYS05068)	$-N(CH_3)_2$	$-CH_2CH_3$	NO_2		30.9	61.9 ± 3.6	2.17 ± 0.62	11.9 ± 2.6	5.2
8a (KYS50040)	–N⬡	—⬡	NH_2		66.8	43.5 ± 4.5	13.02 ± 1.87	11.9 ± 1.3	3.7
8b (KYS05056)	$-N(CH_3)_2$	—⬡	NH_2		**46.7**	**83.8 ± 1.7**	**0.38 ± 0.15**	**2.6 ± 1.4**	**32.2**
8c (KYS05063)	–N⬡	$-CH_2CH_3$	NH_2		**15.1**	**77.5 ± 2.2**	**0.40 ± 0.08**	**6.2 ± 0.4**	**12.5**
8d (KYS05069)	$-N(CH_3)_2$	$-CH_2CH_3$	NH_2		>10	38.6 ± 4.9	12.70 ± 2.50	10.2 ± 1.0	3.8
9a (KYS05041)	–N⬡	—⬡		CH_3	84.7	89.9 ± 1.3	0.17 ± 0.08	14.1 ± 2.1	6.4
9b (KYS05057)	$-N(CH_3)_2$	—⬡		CH_3	30.6	71.2 ± 4.5	0.63 ± 0.04	11.3 ± 1.3	6.3
9c (KYS05064)	–N⬡	$-CH_2CH_3$		CH_3	**92.0**	**91.3 ± 0.6**	**0.96 ± 0.22**	**No blocking**[c]	**>100**
9d (KYS05070)	$-N(CH_3)_2$	$-CH_2CH_3$		CH_3	81.9	81.3 ± 0.8	1.12 ± 0.06	8.9 ± 1.8	9.1
9e (KYS05042)	–N⬡	—⬡		F	80.5	89.0 ± 2.3	0.11 ± 0.06	27.2 ± 1.5	3.3
9f (KYS05058)	$-N(CH_3)_2$	—⬡		F	84.4	73.8 ± 3.7	0.43 ± 0.15	29.1 ± 1.4	2.5
9g (KYS05065)	–N⬡	$-CH_2CH_3$		F	97.2	89.5 ± 2.5	1.04 ± 0.25	10.7 ± 1.1	8.4
9h (KYS05071)	$-N(CH_3)_2$	$-CH_2CH_3$		F	**78.1**	**62.7 ± 2.3**	**4.10 ± 1.08**	**No blocking**[c]	**>100**
Mibefradil					86.0	95.9 ± 1.7	1.34 ± 0.49	67.6 ± 1.2	1.4

[a] % Inhibition value (±SE) was obtained by repeated procedures ($n \geqslant 4$).
[b] IC_{50} value was determined from the dose–response curve.
[c] No blocking means that the inhibition was less than 1%.

^{13}C NMR spectra were recorded on a Varian Unity Plus 300 (300 MHz) spectrometer or a Bruker Avance 300 (300 MHz) spectrometer, using TMS as the internal standard; the chemical shifts (δ) are reported in parts per million and coupling constant (J) values are given in hertz (Hz). Signal multiplicities are represented by: s (singlet), d (doublet), t (triplet), q (quartet), br s (broad singlet), m (multiplet), and dd (double doublet). Low-resolution and high-resolution mass spectra (FABMS, positive ion mode) were obtained using a JEOL 700 mass spectrometer. The progress of all reactions was monitored using TLC on precoated silica gel plates (Merck Silica Gel 60 F_{254}). The chromatograms were viewed under UV light at 254 and 365 nm. For column

chromatography, Merck Silica Gel (230–400 mesh) was used. Chemicals were purchased from Sigma-Aldrich, TCI, Acros, and Fluka.

3.2. Methyl 2-nitrocinnamate (1)

To a solution of 2-nitrocinnamic acid (1.99 g, 10.3 mmol) in CH_3OH (100 mL) was dropped concd H_2SO_4 (0.17 mL, 3.06 mmol) at room temperature, and the reaction mixture was stirred at reflux overnight and then allowed to cool to room temperature. A saturated solution of $NaHCO_3$ was added, and the mixture was extracted with dichloromethane (3×), dried ($MgSO_4$), filtered, and solvent evaporated in vacuo to give the desired product as a colorless solid. Flash column chromatography (*n*-hexane/EtOAc = 5:1) gave 2.07 g (97%) of product (**1**) as a yellow solid: mp 75 °C; IR (KBr) 1718, 1636, 1520, 1432, 1346, 1198, 974, 756 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.10 (1H, d, J = 15.9 Hz, –C*H*=CH-CO_2Me), 8.03 (1H, d, J = 7.5 Hz, Ph), 7.70–7.63 (2H, m, Ph), 7.56 (1H, m, Ph), 6.37 (1H, d, J = 15.9 Hz, –CH=C*H*-CO_2Me), 3.83 (3H, s, –OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.5, 148.6, 140.4, 133.9, 130.9, 130.7, 129.4, 125.2, 123.1, 52.3.

3.3. Methyl 2-aminocinnamate (2)

To a solution of 2-nitrocinnamate (**1**) (202 mg, 0.975 mmol) in EtOAc (20 mL) was added $SnCl_2{\cdot}2H_2O$ (1.11 g, 4.87 mmol) at room temperature, and the solution was stirred at reflux for 1 h. The resulting mixture was basified with $NaHCO_3$ to pH 8–9 and filtered. The filtrate was extracted with EtOAc (3×), dried ($MgSO_4$), filtered, and solvent evaporated in vacuo to give the desired product as a colorless solid. Flash column chromatography (*n*-hexane/EtOAc = 5:1) gave 161 mg (93%) of product (**2**) as a yellow solid: mp 67 °C; IR (KBr) 3365, 2364, 1704, 1622, 1330, 1198, 756 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.86 (1H, d, J = 15.9 Hz, –C*H*=CH-CO_2Me), 7.40 (1H, d, J = 7.5 Hz, Ph), 7.19 (1H, t, J = 7.2 Hz, Ph), 6.78 (1H, t, J = 7.8 Hz, Ph), 6.72 (1H, d, J = 7.5 Hz, Ph), 6.38 (1H, d, J = 15.9 Hz, –CH=C*H*-CO_2Me), 4.02 (2H, br s, –NH_2), 3.82 (3H, s, –OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 145.9, 140.6, 131.6, 128.3, 120.1, 119.2, 117.9, 117.0, 51.9.

3.4. General preparation of compounds (3a–b)

To a solution of methyl 2-aminocinnamate (**2**) (6.35 g, 35.8 mmol) in benzene (150 mL) was added phenyl or ethyl isocyanate (43.0 mmol) at room temperature, and the solution was stirred for 12 hour. The solid residue was washed with ether to give phenyl or ethyl substituted urea (**3a**, **b**) as a white solid.

3.4.1. Compound 3a. 96%; mp 184 °C; IR (KBr) 3346, 3278, 1724, 1650, 1548, 1322, 1172, 758, 672 cm^{-1}; ^1H NMR (300 MHz, DMSO) δ 8.94 (1H, s, –N*H*-CO–), 8.49 (1H, s, –N*H*-CO–), 7.89 (1H, d, J = 15.9 Hz, –C*H*=CH-CO_2Me), 7.76 (2H, d, J = 7.8 Hz, Ph), 7.46 (2H, d, J = 8.4 Hz, Ph), 7.39 (1H, t, J = 8.1 Hz, Ph), 7.28 (2H, t, J = 7.8 Hz, Ph), 7.12 (1H, t, J = 7.5 Hz, Ph), 6.97 (1H, t, J = 7.8 Hz, Ph), 6.58 (1H, d, J = 15.3 Hz, –CH=C*H*-CO_2Me), 3.73 (3H, s, –OCH_3); ^{13}C NMR (75 MHz, DMSO) δ 167.4, 153.5, 140.5, 140.3, 138.5, 131.4, 129.5, 127.8, 126.8, 124.6, 124.4, 122.7, 119.5, 118.9, 52.2.

3.4.2. Compound 3b. 66%; ^1H NMR (300 MHz, DMSO) δ 8.25 (1H, s, –N*H*-CO–), 7.86 (1H, d, J = 15.9 Hz, –C*H*=CH-CO_2Me), 7.74 (2H, dd, J = 7.8 and 6.9 Hz, Ph), 7.35 (1H, t, J = 7.7 Hz, Ph), 7.06 (1H, t, J = 7.5 Hz, Ph), 6.55 (1H, d, J = 15.3 Hz, –CH=C*H*-CO_2Me), 3.75 (3H, s, –OCH_3), 3.18–3.09 (2H, m, –HN-CH_2-CH_3), 1.11–1.06 (3H, m, –HN-CH_2-CH_3); ^{13}C NMR (75 MHz, DMSO) δ 167.5, 155.9, 140.8, 139.5, 131.4, 127.7, 127.1, 123.8, 123.7, 118.9, 52.2, 34.8, 16.1.

3.5. General preparation of compounds (4a–b)

To a solution of urea (**3a** or **b**) (20.4 mmol) and triethylamine (61.2 mmol) in CH_2Cl_2 (100 mL) was added dibromotriphenylphosphorane (30.6 mmol) at 0 °C and stirred at the same temperature for 1 h. The mixture was extracted with dichloromethane (3×), dried ($MgSO_4$), filtered, and the solvent evaporated in vacuo. Flash column chromatography (*n*-hexane/EtOAc) gave product (**4a** or **b**).

3.5.1. Compound 4a. 75%; mp 54 °C; IR (KBr) 2142, 1716, 1484, 1172, 756, 59 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.18 (1H, d, J = 16.2 Hz, –C*H*=CH-CO_2Me), 7.56 (1H, d, J = 7.8 Hz, Ph), 7.36–7.29 (3H, m, Ph), 7.25 (1H, d, J = 7.8 Hz, Ph), 7.20–7.13 (4H, m, Ph), 6.52 (1H, d, J = 16.2 Hz, –CH=C*H*-CO_2Me), 3.80 (3H, s, –OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.5, 140.5, 138.4, 138.0, 134.3, 131.3, 129.8, 129.0, 127.8, 126.1, 126.0, 125.9, 124.6, 119.6, 52.0.

3.5.2. Compound 4b. 66%; ^1H NMR (300 MHz, $CDCl_3$) δ 8.11 (1H, d, J = 16.5 Hz, –C*H*=CH-CO_2Me), 7.55–7.52 (m, 1H, Ph), 7.34–7.29 (m, 3H, Ph), 6.48 (1H, d, J = 15.9 Hz, –CH=C*H*-CO_2Me), 3.81 (3H, s, –OCH_3), 3.49 (2H, q, J = 7.2 Hz, –N=C=N-CH_2-CH_3), 1.37 (3H, t, J = 7.2 Hz, –N=C=N-CH_2-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.5, 140.7, 140.5, 130.9, 128.3, 127.5, 124.7, 118.7, 51.6, 41.8, 17.0.

3.6. General preparation of compounds (5a–d)

To a solution of carbodiimide (**4a**, **b**) (2.17 mmol) in benzene (20 mL) was added piperidine or dimethylamine (2.60 mmol) at room temperature, and the solution was stirred for 2 h. The mixture was extracted with dichloromethane (3×), dried ($MgSO_4$), filtered, and solvent evaporated in vacuo. Flash column chromatography (CH_2Cl_2/MeOH) gave product (**5a**–**d**).

3.6.1. Compound 5a. 80%; mp 109 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.28–7.17 (4H, m, Ph), 7.09–7.01 (3H, m, Ph), 6.97–6.89 (2H, m, Ph), 5.10 (1H, dd, J = 10.8 and 4.5 Hz, –CH_2-C*H*-N–), 3.79 (3H, s, –OCH_3), 3.42 (4H, s, piperidinyl), 2.85 (1H, dd, J = 15.3

and 10.8 Hz, –CO-CH_2–), 2.52 (1H, dd, J = 15.5 and 4.7 Hz, –CO-CH_2–), 1.55–1.50 (2H, m, piperidinyl), 1.43–1.40 (4H, m, piperidinyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 153.2, 146.3, 144.4, 129.4, 128.6, 126.1, 124.9, 124.1, 123.1, 122.6, 122.4, 61.2, 52.0, 47.0, 39.8, 25.7, 25.0; HRMS (FAB, M+H) calcd for $C_{22}H_{26}N_3O_2$: 364.2025, found: 364.2019.

3.6.2. Compound 5b. 88%: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26–7.18 (4H, m, Ph), 7.04–6.99 (3H, m, Ph), 6.94–6.88 (2H, m, Ph), 5.10 (1H, dd, J = 10.7 and 4.6 Hz, –CH_2-CH-N–), 3.75 (3H, s, –OCH_3), 2.88 (6H, s, –N-Me_2), 2.83 (1H, dd, J = 15.1 and 10.7 Hz, –CO-CH_2–), 2.50 (1H, dd, J = 15.0 and 4.5 Hz, –CO-CH_2–); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 153.5, 145.8, 144.0, 129.3, 128.3, 125.3, 124.7, 123.8, 122.8, 122.0, 121.8, 61.2, 51.8, 39.7, 37.6.

3.6.3. Compound 5c. 68%: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (1H, m, Ph), 7.21–7.09 (2H, m, Ph), 7.02–6.91 (2H, m, Ph), 4.62 (1H, dd, J = 10.2 and 4.5 Hz, –CH_2-CH-N–), 3.67 (3H, s, –OCH_3), 3.36–3.27 (5H, m, piperidinyl and –N-CH_2-CH_3), 3.08 (1H, m, –N-CH_2-CH_3), 2.61 (1H, dd, J = 14.7 and 9.9 Hz, –CO-CH_2–), 2.34 (1H, dd, J = 15.1 and 4.6 Hz, –CO-CH_2–), 1.63 (6H, br, piperidinyl), 1.00 (3H, t, J = 7.2 Hz, –N-CH_2-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.7, 157.2, 144.4, 128.0, 126.3, 124.1, 122.7, 122.4, 55.3, 51.4, 47.2, 39.9, 25.8, 24.8, 14.0.

3.6.4. Compound 5d. 68%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21–7.11 (2H, m, Ph), 7.02–6.91 (2H, m, Ph), 4.61 (1H, dd, J = 10.5 and 4.8 Hz, –CH_2-CH-N–), 3.67 (3H, s, –OCH_3), 3.21 (1H, m, –N-CH_2-CH_3), 3.13 (1H, m, –N-CH_2-CH_3), 2.96 (6H, s, –NMe_2), 2.59 (1H, dd, J = 14.7 and 9.9 Hz, –CO-CH_2–), 2.34 (1H, dd, J = 15.1 and 4.7 Hz, –CO-CH_2–), 1.00 (3H, t, J = 7.2 Hz, –N-CH_2-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 157.4, 144.1, 128.0, 125.7, 124.2, 122.5, 122.2, 55.5, 51.3, 46.9, 39.9, 38.4, 13.8.

3.7. General preparation of compounds (7a–h)

To a solution of ester compound (**5a**–**d**) (0.8 mmol) in THF (5 mL) and H_2O (5 mL) was added LiOH/H_2O (4.00 mmol). The mixture was stirred at 70 °C for 2 h. The volatiles were removed in vacuo and acidified with 1 N HCl to pH 3–4. The resulting mixture was extracted with CH_2Cl_2 (3×), dried ($MgSO_4$), filtered, and solvent evaporated in vacuo to give the intermediate (**6a**–**d**) as a white solid. Benzylamine or 4-nitrobenzylamine (2.543 mmol), HOBt (*N*-hydroxy-benzotriazole) (2.543 mmol), and EDC (*N*-ethyl-*N*′-dimethylaminopropyl-carbodiimide hydrochloride) (2.237 mmol) were added to a solution of **6a**–**d** in CH_2Cl_2 (15 mL) and THF (15 mL) at 0 °C. The solution was allowed to warm to room temperature and stirred overnight. The solvents were removed in vacuo, and the resulting mixture was basified with 1 N NaOH to pH 10–11. The organics were extracted with CH_2Cl_2 (3×), dried ($MgSO_4$), filtered, and solvent evaporated in vacuo to give the desired product as a colorless liquid. Flash column chromatography (CH_2Cl_2/MeOH) gave product (**7a**–**h**).

3.7.1. Compound 7a. 74%; mp 168 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (1H, br s, –CO–NH-CH_2-Ph), 7.35–7.31 (2H, m, Ph), 7.29–7.19 (5H, m, Ph), 7.16–7.03 (5H, m, Ph), 6.96–6.92 (2H, m, Ph), 5.18 (1H, dd, J = 10.1 and 5.0 Hz, –CH_2-CH-N–), 4.53 (1H, dd, J = 14.4 and 6.2 Hz, –NH-CH_2-Ph), 4.42 (1H, dd, J = 14.4 and 6.2 Hz, –NH-CH_2-Ph), 3.17 (4H, br s, piperidinyl), 2.68 (1H, dd, J = 14.0 and 10.1 Hz, –CO-CH_2–), 2.23 (1H, dd, J = 14.1 and 5.0 Hz, –CO-CH_2–), 1.37–1.33 (2H, m, piperidinyl), 1.18 (4H, br s, piperidinyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 153.9, 146.1, 143.2, 138.6, 129.3, 128.8, 128.5, 128.4, 127.7, 127.0, 125.2, 124.9, 124.4, 123.2, 122.8, 122.3, 61.1, 47.4, 43.9, 41.9, 25.3, 24.7; HRMS (FAB, M+H) calcd for $C_{28}H_{31}N_4O$: 439.2498, found: 439.2534.

3.7.2. Compound 7b. 30%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36–7.24 (10H, m, Ph), 7.20–7.08 (4H, m, Ph), 7.00–6.95 (1H, m, Ph), 5.24 (1H, dd, J = 10.5 and 5.0 Hz, –CH_2-CH-N–), 4.54 (1H, d, J = 5.9 Hz, –CH_2-NH–), 4.49 (1H, d, J = 5.9 Hz, –CH_2-NH–), 2.78 (6H, s, –NMe_2), 2.73 (1H, dd, J = 14.3 and 10.5 Hz, –CO-CH_2), 2.30 (1H, dd, J = 14.3 and 5.0 Hz, –CO-CH_2–); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 154.1, 145.1, 140.9, 138.5, 129.8, 128.8, 128.7, 128.6, 127.7, 126.5, 125.1, 125.0, 123.5, 122.9, 121.8, 61.8, 44.0, 42.2, 38.8, 30.0.

3.7.3. Compound 7c. 65%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (1H, br s, –CO-NH-CH_2–), 7.36–7.25 (5H, m, Ph), 7.11–6.82 (4H, m, Ph), 4.81 (1H, dd, J = 9.1 and 5.6 Hz, –CH_2-CH-N–), 4.53 (1H, dd, J = 14.5 and 5.9 Hz, –CH_2-NH–), 4.41 (1H, dd, J = 14.5 and 6.0 Hz, –CH_2-NH–), 3.35–2.97 (6H, m, piperidinyl and –N-C$H_2$$CH_3$), 2.33 (1H, dd, J = 14.3 and 9.1 Hz, –CO-CH_2), 2.10 (1H, dd, J = 14.3 and 5.7 Hz, –CO-CH_2–), 1.43 (6H, br s, piperidinyl), 1.02 (3H, t, J = 7.2 Hz, –N-CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 157.3, 142.5, 138.5, 128.5, 128.2, 128.0, 127.3, 127.1, 124.4, 123.1, 121.8, 55.3, 47.3, 43.5, 41.9, 25.7, 24.6, 14.3.

3.7.4. Compound 7d. 28%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.87 (1H, br s, –CO-NH-CH_2–), 7.30–7.17 (6H, m, Ph), 6.99–6.77 (3H, m, Ph), 4.73 (1H, dd, J = 9.4 and 4.6 Hz, –CH_2-CH-N–), 4.48 (1H, dd, J = 14.5 and 5.5 Hz, –CH_2-NH–), 4.36 (1H, dd, J = 14.5 and 5.5 Hz, –CH_2-NH–), 3.16–3.00 (2H, m, –N-C$H_2$$CH_3$), 2.60 (6H, s, –N$Me_2$), 2.29 (1H, dd, J = 13.9 and 9.4 Hz, –CO-CH_2–), 1.98 (1H, dd, J = 13.9 and 4.6 Hz, –CO-CH_2–), 0.91 (3H, t, J = 6.6 Hz, –N-CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 157.8, 142.6, 138.9, 128.7, 128.6, 128.3, 127.6, 127.1, 124.9, 123.3, 121.8, 55.9, 47.5, 43.8, 42.1, 39.3, 14.5.

3.7.5. Compound 7e. 80%; IR (KBr) 3192, 2932, 2848, 1668, 1552, 1486, 1430, 1344, 1282, 756 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (1H, br s, –CO-NH-CH_2–), 8.15 (2H, d, J = 8.7 Hz, –CH_2-C_4H_4-NO_2), 7.49 (2H, d, J = 8.1 Hz, –CH_2-C_6H_4-NO_2), 7.27–7.20

(2H, m, Ph), 7.15–7.02 (4H, m, Ph), 6.95–6.87 (3H, m, Ph), 5.23 (1H, dd, J = 8.9 and 6.0 Hz, –CH_2-CH-N–), 4.67 (1H, dd, J = 13.7 and 6.2 Hz, –CH_2-NH–), 4.58 (1H, dd, 13.7 and 6.2 Hz, –CH_2-NH–), 3.10 (4H, br s, piperidinyl), 2.58 (1H, dd, J = 14.5 and 8.9 Hz, –CO-CH_2–), 2.32 (1H, dd, J = 14.5 and 6.1 Hz, –CO-CH_2–), 1.35 (2H, br s, piperidinyl), 1.13 (4H, br s, piperidinyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 154.3, 147.4, 146.5, 145.9, 143.1, 129.5, 129.0, 128.5, 127.0, 125.4, 124.7, 124.0, 123.1, 123.0, 122.0, 60.8, 47.5, 43.2, 41.6, 25.2, 24.6; HRMS (FAB, M+H) calcd for $C_{28}H_{30}N_5O_3$: 484.2349, found: 484.2341.

3.7.6. Compound 7f. 88%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14–8.10 (2H, m, Ph), 7.39 (2H, d, J = 8.7 Hz, Ph), 7.30–7.13 (4H, m, Ph), 7.08–7.03 (3H, m, Ph), 6.98–6.89 (2H, m, Ph), 5.20 (1H, dd, J = 9.5 and 6.0 Hz, –CH_2-CH-N–), 4.59 (1H, d, J = 15.5 Hz, –NH-CH_2–), 4.36 (1H, d, J = 15.5 Hz, –NH-CH_2–), 2.78 (6H, s, –NMe_2), 2.63 (1H, dd, J = 13.8 and 9.5 Hz, –CO-CH_2–), 2.43 (1H, dd, J = 13.8 and 6.0 Hz, –CO-CH_2–); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 154.4, 147.0, 145.8, 145.0, 142.7, 129.4, 128.4, 128.3, 126.3, 125.0, 124.4, 123.6, 122.7, 122.2, 121.9, 61.1, 42.7, 41.2, 37.8.

3.7.7. Compound 7g. 67%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.19 (1H, t, J = 5.4 Hz, –CO-NH-CH_2–), 8.11 (2H, d, J = 8.7 Hz, –CH_2-C_4H_4-NO_2), 7.57 (2H, d, J = 9.0 Hz, –CH_2-C_6H_4-NO_2), 7.18–7.07 (4H, m, Ph), 4.89 (1H, dd, J = 10.7 and 4.1 Hz, –CH_2-CH-N–), 4.49 (1H, dd, J = 13.4 and 4.7 Hz, –CH_2-NH–), 4.43 (1H, dd, J = 13.4 and 4.7 Hz, –CH_2-NH–), 3.48–3.20 (6H, m, piperidinyl and –N-C$H_2$$CH_3$), 2.89 (1H, dd, J = 14.8 and 10.9 Hz, –CO-CH_2–), 2.08 (1H, dd, J = 14.6 and 4.1 Hz, –CO-CH_2–), 1.52 (6H, br s, piperidinyl), 1.07 (3H, t, J = 7.2 Hz, –N-CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.3, 170.1, 156.7, 146.3, 138.4, 128.6, 128.5, 127.2, 124.4, 124.3, 123.5, 120.2, 55.1, 47.5, 42.6, 41.2, 25.3, 24.1, 23.3, 14.2.

3.7.8. Compound 7h. 25%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.26 (1H, t, J = 6.0 Hz, –CO-NH-CH_2–), 8.12 (2H, d, J = 8.7 Hz, Ph), 7.62–7.55 (3H, m, Ph), 7.17–7.09 (3H, m, Ph), 4.85 (1H, dd, J = 10.8 and 4.1 Hz, –CH_2-CH-N–), 4.49–4.46 (2H, m, –CH_2-NH–), 3.28–3.22 (2H, m, –N-C$H_2$$CH_3$), 3.05 (6H, s, –N$Me_2$), 2.83 (1H, dd, J = 14.3 and 10.9 Hz, –CO-CH_2), 2.05 (1H, dd, J = 14.4 and 4.1 Hz, –CO-CH_2–), 1.06 (3H, t, J = 7.2 Hz, –N-CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.6, 155.9 147.0 146.3, 134.3, 129.1, 128.6, 126.4, 125.7, 124.4, 123.4, 119.1, 55.9, 48.1, 42.6, 41.3, 40.6, 14.0.

3.8. General preparation of compounds (8a–d)

To a solution of 4-nitrobenzylcarbamoyl-substituted 3,4-dihydroquinazoline compounds (**7e**–**h**) (2.87 mmol) in MeOH (40 mL) was added 10% Pd (C) (0.28 g), and the mixture was stirred for 2 h under H_2 atmosphere. The resulting mixture was filtered with Celite 545 and the solvent was evaporated in vacuo. Flash column chromatography (CH_2Cl_2/MeOH) of the residue gave product (**8a**–**d**).

3.8.1. Compound 8a. 97%; IR (KBr) 3218, 2930, 2850, 1648, 1550, 1480, 1430, 1350, 1282, 732 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26–7.22 (2H, m, Ph), 7.20–7.11 (4H, m, Ph), 7.07–7.02 (3H, m, Ph), 6.96–6.90 (2H, m, Ph), 6.60–6.56 (2H, m, Ph), 6.37 (1H, br s, –CO-NH-CH_2–), 5.17 (1H, dd, J = 9.9 and 5.3 Hz, –CH_2-CH-N–), 4.32 (2H, d, J = 5.7 Hz, –CH_2-NH–), 3.51 (2H, br s, –C_4H_4-NH_2), 3.26 (4H, br s, piperidinyl), 2.57 (1H, dd, J = 14.1 and 9.9 Hz, –CO-CH_2), 2.31 (1H, dd, J = 14.1 and 5.3 Hz, –CO-CH_2–), 1.43 (2H, br s, piperidinyl), 1.26 (4H, br s, piperidinyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.7, 153.3, 145.7, 145.2, 141.0, 129.5, 129.2, 128.2, 128.1, 126.7, 124.8, 124.6, 123.2, 123.1, 121.5, 115.0, 61.2, 47.6, 43.2, 41.7, 24.8, 24.2; HRMS (FAB, M+H) calcd for $C_{28}H_{32}N_5O$: 454.2607, found: 454.2654.

3.8.2. Compound 8b. 95%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (1H, t, J = 5.6 Hz, –CO-NH-CH_2), 7.26–7.20 (2H, m, Ph), 7.14–6.99 (6H, m, Ph), 6.92–6.83 (2H, m, Ph), 6.57–6.54 (2H, m, Ph), 5.20 (1H, dd, J = 9.9 and 5.1 Hz, –CH_2-CH-N–), 4.41 (1H, dd, J = 14.3 and 5.6 Hz, –CH_2-NH–), 4.32 (1H, dd, J = 14.3 and 5.6 Hz, –CH_2-NH–), 3.67 (2H, br s, –C_6H_4-NH_2), 2.66 (6H, s, –NMe_2), 2.50 (1H, dd, J = 14.3 and 9.9 Hz, –CO-CH_2), 2.25 (1H, dd, J = 14.3 and 5.1 Hz, –CO-CH_2–); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 153.9, 145.7, 145.6, 143.1, 129.4, 129.2, 128.0, 126.2, 124.9, 123.9, 122.3, 122.2, 121.9, 115.0, 61.2, 43.3, 41.6, 37.9.

3.8.3. Compound 8c. 83%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (1H, br s, –CH_2-NH-CO–), 7.08–7.03 (3H, m, Ph), 6.97–6.84 (3H, m, Ph), 6.57 (2H, d, J = 8.1 Hz, –CH_2-C_6H_4-NO_2), 4.78 (1H, dd, J = 9.2 and 5.6 Hz, –CH_2-CH-N–), 4.37 (1H, dd, J = 14.3 and 6.1 Hz, –CH_2-NH–), 4.24 (1H, dd, J = 14.3 and 5.6 Hz, –CH_2-NH–), 3.27 (1H, m, –N-C$H_2$$CH_3$), 3.18–3.01 (5H, m, piperidinyl and –N-C$H_2$$CH_3$), 2.26 (1H, dd, J = 14.3 and 9.2 Hz, –CO-CH_2–), 2.01 (1H, dd, J = 14.3 and 5.6 Hz, –CO-CH_2–), 1.43 (6H, br s, piperidinyl), 0.98 (3H t, J = 6.9 Hz, –N-CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.5, 157.0, 145.3, 142.0, 129.0, 128.1, 127.4, 127.0, 123.9, 122.6, 121.1, 114.5, 76.4, 54.6, 46.8, 42.6, 41.3, 25.2, 24.1, 13.9.

3.8.4. Compound 8d. 71%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (1H, br s, –CO-NH-CH_2–), 7.14–7.07 (4H, m, Ph), 6.97 (2H, d, J = 4.1 Hz, Ph), 6.50 (2H, d, J = 8.3 Hz, Ph), 4.78 (1H, dd, J = 10.7 and 4.4 Hz, –CH_2-CH-N–), 4.36 (1H, dd, J = 14.1 and 6.2 Hz, –CH_2-NH–), 4.19 (1H, dd, J = 14.1 and 5.5 Hz, –CH_2-NH–), 3.19–3.13 (2H, m, –N-C$H_2$$CH_3$), 2.76 (6H, s, –N$Me_2$), 2.41 (1H, dd, J = 14.2 and 10.9 Hz, –CO-CH_2–), 2.02 (1H, dd, J = 14.2 and 4.4 Hz, –CO-CH_2–), 0.98 (3H, t, J = 7.1 Hz, –N-CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 157.9, 146.0, 142.9, 129.8, 128.5, 128.3, 127.1, 124.9, 123.1, 121.9, 115.3, 55.9, 47.5, 43.4, 42.2, 39.3, 14.5.

3.9. General preparation of compounds (9a–h)

To a stirred solution of 4-aminobenzylcarbamoyl-substituted 3,4-dihydroquinazoline compounds (**8a**–**d**)

(0.446 mmol) in CH_2Cl_2(10 mL) were added pyridine (1.34 mmol) and *p*-toluenesulfonyl chloride or 4-fluorobenzensulfonyl chloride (0.535 mmol) sequentially. The mixture was allowed to stir at room temperature for 24 h and then H_2O was added. The resulting mixture was extracted with CH_2Cl_2 (3×), dried ($MgSO_4$), filtered, and solvent evaporated in vacuo. Flash column chromatography (CH_2Cl_2/MeOH) gave product (**9a**–**h**).

3.9.1. Compound 9a. 94%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (1H, d, J = 8.4 Hz, Ph), 7.58–7.73 (3H, m, Ph), 7.28–7.21 (3H, m, Ph), 7.18–6.95 (12H, m, Ph), 5.19 (1H, dd, J = 10.3 and 5.2 Hz, –CH_2-C*H*-N–), 4.35 (1H, dd, J = 14.5 and 5.8 Hz, –CH_2-NH–), 4.24 (1H, dd, J = 14.5 and 5.8 Hz, –CH_2-NH–), 3.28 (4H, br s, piperidinyl), 2.82 (1H, dd, J = 14.2 and 10.3 Hz, –CO-CH_2–), 2.36 (1H, dd, J = 14.2 and 5.2 Hz, –CO-CH_2–), 2.29 (3H, s, –SO_2-C_6H_4-CH_3), 1.33 (2H, br s, piperidinyl), 1.20 (4H, br s, piperidinyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 154.0, 144.9, 143.6, 138.9, 136.9, 136.7, 134.7, 129.8, 129.2, 129.0, 128.9, 127.3, 126.2, 125.9, 125,4, 124.4, 124.0, 121.1, 121.0, 61.7, 48.6, 43.2, 41.9, 24.8, 24.2, 21.7; HRMS (FAB, M+H) calcd for $C_{35}H_{38}N_5O_3S$: 608.2695, found: 608.2680.

3.9.2. Compound 9b. 60%; ^{1}H NMR (300 MHz, DMSO) δ 10.2 (1H, br s, Ts-N*H*–), 8.52 (1H, t, J = 5.6 Hz, –CO-N*H*-CH_2), 7.62 (2H, d, J = 8.1 Hz, Ph), 7.33–7.23 (m, 4H, Ph), 7.15–7.05 (3H, m, Ph), 7.01–6.96 (7H, m, Ph), 6.82 (1H, m, Ph), 5.08 (1H, dd, J = 10.4 and 4.3 Hz, –CH_2-C*H*-N–), 4.26 (1H, dd, J = 14.7 and 5.8 Hz, –NH-CH_2–), 4.17 (1H, dd, J = 14.8 and 5.8 Hz, –NH-CH_2–), 2.63 (6H, s, –NMe_2), 2.52 (1H, m, –CO-CH_2), 2.33 (3H, s, –SO_2-C_6H_4-CH_3), 2.24 (1H, dd, J = 14.0 and 4.3 Hz, –CO-CH_2–); ^{13}C NMR (75 MHz, DMSO) δ 169.4, 152.9, 145.8, 143.9, 143.1, 136.7, 136.4, 134.8, 129.6, 129.2, 128.5, 127.7, 126.6, 126.2, 124.8, 123.2, 122.0, 121.5, 121.1, 119.7, 60.8, 41.7, 40.8, 37.2, 20.9.

3.9.3. Compound 9c. 38%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (1H, t, J = 6.0 Hz, –CO-N*H*-CH_2–), 7.72 (2H, d, J = 8.4 Hz, Ph), 7.63 (1H, d, J = 7.8 Hz, Ph), 7.27–7.02 (9H, m, Ph), 4.99 (1H, dd, J = 10.5 and 4.8 Hz, –CH_2-C*H*-N–), 4.22 (2H, d, J = 6.0 Hz, –Ph-CH_2-NH–), 3.39–3.22 (6H, m, piperidinyl-2H_2, 2H_6 and –N-C$H_2$$CH_3$), 2.83 (1H, dd, J = 14.2 and 10.5 Hz, –CO-CH_2–), 2.29 (3H, s, –SO_2-C_6H_4-CH_3), 2.22 (1H, dd, J = 14.2 and 4.8 Hz, –CO-CH_2–), 1.60–1.35 (6H, m, piperidinyl-2H_3, 2H_4, 2H_5), 1.09 (3H, t, J = 7.0 Hz, –N-CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.3, 155.2, 143.3, 136.5, 136.4, 134.5, 133.8, 129.4, 128.8, 127.2, 126.4, 125.9, 124.4, 120.8, 119.1, 77.2, 55.4, 48.2, 42.6, 41.3, 25.1, 23.5, 21.4, 14.1; HRMS (FAB, M+H) calcd for $C_{31}H_{38}N_5O_3S$: 560.2695, found: 560.2720.

3.9.4. Compound 9d. 55%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (1H, br s, –CO-N*H*-CH_2–), 7.68 (2H, d, J = 8.1 Hz, Ph), 7.56 (1H, d, J = 7.2 Hz, Ph), 7.11–6.94 (9H, m, Ph), 4.85 (1H, m, –CH_2-C*H*-N–), 4.31–4.00 (2H, m, –CO-NH-CH_2–), 3.02–3.00 (2H, m, –N-C$H_2$$CH_3$), 2.73 (6H, s, –N$Me_2$), 2.76 (1H, m, –CO-C$H_2$–), 2.17 (3H, s, –$SO_2$-$C_6H_4$-C$H_3$), 2.00 (1H, m, –CO-C$H_2$–), 0.89 (3H, t, J = 6.9 Hz, –N-CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 155.1, 143.0, 136.3, 134.5, 132.7, 129.2, 129.0, 128.5, 127.0, 126.0, 125.7, 124.2, 120.6, 118.5, 55.6, 47.9, 42.4, 41.2, 40.3, 21.1, 13.7.

3.9.5. Compound 9e. 73%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66–7.62 (2H, m, Ph), 7.28–7.23 (2H, m, Ph), 7.20–7.06 (9H, m, Ph), 7.04–6.90 (4H, m, Ph), 6.72 (1H, br, –CO-N*H*-CH_2–), 5.19 (1H, dd, J = 9.8 and 6.0 Hz, –CH_2-C*H*-N–), 4.41 (1H, dd, J = 14.8 and 6.1 Hz, –Ph-CH_2-NH–), 4.24 (1H, dd, J = 14.8 and 5.5 Hz, –Ph-CH_2-NH–), 3.28 (4H, br s, piperidinyl), 2.74 (1H, dd, J = 14.1 and 9.8 Hz Hz, –CO-CH_2), 2.44 (1H, dd, J = 14.1 and 6.0 Hz, –CO-CH_2–), 1.39 (2H, br s, piperidinyl), 1.25 (4H, br s, piperidinyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 166.7, 153.9, 145.3, 141.7, 135.9, 135.0, 129.9, 129.7, 129.3, 128.9, 128.4, 126.7, 125.2, 124.8, 123.2, 121.5, 116.3, 116.0, 61.4, 47.9, 43.3, 42.0, 25.2, 24.6; HRMS (FAB, M+H) calcd for $C_{34}H_{35}FN_5O_3S$: 612.2445, found: 612.2436.

3.9.6. Compound 9f. 38%; ^{1}H NMR (300 MHz, DMSO) δ 10.3 (1H, br s, Ts-N*H*–), 8.56 (1H, t, J = 5.3 Hz, –CO-N*H*-CH_2), 7.82–7.77 (2H, m, Ph), 7.41–7.35 (2H, m, Ph), 7.30–7.25 (2H, m, Ph), 7.19–6.98 (10H, m, Ph), 6.89 (1H, m, Ph), 5.10 (1H, dd, J = 10.5 and 3.9 Hz, –CH_2-C*H*-N–), 4.26 (1H, dd, J = 14.5 and 5.0 Hz, –Ph-CH_2-NH–), 4.17 (1H, dd, J = 14.4 and 5.0 Hz, –Ph-CH_2-NH–), 2.66 (6H, s, –NMe_2), 2.55 (1H, m, –CO-CH_2–), 2.27 (1H, dd, J = 14.4 and 3.9 Hz, –CO-CH_2–); ^{13}C NMR (75 MHz, DMSO) δ 169.3, 165.9, 162.6, 152.9, 145.4, 136.1, 135.9, 135.2, 129.8, 129.6, 129.3, 128.6, 127.9, 126.2, 125.0, 123.7, 121.4, 120.2, 116.6, 116.3, 60.9, 41.8, 40.9, 37.5.

3.9.7. Compound 9g. 12% ; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (1H, br, –CO-N*H*-CH_2–), 7.89–7.84 (2H, m, Ph), 7.56 (1H, d, J = 8.4 Hz, Ph), 7.14–7.05 (7H, m, Ph), 7.01–6.95 (2H, m, Ph), 4.94 1H, (dd, J = 10.0 and 5.2 Hz, –CH_2-C*H*-N–), 4.30 (1H, dd, J = 14.7 and 6.1 Hz, –Ph-CH_2-NH–), 4.23 (1H, dd, J = 14.6 and 6.1 Hz, –Ph-CH_2-NH–), 3.36–3.22 (6H, m, piperidinyl and –N-C$H_2$$CH_3$), 2.67 (1H, dd, J = 14.3 and 9.9 Hz, –CO-CH_2–), 2.24 (1H, dd, J = 14.3 and 5.1 Hz, –CO-CH_2–), 1.45 (6H, br, piperidinyl), 1.10 (3H, t, J = 6.9 Hz, –N-CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.3, 166.5, 163.1, 155.1, 136.2, 135.4, 134.8, 133.6, 129.9, 128.8, 126.5, 125.9, 124.4, 121.1, 118.9, 116.1, 55.3, 48.2, 42.6, 41.4, 25.1, 23.5, 14.0.

3.9.8. Compound 9h. 22%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.0 (1H, br s, Ts-N*H*–), 8.18 (1H, m, –CO-N*H*-CH_2–), 7.91–7.79 (3H, m, Ph), 7.51 (1H, d, J = 7.8 Hz, Ph), 7.30–6.95 (8H, m, Ph), 5.02 (1H, dd, J = 10.2 and 4.5 Hz, –CH_2-C*H*-N–), 4.30–4.26 (2H, m, –Ph-CH_2-NH–), 3.29 (2H, q, J = 7.2 Hz, –N-C$H_2$$CH_3$), 2.95 (6H, s, N($CH_3$)$_2$), 2.86 (1H, dd, J = 14.0 and 10.2 Hz, –CO-CH_2–), 2.35 (1H, dd, J = 14.0 and 4.5 Hz, –CO-CH_2–), 1.13 (3H, t, J = 7.2 Hz, –N-CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) 169.3, 166.5, 163.1, 155.1, 136.2, 135.5, 134.8, 133.6, 130.0, 129.9, 128.8, 126.5,

125.9, 124.4, 121.1, 118.9, 116.1, 115.8, 56.1, 48.5, 43.0, 41.8, 40.7, 14.4; HRMS (FAB, M+H) calcd for $C_{27}H_{31}FN_5O_3S$: 524.2132, found: 524.2141.

4. Biological data

4.1. Preparation of unfertilized *Xenopus* oocytes and cRNA synthesis of α_{1H} T-type calcium channel[21]

In order to express a gene encoding T-type calcium channel α_{1H} ($Ca_v3.2$) in unfertilized *Xenopus* oocytes, vector (pGEM-HEA) was treated with restriction enzyme AflII to obtain a DNA fragment containing 5′-terminal region having the T-type calcium channel cDNA (AF051946), and cRNA having a corresponding sequence to that of the fragment was synthesized using T7 RNA polymerase according to the manufacturer's instruction (mMESSAGE mMACHINE kit, Ambion, Austin, U.S.A.). The synthesized cRNA was quantified by measuring the OD value with a spectrophotometer. At this time, unfertilized oocytes were prepared from female *Xenopus laevis* (Xenopus I, U.S.A.) according to the following method. After the frog's abdomen was incised by about 1 cm, three to four lobes were detached therefrom with scissors and separated into small pieces to which several oocytes attached. The small pieces were hydrolyzed in OR solution (82.5 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.6) supplemented with collagenase type IA (Sigma, U.S.A.) to remove defolliculation. After selecting healthy oocytes with a dissecting microscope, they were soaked in SOS solution (100 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, 2.5 mM pyruvate, and 50 μM/mL gentamicin, pH 7.6) for 3 to 4 h to revitalize them. 2 to 5 ng of cRNA was injected into each oocyte using a nano-injector, and the oocytes were subjected to test for examining the electrical properties of the channel expressed therefrom 4 to 5 days after the injection with maintaining at 18 °C.

4.2. Examination of electrophysiological property of α_{1H} T-type calcium channel using a two-electrode voltage clamping method[21]

Current of the calcium channel expressed from the *Xenopus* unfertilized oocytes was measured according to a two-electrode voltage clamp method. Current was measured in 10 mM Ba^{2+} solution [10 mM $Ba(OH)_2$, 90 mM NaOH, 1 mM KCl, 0.1 mM EDTA, and 5 mM HEPES, pH was adjusted to 7.4 with methanesulfonic acid]. At this time, voltage clamp and current measurements were regulated with an amplifier for unfertilized oocytes (Model OC-725C, Warner Instrument Corp., U.S.A.), analog signals were converted into digital signals using Digidata 2000A (Analog-Digital converter, Axon Instrument), and acquisition, storage, and analysis of all data were recorded in Pentium IV computer via pCLAMP8. The data were mainly collected at 5 KHz and filtered at 1 KHz (Model 902 filter; Frequency devices located at Harverhill, MA, U.S.A.). The generation of T-type current was occurred by imposing test electric potential of −20 mV every 15 s on the unfertilized oocytes whose potential was fixed at −90 mV, and a blocking percentage was calculated by comparing the potentials before and after the drug treatment.

4.3. Methods for culturing HEK293 cells and measuring T- and N-type calcium channel activity using an electrophysiological method[22]

HEK293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (v/v) in 36.5 °C humidified incubator (95% air–5% CO_2). The culture solution was replaced with a fresh medium every 3 to 4 days, and the cultured cells were subjected to subculture every week. At this time, the culture solution was treated with G-418 (0.5 mg/mL) solution so that only HEK293 cells expressing α_{1G} T-type calcium channel can grow. The cells used for T-type calcium channel activity assay were cultured on a coverslip coated with poly-L-lysine (0.5 mg/mL) whenever sub-cultured, and their calcium channel activity was recorded 2 to 7 days after the cultivation. Current of the T-type calcium channel at a single cell level was measured according to an electrophysiological whole-cell patch-clamp method using EPC-9 amplifier (HEKA, Germany). At this time, a cell exterior solution [140 mM NaCl, 2 mM $CaCl_2$, and 10 mM HEPES (pH 7.4)] and a cell interior solution [KCl 130 mM, HEPES 10 mM, EGTA 11 mM, and MgATP 5 mM (pH 7.4)] were employed. Inward current caused by the T-type calcium channel activation which occurred when the cells were converted into a whole-cell recording mode by stabbing a microglass electrode having 3–4 MΩ resistance which was filled with the cell interior solution into a single cell and depolarized at −30 mV (50 ms duration period) every 10 s with fixing membrane potential to −100 mV was measured according to a T-type calcium channel protocol activated at low current. In the case of N-type, Ca^{2+} currents were measured in HEK293 cells expressing α_{1B} Ca^{2+} channel using a cell exterior solution [151 mM TEACl, 5 mM $BaCl_2$, 10 mM HEPES, 1 mM $MgCl_2$, and 10 mM glucose (pH 7.4)] and a cell interior solution [100 mM CsCl, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM BAPTA, 3.6 mM MgATP, 0.1 mM GTP, 14 mM phosphocreatine and 50 U/ml creatine phosphokinase (pH 7.4)]. The currents were evoked every 15 s by a 200 ms depolarizing voltage step from −80 to 0 mV.

4.4. Method for screening T-type calcium channel blockers using an electrophysiological method

In order to confirm whether the cells and methods used in 4.3 are a suitable screening system for selecting T-type calcium channel blockers, the results obtained in 4.3 were compared with those of α_{1G} T-type calcium channel study reported in a public document.[22] As a result, it has been confirmed that since the screening system of the present invention showed: (1) the maximum activation at low voltage of −30 mV, (2) the fast activation-inactivation of the activated current, and (3) the same IC_{50} as those of Ni^{2+} and Mibefradil known as T-type calcium channel blockers, it is suitable for screening

T-type calcium channel blockers. Thus, the candidate compounds were subjected to test for their inhibitory effects on the T-type calcium channel according to the screening system of the present invention as follows: each compound was dissolved in 100% dimethylsulfoxide (DMSO) to prepare 10 mM stock solution, and the inhibitory effect on the T-type calcium channel current was examined in 10 μM sample solution (containing 0.1% DMSO) prepared by diluting the stock solution by 1000-fold. The cells were treated with each compound at a concentration of 10 μM for 30–60 s with the cell exterior solution. Then, the inhibition level of peak current caused by the compound was calculated as a percentage.

4.5. Analysis for cytotoxicities of T-type calcium channel blockers using MTT assay[23]

In order to analyze cytotoxicities of the T-type calcium channel blockers in HEK293 cells, MTT assay was conducted as follows: the cultured HEK293 cells were treated with each compound at a concentration of 10 and 100 μM, respectively. At this time, the cells treated with a solvent, that is, 0.1% DMSO, were used as a negative control and the cells treated with H_2O_2 (125 μM) inducing cytotoxicity were used as a positive control. 6 h after the drug treatment, the cells were treated with 50 μL MTT (1 mg/mL) dissolved in PBS for 4 h. Then, the reaction mixture was centrifuged to remove a supernatant, and formazan crystals thus obtained were dissolved in 100 μL DMSO. The solution's absorbance was measured at 560 nm with an automated spectrophotometric plate reader.

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