

Article

Design, Synthesis and Biological Evaluation of *N,N*-Substituted Amine Derivatives as Cholesteryl Ester Transfer Protein Inhibitors

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Abstract: *N,N*-Substituted amine derivatives were designed by utilizing a bioisosterism strategy. Consequently, twenty-two compounds were synthesized and evaluated for their inhibitory activity against CETP. Structure-activity relationship (SAR) studies indicate that hydrophilic groups at the 2-position of the tetrazole and 3,5-bistrifluoromethyl groups on the benzene ring provide important contributions to the potency. Among these compounds, compound 17 exhibited excellent CETP inhibitory activity ($IC_{50} = 0.38 \pm 0.08 \mu\text{M}$) in vitro. Furthermore, compound 17 was selected for an in vitro metabolic stability study.

Keywords: synthesis; *N,N*-substituted amine derivatives; CETP inhibitors; HDL-C

1. Introduction

Cholesteryl ester transfer protein (CETP) is a hydrophobic glycoprotein secreted predominately by the liver. CETP facilitates the movement of cholesteryl esters (CEs) from high-density lipoprotein (HDL) to low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL), in exchange for triglycerides (TGs) [1,2]. Epidemiological studies have provided compelling evidence to demonstrate an inverse association between HDL-C and cardiovascular events [3–5]. Therefore, CETP inhibitors can provide benefits to high risk coronary heart disease (CHD) patients by increasing HDL-C plasma levels [1,6–8].

Torcetrapib (**1**, Figure 1) was the first CETP inhibitor to undergo advanced clinical development. Although early studies have demonstrated that torcetrapib exhibited a significant lipid-regulating ability, subsequent trials indicated that this inhibitor possessed off-target effects affecting blood pressure and aldosterone levels, and failed to demonstrate any favourable impact on CHD patients [9–13]. Accordingly, torcetrapib's phase III trial was halted in 2006. Dalcetrapib (**2**, Figure 1) was a modest inhibitor without the off-targets effect seen with torcetrapib, elevating HDL-C levels by up to 30% and with a minimal effect on LDL-C levels [14,15]. However, dal-OUTCOMES study was stopped due to lack of clinical benefit [16]. More recently, a Canadian study found that dalcetrapib exhibited potentially clinical effects on atherosclerotic patients with polymorphisms in the ADCY9 gene using a genome-wide approach [17]. Evacetrapib (**3**, Figure 1) possessed profound lipid-regulating effects, raising HDL-C levels by more than 129% and decreasing LDL-C by up to 36%. Evacetrapib did not exhibit torcetrapib-like side-effects, but its phase III ACCELERATE study was terminated due to clinical futility [18,19]. Anacetrapib (**4**, Figure 1) a potent CETP inhibitor currently in phase

III, could raise HDL-C levels to 130% and decrease LDL-C levels to 40%. Researchers found that anacetrapib demonstrated potential clinical benefits and would not produce adverse clinical effects similar to those observed with torcetrapib in the DEFINE study [20,21]. TA-8995 (5, Figure 1), a novel CETP inhibitor, was well tolerated and had beneficial effects on lipids, raising HDL-C levels by up to 179% and decreasing LDL-C by up to 45% [22,23]. It remains to be seen whether these potent CETP inhibitors will proceed further in the future.

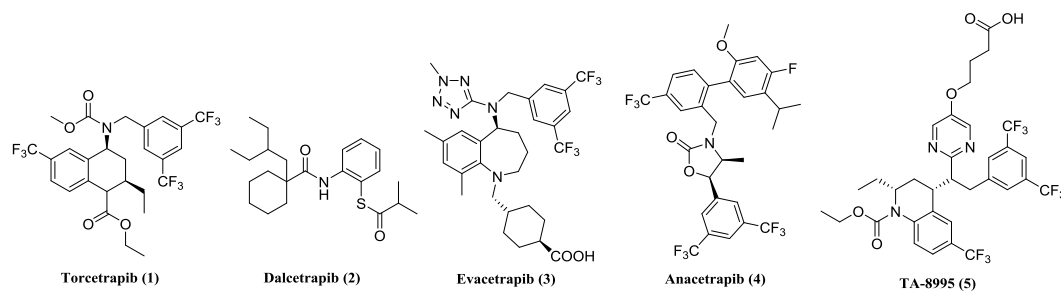


Figure 1. Representative CETP inhibitors.

In a previous study, compound 6 (Figure 2) was identified to show weak micromolar activity ($IC_{50} = 20.97 \mu M$) [24]. Our initial approach was to build a ring to replace the carbamate part to investigate the effect of steric hindrance on activity. Considering that tetrazole is a bioisoster of the carboxylic acid and the carbamate part of compound 6 could be treated as a carboxylic acid methyl ester, we hold that 2-methyltetrazole is a bioisoster of carbamate. In this paper, our group investigated the cyclization of the carbamate using a bioisosterism strategy to afford a 2-methyltetrazole compound. Fortunately, the compound containing the tetrazole moiety (40, Figure 2, $IC_{50} = 0.81 \pm 0.03 \mu M$) showed increased inhibitory activity. A series of novel *N,N*-substituted amine derivatives were then synthesized. Optimization efforts on this scaffold are discussed in this study.

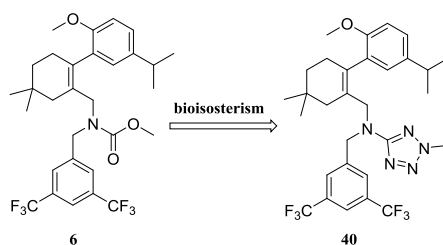


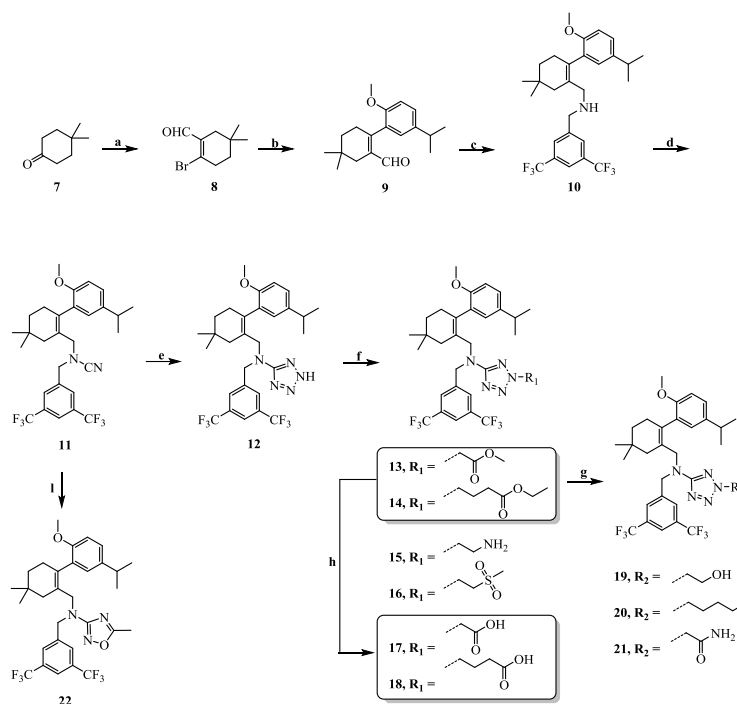
Figure 2. Design of new structures based on compound 6.

2. Results and Discussion

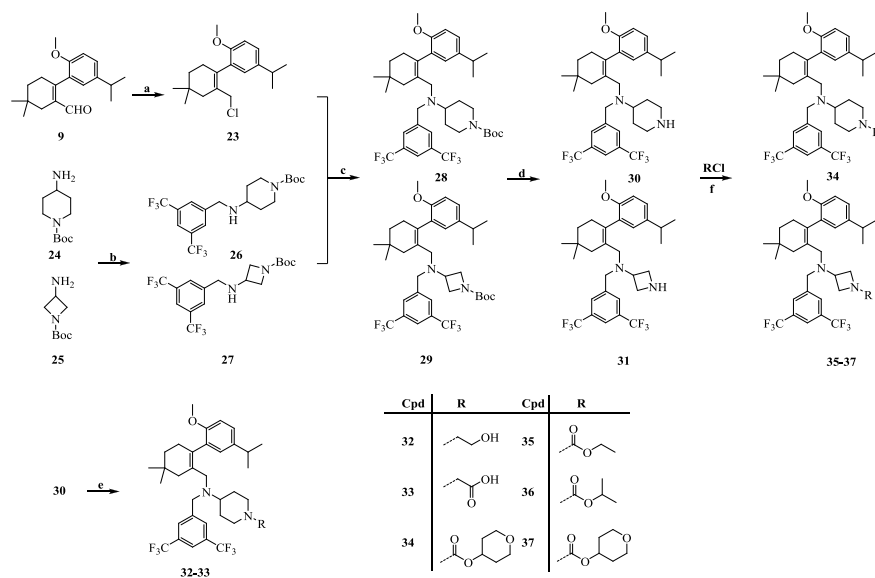
2.1. Chemistry

Compounds 12–22 were prepared according to the procedure shown in Scheme 1. The intermediate 10 was obtained in a manner similar to that described in the previously published paper [24]. The treatment of 10 with cyanogen bromide under basic conditions furnished 11 in good yield. Then, the -CN of the resulting intermediate was subjected to cycloaddition with sodium azide to produce 12. Compound 12 was subjected to substitution reactions with various substituted alkyl bromides to yield compounds 13–15. Alternatively, treatment of 12 with 2-(methylsulfonyl) ethanol under Mitsunobu conditions afforded 16. Carboxylic acid derivatives 17 and 18 were prepared by hydrolysis of the ester groups of compounds 13 and 14, respectively. Subsequent reduction or aminolysis reaction of compounds 13 and 14 produced compounds 19–21. Compound 11 reacted with hydroxylamine hydrochloride and subsequently, acetic anhydride, to obtain compound 22.

Compounds **32–37** were prepared according to the procedure in Scheme 2. The resulting **9** was reacted with NaBH_4 and subsequently, SOCl_2 to afford intermediate **23**.

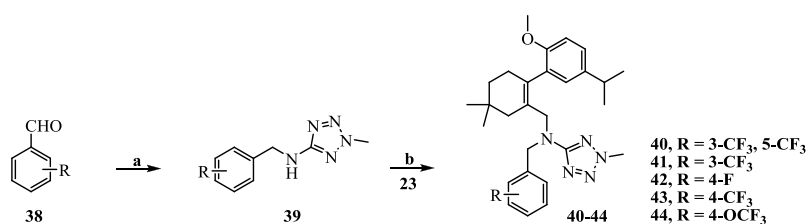


Scheme 1. Synthesis of target compounds **12–22**. *Reagents and conditions:* (a) DMF, PBr_3 , CHCl_3 , rt; (b) 5-isopropyl-2-methoxyphenylboronic acid, $\text{Pd}(\text{OAc})_2$, K_2CO_3 , acetylacetonate, EtOH, 80°C ; (c) 3,5-bis(trifluoromethyl)benzyl amine, $\text{NaBH}(\text{OAc})_3$, 1,2-dichloroethane, rt; (d) CNBr , DIEA, THF, rt; (e) NaN_3 , NH_4Cl , DMF, 100°C ; (f) $\text{R}_1\text{-X}$, Et_3N , acetonitrile, reflux; or (i) $\text{Boc-R}_1\text{-X}$, Et_3N , acetonitrile, reflux, (ii) TFA, DCM, rt; or $\text{R}_1\text{-OH}$, PPh_3 , DIAD, rt; (g) $\text{NaBH}_4/\text{MeOH}$, THF, reflux; or $\text{NH}_3\text{-EtOH}$, rt; (h) (i) 1 M aq. NaOH , (ii) 1 M aq. HCl ; (l) $\text{NH}_2\text{OH}\cdot\text{HCl}$, Ac_2O , Et_3N , EtOH, reflux.



Scheme 2. Synthesis of target compounds **32–37**. *Reagents and conditions:* (a) (i) NaBH_4 , MeOH, rt, (ii) SOCl_2 , DMF, rt; (b) 3,5-bis(trifluoromethyl)benzaldehyde, NaBH_4 , MeOH, rt; (c) NaH , DMF, 0°C ; (d) TFA/ CH_2Cl_2 (1:1), rt; (e) (i) methyl 2-bromoacetate, TEA, DMF, rt, (ii) $\text{NaBH}_4/\text{MeOH}$, THF, reflux; or 1 M aq. NaOH , 1 M aq. HCl ; (f) TEA, DCM, rt.

The starting materials **24** and **25** were subjected to reductive amination with 3,5-bis(trifluoromethyl)benzaldehyde, which provided **26** and **27**. Intermediates **28** and **29** were obtained by the nucleophilic substitution of **23** by **26** and **27** in the presence of NaH at room temperature. The key intermediates **30** and **31** were obtained by deprotection of **28** and **29** under the condition of TFA/DCM (*v:v* = 1:1). Intermediate **30** was reacted with methyl 2-bromoacetate, then the resultant ester intermediate was subjected to reduction conditions to yield compound **32** or hydrolysis conditions to furnish compound **33**. The obtained intermediates **30** and **31** were treated with corresponding chloroformates to give compounds **34–37**. As illustrated in Scheme 3, compounds **40–44** were synthesized from the corresponding starting benzaldehydes through reductive amination and substitution reactions.



Scheme 3. Synthesis of target compounds **40–44**. Reagents and conditions: (a) 2-methyl-2H-tetrazol-5-amine, NaBH₄, MeOH, rt; (b) NaH, DMF, 0 °C.

2.2. In Vitro Activity Against Cholesteryl Ester Transfer Protein

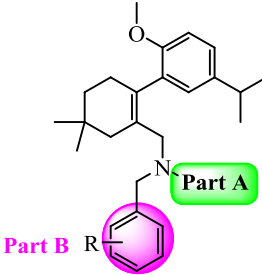
The *N,N*-substituted amine derivatives and the reference compound anacetrapib (**4**) were screened for their in vitro activity against CETP by a BODIPY-CE fluorescence assay with the CETP RP Activity Assay Kit (Catalogue # RB-RPAK; Roar, New York, NY, USA). The results are shown in Table 1. As seen in the table, replacement of a carbamate (**6**, IC₅₀ = 20.97 μM) with an *N*-2-methyltetrazole (**40**, IC₅₀ = 0.81 ± 0.03 μM) caused a significant increase in the activity. Subsequently, we investigated the relationship between various substituents at the 2-position of the tetrazole (Part A) and the CETP inhibitory activity. The replacement of –CH₃ by –H (**12**, IC₅₀ = 2.84 ± 0.04 μM) was detrimental for activity. Introduction of carboxylic esters (e.g., compounds **13** and **14**) at the 2-position of the tetrazole dramatically reduced the activity, however potency was recovered in the corresponding carboxylic acids (**17** and **18**) and straight chain saturated alcohols (**19** and **20**), where especially two atoms alkyl chains were better. We observed that amine and sulfone groups (**15** and **16**) were tolerated and an amide group (**21**) caused a weak decrease in the activity. Changing *N*-2-methyltetrazole (**40**) to 3-methoxydiazole (**22**, IC₅₀ = 0.72 ± 0.06 μM) showed no advantage. We speculate that carboxylic acids and saturated alcohols at the 2-position of tetrazole provide an important contribution to the potency.























Next, we investigated the relationship between an aliphatic heterocycle (Part A) and the CETP inhibitory activity. Unfortunately, replacement of *N*-2-substituted tetrazole with azetidine/ piperidine derivatives (compounds **32–37**) caused a dramatic decrease in the activity. These results indicate that a flexible fragment in Part A is unfavourable for inhibitory activity.

To further study the relationship between Part B and the CETP inhibitory activity, another five compounds **40–44** were prepared and evaluated for their activity. Introduction of trifluoromethyl groups on the 3-position and the 5-position of benzene ring (**40**, IC₅₀ = 0.81 ± 0.03 μM) were more beneficial for CETP inhibition. A single trifluoromethyl group on the *meta*-position of the benzene ring (**41**, IC₅₀ = 10.05 ± 0.06 μM) caused a severe decrease in the activity. A trifluoromethyl group (**43**), fluorine atom (**42**), and trifluoromethoxy group (**44**) on the *para*-position of the benzene ring resulted in no CETP inhibition.

Half of the compounds showed excellent activities, and four of them exhibited submicromolar activities, but the activity of the most outstanding compound **17** ($IC_{50} = 0.38 \pm 0.08 \mu M$) was still 10-fold weaker than the reference compound.

Table 1. Structures and activities of compounds **12–22**, **32–37**, **40–44**.



NO.	Part A	R (Part B)	IC ₅₀ (μM)	NO.	Part A	R (Part B)	IC ₅₀ (μM)
12		3-CF ₃ , 5-CF ₃	2.84 ± 0.04	33		3-CF ₃ , 5-CF ₃	>50 ^b
13		3-CF ₃ , 5-CF ₃	>50 ^b	34		3-CF ₃ , 5-CF ₃	>50 ^b
14		3-CF ₃ , 5-CF ₃	>50 ^b	35		3-CF ₃ , 5-CF ₃	>50 ^b
15		3-CF ₃ , 5-CF ₃	1.92 ± 0.10	36		3-CF ₃ , 5-CF ₃	>50 ^b
16		3-CF ₃ , 5-CF ₃	1.20 ± 0.01	37		3-CF ₃ , 5-CF ₃	>50 ^b
17		3-CF ₃ , 5-CF ₃	0.38 ± 0.08	40		3-CF ₃ , 5-CF ₃	0.81 ± 0.03
18		3-CF ₃ , 5-CF ₃	1.60 ± 0.02	41		3-CF ₃	10.05 ± 0.06
19		3-CF ₃ , 5-CF ₃	0.73 ± 0.09	42		4-F	>50 ^b
20		3-CF ₃ , 5-CF ₃	5.76 ± 0.03	43		4-CF ₃	>50 ^b
21		3-CF ₃ , 5-CF ₃	20.06 ± 0.12	44		4-OCF ₃	>50 ^b
22		3-CF ₃ , 5-CF ₃	0.72 ± 0.06				
32		3-CF ₃ , 5-CF ₃	>50 ^b	Anacetrapib ^a			0.04 ± 0.01

^a Used as a positive control. ^b Considered with no CETP inhibition activity.

2.3. In Vitro Metabolic Stability Study

Based on the result of the in vitro CETP inhibitory assay, the potent inhibitor **17** was selected for the in vitro metabolic stability study. As shown in Table 2, compound **17** showed weak stability, with a clearance rate of 119.0 and 146.1 μL/min/mg in human and rat liver microsomes.

Table 2. In vitro DMPK profile.

Compound	HLM Stability ^a (μL/min/mg)	RLM Stability ^b (μL/min/mg)	CYPs Direct Inhibition Mean (10 μM)				
			3A4	2D6	2C9	1A2	2C19
17	119.0	146.1	17.7%	No inhibition	14.3%	No inhibition	3.1%

^a Human liver microsomal intrinsic clearance. ^b Rat liver microsomal intrinsic clearance.

In addition, five cytochrome P450 (CYP) enzymes that commonly metabolize exogenous chemicals were used to test the direct inhibition of compound **17**. Compound **17** possessed favourable metabolic

properties, as the inhibition ratios for five CYPs were less than 20% even at the compound concentration of 10 μ M.

3. Experimental

3.1. General Information

All chemicals were obtained from commercial sources and were used without purification unless otherwise specified. Solvents were distilled and dried using standard methods. TLC was performed on silica gel plates with F-254 indicator and visualized by UV-light. The purities of target compounds (all \geq 95%) were detected by HPLC, performed on a Waters 1525–2489 system (Waters, Milford, MA, USA). The method conditions were as follows: 100% CH₃OH or a mixture of solvents H₂O (A) and MeOH (B) (VA:VB = 5:95) as eluent, flow rate at 1.0 mL/min. Peaks were detected at λ = 254 nm. NMR spectra were recorded on 400 MHz and 600 MHz instruments (Bruker, Karlsruhe, Germany) and the chemical shifts were reported in terms of parts per million with TMS as the internal reference. High-resolution accurate mass determinations (HRMS) for all final target compounds were obtained on a Bruker Micromass Time of Flight mass spectrometer equipped with electrospray ionisation (ESI). Column chromatography was performed with silica gel (200–300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd. (Qingdao, China)

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)cyanamide (**11**): Intermediate **10** (0.3 g, 0.6 mmol) was dissolved in THF (5 mL) and cyanogen bromide (0.2 g, 1.8 mmol) and *N,N*-diisopropylethylamine (0.4 mL, 2.4 mmol) were added. The reaction mixture was stirred at room temperature for 5 h and then poured into H₂O (10 mL) and extracted with EtOAc (10 mL \times 3). The combined organic layers were washed with H₂O (10 mL \times 3) and brine (10 mL \times 3), dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether:EtOAc = 15:1) to give **11** (0.3 g, 92.8%) as a pale yellow oil. ¹H-NMR (400 MHz, DMSO-*d*₆) δ 8.06 (s, 1H), 7.90 (s, 2H), 7.05 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.91–6.77 (m, 2H), 4.29 (s, 2H), 3.64 (s, 3H), 3.49–3.35 (m, 2H), 2.72 (dt, *J* = 13.7, 6.9 Hz, 1H), 2.40–2.28 (m, 1H), 2.11–1.99 (m, 1H), 1.90 (m, 2H), 1.40 (t, *J* = 6.4 Hz, 2H), 1.08 (dd, *J* = 6.9, 1.6 Hz, 6H), 0.98 (d, *J* = 2.8 Hz, 6H). HPLC: *t*_R = 13.710 min, 99.27%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)-2H-tetrazol-5-amine (**12**): Intermediate **11** (0.1 g, 0.2 mmol) was dissolved in DMF (5 mL) and ammonium chloride (0.01 g, 0.8 mmol) and sodium azide (0.05 g, 0.2 mmol) were added. After being stirred at 100 °C for 5 h, the reaction mixture was cooled to room temperature, and H₂O (20 mL) was added. The mixture was extracted with CH₂Cl₂ (20 mL \times 3) and the combined organic layers were washed with water (20 mL \times 3) and brine (20 mL \times 3), dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether:EtOAc = 2:1) to give **12** (0.1 g, 96.3%) as a white solid. Mp 150.6–152.6 °C. ¹H-NMR (600 MHz, DMSO-*d*₆) δ 14.88 (s, 1H), 7.94 (s, 1H), 7.66 (s, 2H), 6.99 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 1H), 6.71 (d, *J* = 2.2 Hz, 1H), 4.55 (s, 2H), 3.91 (m, 2H), 3.61 (s, 3H), 2.61 (dt, *J* = 13.8, 6.9 Hz, 1H), 2.36–2.27 (m, 1H), 2.06–2.03 (m, 1H), 1.77–1.69 (m, 2H), 1.38 (s, 2H), 1.36 (t, *J* = 6.4 Hz, 2H), 1.00 (d, *J* = 6.9 Hz, 6H), 0.91 (d, *J* = 10.3 Hz, 6H). ¹³C-NMR (150 MHz, CDCl₃) δ : 151.95, 142.58, 139.79, 136.20, 132.01 (\times 2), 129.76, 128.36, 127.92 (\times 2), 127.27, 126.44, 123.95, 122.14, 121.79, 111.11 (\times 2), 55.84 (\times 2), 53.23, 39.75, 34.93, 33.16, 30.00, 28.75, 28.44, 27.24, 24.07, 23.99. HRMS calcd for C₂₉H₃₄F₆N₅O, [M + H]⁺, 582.2589; found 582.2668. HPLC: *t*_R = 14.640 min, 96.47%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)-2H-tetrazol-5-amine-2-carboxylic acid methyl ester (**13**): Compound **12** (0.1 g, 0.2 mmol) and triethylamine (0.1 mL, 0.8 mmol) were dissolved in acetonitrile (2 mL) followed by the addition of methyl 2-bromoacetate (0.03 mL, 0.4 mmol). After being stirred at 80 °C for 2 h, the reaction mixture was cooled to room temperature, and H₂O (10 mL) was added. The aqueous layer was extracted

with EtOAc (5 mL \times 3) and the combined organic layers were washed with H₂O (5 mL \times 3) and brine (5 mL \times 3), dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether:EtOAc = 4:1) to give **13** (0.09 g, 68.4%) as a colourless oil. ¹H-NMR (400 MHz, CDCl₃) δ 7.67 (s, 1H), 7.54 (s, 2H), 7.03 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.76 (d, *J* = 2.3 Hz, 1H), 6.72 (d, *J* = 8.5 Hz, 1H), 5.18 (s, 2H), 4.58–4.38 (m, 2H), 4.19 (d, *J* = 14.5 Hz, 1H), 4.00 (d, *J* = 14.4 Hz, 1H), 3.76 (s, 3H), 3.68 (s, 3H), 2.76 (dt, *J* = 13.8, 6.9 Hz, 1H), 2.54–2.37 (m, 1H), 2.16–2.00 (m, 1H), 1.83 (s, 2H), 1.50–1.33 (m, 2H), 1.15 (d, *J* = 6.9 Hz, 6H), 0.94 (d, *J* = 11.9 Hz, 6H). ¹³C-NMR (150 MHz, CDCl₃) δ : 169.64, 165.70, 154.12, 141.11, 140.87, 135.34, 131.28(\times 2), 130.52, 128.06(\times 2), 127.76(\times 2), 127.63, 125.66(\times 2), 110.64(\times 2), 55.24, 53.01, 52.91, 51.69, 49.38, 40.57, 35.42, 33.03, 29.10, 28.99, 28.03(\times 2), 23.99(\times 2). HRMS calcd for C₃₂H₃₈F₆N₅O₃, [M + H]⁺, 654.2801; found 654.2877. HPLC: *t*_R = 8.753 min, 95.8%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)-2*H*-tetrazol-5-amine-2-butyrac acid ethyl ester (**14**): Colourless oil; yield 71.3%; ¹H-NMR (400 MHz, CDCl₃) δ 7.66 (s, 1H), 7.54 (s, 2H), 7.03 (d, *J* = 7.6 Hz, 1H), 6.82–6.65 (m, 2H), 4.46 (d, *J* = 3.5 Hz, 4H), 4.18–4.11(m, 3H), 3.98 (d, *J* = 14.5 Hz, 1H), 3.67 (s, 3H), 2.76 (s, 1H), 2.46 (d, *J* = 19.3 Hz, 1H), 2.30 (d, *J* = 4.5 Hz, 2H), 2.23 (s, 2H), 2.12–2.03 (m, 1H), 1.82 (s, 2H), 1.42 (s, 2H), 1.25–1.24 (m, 3H), 1.14 (s, 6H), 0.94 (d, *J* = 11.1 Hz, 6H). ¹³C-NMR (150 MHz, CDCl₃) δ : 172.11, 169.37, 154.14, 141.34, 140.85, 135.18, 131.24(\times 2), 130.57, 128.08, 127.77(\times 2), 125.63, 124.16, 122.35, 120.60, 110.62(\times 2), 60.54, 55.22, 51.77, 51.65, 49.36, 40.61, 35.43, 33.03, 30.60, 29.10, 29.00, 28.04, 28.02, 24.12, 23.99, 23.98, 14.04. HRMS calcd for C₃₅H₄₄F₆N₅O₃, [M + H]⁺, 696.3270; found 656.3361. HPLC: *t*_R = 7.653 min, 96.4%.

2-(2-Aminoethyl)-*N*-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)methyl)-2*H*-tetrazol-5-amine (**15**): Compound **12** (0.5 g, 0.9 mmol) and triethylamine (1.8 mL, 13.0 mmol) were dissolved in acetonitrile (10 mL), followed by the addition of tert-butyl 2-bromoethylcarbamate (0.6 mL, 2.6 mmol). After being stirred at 80 °C for 2 h, the reaction mixture was cooled to room temperature, and H₂O (10 mL) was added. The aqueous layer was extracted with EtOAc (5 mL \times 3) and the combined organic layers were washed with H₂O (5 mL \times 3) and brine (5 mL \times 3), dried over Na₂SO₄, and concentrated in vacuo. The residue was immediately dissolved in a trifluoroacetic acid–dichloromethane (1:1) solution (2 mL) and stirred at room temperature overnight. After concentration, the residue was dissolved in EtOAc (5 mL), washed with H₂O (5 mL \times 3) and brine (5 mL \times 3), dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether:EtOAc = 2:1) to give **15** (0.35 g, 62.2%) as a colourless oil. ¹H-NMR (400 MHz, DMSO-*d*₆) δ 7.93 (s, 1H), 7.66 (s, 2H), 7.00 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.79–6.76 (m, 2H), 4.49 (s, 2H), 4.35 (t, *J* = 6.1 Hz, 2H), 4.02–3.89 (m, 2H), 3.62 (s, 3H), 2.94 (t, *J* = 6.2 Hz, 2H), 2.67 (dt, *J* = 13.8, 6.9 Hz, 1H), 2.54–2.37 (m, 1H), 2.16–2.00 (m, 1H), 1.76 (s, 2H), 1.35 (t, *J* = 6.3 Hz, 2H), 1.04 (d, *J* = 6.9 Hz, 6H), 0.89 (d, *J* = 10.2 Hz, 6H). ¹³C-NMR (150 MHz, CDCl₃) δ : 169.41, 154.13, 141.31, 140.86, 135.29, 131.25(\times 2), 130.55, 128.07, 127.80, 127.70, 125.65, 124.14, 122.34, 120.63, 110.62(\times 2), 55.81, 55.22, 51.72, 49.39, 40.93, 40.64, 35.42, 33.03, 29.11, 29.01, 28.06, 28.03, 24.00, 23.98. HRMS calcd for C₃₁H₃₉F₆N₆O, [M + H]⁺, 625.3011; found 625.3070. HPLC: *t*_R = 18.895 min, 96.6%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)-2-(2-(methylsulfonyl)ethyl)-2*H*-tetrazol-5-amine (**16**): 2-(methylsulfonyl)ethanol (0.04 mL, 0.4 mmol) was added to a solution of compound **12** (0.1 g, 0.2 mmol) in THF (2 mL) and cooled to 0 °C, followed by the addition of Ph₃P (0.07 g, 0.3 mmol) and DIAD (0.05 mL, 0.3 mmol). After being stirred at room temperature for 4 h, the reaction mixture was poured into H₂O (10 mL) and extracted with EtOAc (5 mL \times 3), and the combined organic layers were washed with H₂O (5 mL \times 3) and brine (5 mL \times 3), dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether:EtOAc = 1:1) to give **16** (0.09 g, 65.3%) as a colourless oil. ¹H-NMR (400 MHz, CDCl₃) δ 7.68 (s, 1H), 7.50 (s, 2H), 7.03 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.76 (d, *J* = 2.3 Hz, 1H), 6.71 (d, *J* = 8.5 Hz, 1H), 4.90 (t, *J* = 6.9 Hz, 2H), 4.48 (s, 2H), 4.17 (d, *J* = 15.4 Hz, 1H), 3.99 (d, *J* = 14.4 Hz, 1H), 3.70–3.60 (m, 5H), 2.82–2.71 (m, 4H), 2.46 (d, *J* = 18.1 Hz, 1H), 2.10 (d, *J* = 18.4 Hz, 1H), 1.79 (s, 2H),

1.43 (dd, $J = 9.7, 6.4$ Hz, 2H), 1.15 (d, $J = 6.9$ Hz, 6H), 0.94 (d, $J = 11.3$ Hz, 6H). $^{13}\text{C-NMR}$ (150 MHz, CDCl_3) δ : 169.58, 154.08, 140.96, 135.68, 131.36($\times 2$), 130.40, 128.01, 127.69, 127.33, 125.74($\times 2$), 124.10, 122.29, 120.77, 110.66($\times 2$), 55.25, 52.90, 51.66, 49.20, 46.20, 41.37, 40.66, 35.36, 33.03, 29.10, 29.02, 28.08, 28.03, 24.00, 23.98. HRMS calcd for $\text{C}_{32}\text{H}_{40}\text{F}_6\text{N}_5\text{O}_3\text{S}$, $[\text{M} + \text{H}]^+$, 688.2678; found 688.2754. HPLC: $t_{\text{R}} = 13.667$ min, 95.6%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)-2*H*-tetrazol-5-amine-2-acetic acid (**17**): Compound **13** (0.2 g, 0.3 mmol) was dissolved in MeOH (5 mL) and 1 mol/L NaOH (5 mL) was added and the mixture was stirred at room temperature for 2 h. After concentration, the residue was dissolved in H_2O (10 mL). Then, 1 mol/L HCl (5 mL) was added to the mixture, the mixture was extracted with EtOAc (10 mL $\times 3$), and the combined organic layers were washed with H_2O (10 mL $\times 3$) and brine (10 mL $\times 3$), dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by chromatography on silica gel (DCM:MeOH = 5:1) to give **17** (0.17 g, 86.4%) as a colourless oil. $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ 7.92 (s, 1H), 7.65 (s, 2H), 7.00 (dd, $J = 8.5, 2.2$ Hz, 1H), 6.77 (dd, $J = 7.9, 5.4$ Hz, 2H), 5.35 (s, 2H), 4.57–4.45 (m, 2H), 3.97 (t, $J = 10.4$ Hz, 2H), 3.63 (s, 3H), 2.67 (dt, $J = 13.7, 6.9$ Hz, 1H), 2.33 (d, $J = 17.9$ Hz, 1H), 2.02 (d, $J = 18.0$ Hz, 1H), 1.76 (s, 2H), 1.36 (t, $J = 6.4$ Hz, 2H), 1.04 (d, $J = 6.9$ Hz, 6H), 0.90 (d, $J = 14.3$ Hz, 6H). $^{13}\text{C-NMR}$ (150 MHz, $\text{DMSO-}d_6$) δ : 169.21, 167.87, 154.37, 142.28, 140.54, 134.58, 130.63($\times 2$), 130.29, 127.81, 127.74, 127.62, 125.85, 124.50, 122.69, 120.98, 111.32($\times 2$), 55.70, 53.85, 51.65, 49.55, 40.57, 35.36, 32.75, 29.24, 29.07, 28.46, 28.09, 24.27, 24.21. HRMS calcd for $\text{C}_{31}\text{H}_{36}\text{F}_6\text{N}_5\text{O}_3$, $[\text{M} + \text{H}]^+$, 640.2644; found 640.2709. HPLC: $t_{\text{R}} = 19.013$ min, 97.3%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)-2*H*-tetrazol-5-amine-2-butyric acid (**18**): Colourless oil; yield 81.3%; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 12.18 (s, 1H), 7.90 (s, 1H), 7.65 (s, 2H), 6.99 (d, $J = 8.5$ Hz, 1H), 6.78 (d, $J = 8.5$ Hz, 2H), 4.50 (s, 2H), 4.44 (t, $J = 6.8$ Hz, 2H), 4.00–3.90 (m, 2H), 3.62 (s, 3H), 2.66 (dt, $J = 13.7, 6.8$ Hz, 1H), 2.32 (d, $J = 17.7$ Hz, 1H), 2.19–2.15 (m, 2H), 2.07–1.98 (m, 3H), 1.75 (s, 2H), 1.35 (t, $J = 5.9$ Hz, 2H), 1.03 (d, $J = 6.9$ Hz, 6H), 0.88 (d, $J = 7.5$ Hz, 6H). $^{13}\text{C-NMR}$ (150 MHz, $\text{DMSO-}d_6$) δ : 173.76, 169.19, 154.39, 142.35, 140.54, 134.46, 130.61($\times 2$), 127.88, 127.74, 127.72($\times 2$), 125.84, 124.51, 122.70, 120.94, 111.33($\times 2$), 55.69, 51.88, 51.70, 49.66, 40.63, 35.36, 32.76, 30.40, 29.25, 29.05, 28.40, 28.13, 24.44, 24.25, 24.21. HRMS calcd for $\text{C}_{33}\text{H}_{40}\text{F}_6\text{N}_5\text{O}_3$, $[\text{M} + \text{H}]^+$, 668.2957; found 668.3050. HPLC: $t_{\text{R}} = 18.767$ min, 97.1%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)-2*H*-tetrazol-5-amine-2-ethyl alcohol (**19**): Compound **13** (0.4 g, 0.6 mmol) was dissolved in THF (5 mL) and NaBH_4 (0.05 g, 1.2 mmol) and 2 drops of MeOH were added and the mixture was stirred at 70 $^\circ\text{C}$ for 2 h and then cooled to room temperature. After concentration, the residue was dissolved in EtOAc (10 mL), washed with H_2O (10 mL $\times 3$) and brine (10 mL $\times 3$), dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether:EtOAc = 4:1) to give **19** (0.3 g, 79.8%) as a colourless oil. $^1\text{H-NMR}$ (600 MHz, $\text{DMSO-}d_6$) δ 7.92 (s, 1H), 7.67 (s, 2H), 7.00 (dd, $J = 8.5, 2.2$ Hz, 1H), 6.78 (d, $J = 8.5$ Hz, 1H), 6.77 (d, $J = 2.3$ Hz, 1H), 4.95 (t, $J = 5.6$ Hz, 1H), 4.49 (s, 2H), 4.42 (t, $J = 5.4$ Hz, 2H), 3.97 (q, $J = 14.4$ Hz, 2H), 3.78 (dd, $J = 10.8, 5.5$ Hz, 2H), 3.62 (s, 3H), 2.67 (dt, $J = 13.8, 6.9$ Hz, 1H), 2.33 (d, $J = 18.0$ Hz, 1H), 2.02 (d, $J = 18.0$ Hz, 1H), 1.76 (s, 2H), 1.35 (t, $J = 6.4$ Hz, 2H), 1.04 (d, $J = 6.9$ Hz, 6H), 0.89 (d, $J = 16.3$ Hz, 6H). $^{13}\text{C-NMR}$ (150 MHz, CDCl_3) δ : 169.30, 154.11, 141.18, 140.89, 135.43, 131.32($\times 2$), 130.51, 128.04, 127.72, 127.55, 125.67, 124.13, 122.32, 120.71, 110.63($\times 2$), 60.30, 55.23, 54.95, 51.80, 49.41, 40.68, 35.40, 33.03, 29.12, 29.02, 28.04($\times 2$), 23.99, 23.98. HRMS calcd for $\text{C}_{31}\text{H}_{38}\text{F}_6\text{N}_5\text{O}_2$, $[\text{M} + \text{H}]^+$, 626.2851; found 626.2920. HPLC: $t_{\text{R}} = 16.776$ min, 95.8%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)-2*H*-tetrazol-5-amine-2-butanol (**20**): Colourless oil; yield 73.8%; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 7.92 (s, 1H), 7.67 (s, 2H), 7.02 (dd, $J = 8.5, 2.1$ Hz, 1H), 6.83–6.76 (m, 2H), 4.52 (s, 2H), 4.48–4.39 (m, 3H), 4.03–3.92 (m, 2H), 3.64 (s, 3H), 3.37 (d, $J = 5.4$ Hz, 2H), 2.68 (dt, $J = 13.7, 6.8$ Hz, 1H), 2.40–2.29

(m, 1H), 2.06–2.00 (m, 1H), 1.89–1.80 (m, 2H), 1.77 (s, 2H), 1.40–1.28 (m, 4H), 1.05 (d, $J = 6.9$ Hz, 6H), 0.90 (d, $J = 7.5$ Hz, 6H). ^{13}C -NMR (150 MHz, CDCl_3) δ : 169.27, 154.14, 141.37, 140.85, 135.19, 131.22($\times 2$), 130.58, 128.09, 127.77($\times 2$), 125.63, 124.16, 122.36, 120.59, 110.62($\times 2$), 61.78, 55.22, 52.52, 51.64, 49.35, 40.60, 35.43, 33.03, 29.12, 29.10, 29.00, 28.04($\times 2$), 25.53, 23.99, 23.98. HRMS calcd for $\text{C}_{33}\text{H}_{42}\text{F}_6\text{N}_5\text{O}_2$, $[\text{M} + \text{H}]^+$, 654.3164; found 654.3257. HPLC: $t_{\text{R}} = 15.755$ min, 96.4%.

2-(5-((3,5-Bis(trifluoromethyl)benzyl)((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)amino)-2H-tetrazol-2-yl)acetamide (21): Compound **13** (0.2 g, 0.3 mmol) was dissolved in a saturated NH_3 -EtOH solution (5 mL). After being stirred at room temperature for 12 h, the reaction mixture was concentrated in vacuo. The residue was dissolved in EtOAc (10 mL), washed with H_2O (10 mL $\times 3$) and brine (10 mL $\times 3$), dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether:EtOAc = 4:1) to give **21** (0.12 g, 62.6%) as a colourless oil. ^1H -NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.91 (s, 1H), 7.70 (s, 1H), 7.66 (s, 2H), 7.42 (s, 1H), 6.99 (dd, $J = 8.5, 2.2$ Hz, 1H), 6.78–6.75(m, 2H), 5.12 (s, 2H), 4.50 (s, 2H), 3.97 (d, $J = 4.6$ Hz, 2H), 3.62 (s, 3H), 2.66 (dt, $J = 13.7, 6.8$ Hz, 1H), 2.33 (d, $J = 18.0$ Hz, 1H), 2.01 (d, $J = 17.7$ Hz, 1H), 1.76 (s, 2H), 1.35 (t, $J = 6.2$ Hz, 2H), 1.04 (d, $J = 6.9$ Hz, 6H), 0.89 (d, $J = 10.9$ Hz, 6H). ^{13}C -NMR (150 MHz, CDCl_3) δ : 169.77, 154.08, 140.96, 140.88, 131.43($\times 2$), 130.42, 127.99($\times 2$), 127.73, 127.29, 125.77($\times 2$), 124.07, 122.26, 120.84, 110.68($\times 2$), 55.24, 54.83, 51.87, 49.43, 40.79, 35.35, 33.04, 29.13, 29.03, 28.10, 28.00, 24.01, 23.98. HRMS calcd for $\text{C}_{31}\text{H}_{37}\text{F}_6\text{N}_6\text{O}_2$, $[\text{M} + \text{H}]^+$, 639.2804; found 639.2909. HPLC: $t_{\text{R}} = 12.757$ min, 98.2%.

N-(3,5-Bis(trifluoromethyl)benzyl)-N-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)-5-methyl-1,2,4-oxadiazol-3-amine (22): Intermediate **11** (0.3 g, 0.6 mmol) was dissolved in EtOH (5 mL) and triethylamine (0.08 mL, 0.6 mmol) and hydroxylamine hydrochloride (0.04 g, 0.6 mmol) were added. After being stirred at 80 °C for 2 h, the reaction mixture was cooled to room temperature and concentrated in vacuo. Then, the residue was added pyridine (5 mL) and acetic anhydride (0.07 mL, 0.7 mmol). After being stirred at 80 °C for 12 h, the reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was dissolved in EtOAc (10 mL), washed with H_2O (10 mL $\times 3$) and brine (10 mL $\times 3$), dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether:EtOAc = 4:1) to give **22** (0.15 g, 41.9%) as a colourless oil. ^1H -NMR (600 MHz, CDCl_3) δ 7.66 (s, 1H), 7.50 (s, 2H), 7.00 (dd, $J = 8.4, 2.1$ Hz, 1H), 6.72 (d, $J = 2.0$ Hz, 1H), 6.67 (d, $J = 8.4$ Hz, 1H), 4.43 (q, $J = 16.2$ Hz, 2H), 4.06 (d, $J = 14.5$ Hz, 1H), 3.90 (d, $J = 14.5$ Hz, 1H), 3.65 (s, 3H), 2.73 (dt, $J = 13.8, 6.9$ Hz, 1H), 2.47–2.43 (m, 4H), 2.08 (d, $J = 18.2$ Hz, 1H), 1.81 (s, 2H), 1.42 (dq, $J = 13.0, 6.5$ Hz, 2H), 1.13 (d, $J = 6.9$ Hz, 6H), 0.95 (d, $J = 13.1$ Hz, 6H). ^{13}C -NMR (150 MHz, CDCl_3) δ : 175.39, 170.36, 154.07, 140.92, 140.85, 135.59, 131.31($\times 2$), 130.37, 128.00, 127.54, 127.35, 125.66, 124.13, 122.32, 120.70, 110.62($\times 2$), 55.22, 51.08, 48.61, 40.40, 35.41, 33.01, 29.04, 29.01, 28.10, 27.97, 23.96($\times 2$), 12.63. HRMS calcd for $\text{C}_{31}\text{H}_{36}\text{F}_6\text{N}_3\text{O}_2$, $[\text{M} + \text{H}]^+$, 596.2633; found 596.2716. HPLC: $t_{\text{R}} = 9.953$ min, 95.8%.

2-(2-(Chloromethyl)-4,4-dimethylcyclohex-1-enyl)-4-isopropyl-1-methoxybenzene (23): Intermediate **9** (114.5 mg, 0.4 mmol) was dissolved in ethanol (5 mL). Sodium borohydride (19.0 mg, 0.5 mmol) was added to the mixture. After being stirred at room temperature for 30 min, the reaction mixture was poured into H_2O (20 mL) and extracted with EtOAc (10 mL $\times 3$). The combined organic layers were washed with H_2O (10 mL $\times 3$) and brine (10 mL $\times 3$), dried over Na_2SO_4 , and concentrated in vacuo. SOCl_2 (0.1 mL, 1.4 mmol) was added to a solution of the above residue in DMF (2 mL) cooled to 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was poured into H_2O (10 mL) and was extracted with EtOAc (5 mL $\times 3$). The combined organic layers were washed with H_2O (10 mL $\times 3$) and brine (10 mL $\times 3$), dried over Na_2SO_4 , and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether:EtOAc = 10:1) to give **23** (92.5 mg, 75.3% in two steps), which was used immediately for the next step because of its instability. HPLC: $t_{\text{R}} = 7.706$ min, 96.8%.

tert-Butyl 4-(3,5-bis(trifluoromethyl)benzylamino)piperidine-1-carboxylate (26): *tert*-butyl 4-amino-piperidine-1-carboxylate (1.0 g, 5.0 mmol) and 3,5-bis(trifluoromethyl) benzaldehyde (1.0 g, 4.1 mmol)

were dissolved in MeOH (15 mL). After stirring for 3 h at room temperature, NaBH₄ (0.3 g, 7.0 mmol) was added to the mixture. The reaction mixture was stirred at room temperature for 30 min and then poured into a saturated sodium bicarbonate solution (20 mL). The mixture was extracted with CH₂Cl₂ (20 mL × 3) and the combined organic layers were washed with water (20 mL × 3) and brine (20 mL × 3), dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether:EtOAc = 4:1) to give **26** (1.4 g, 80.0%) as a pale yellow oil. ¹H-NMR (600 MHz, CDCl₃) δ 7.83 (s, 2H), 7.76 (s, 1H), 4.04 (s, 2H), 3.96 (s, 2H), 2.82 (s, 2H), 2.71–2.63 (m, 1H), 1.88 (d, *J* = 11.2 Hz, 2H), 1.46 (s, 9H), 1.35–1.26 (m, 2H). HPLC: *t*_R = 13.657 min, 95.7%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)piperidine-4-amine-1-carboxylic acid tert-butyl ester (**28**): NaH (30.0 mg, 0.7 mmol, 60% in oil) was added to a solution of intermediate **26** (213.1 mg, 0.5 mmol) in DMF (5 mL) cooled to 0 °C. After stirring at 0 °C for 30 min, a solution of intermediate **23** (153.4 mg, 0.5 mmol) in DMF (5 mL) was added. The reaction mixture was allowed to warm to room temperature and stirred for 30 min, and then was poured onto crushed ice. The mixture was diluted with EtOAc (15 mL) and the layers were separated. The aqueous layer was extracted with EtOAc (5 mL × 3), and the combined organic layers were washed with H₂O (10 mL × 3) and brine (10 mL × 3), dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether:EtOAc = 20:1) to give **28** (235.9 mg, 67.7%) as a colourless oil. ¹H-NMR (400 MHz, CDCl₃) δ 7.83 (s, 2H), 7.73 (s, 1H), 7.08 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.79 (d, *J* = 8.4 Hz, 1H), 6.74 (d, *J* = 2.3 Hz, 1H), 4.11 (s, 2H), 3.70 (s, 3H), 3.50 (q, *J* = 15.0 Hz, 2H), 2.90–2.78 (m, 2H), 2.74–2.48 (m, 4H), 2.44–2.31 (m, 1H), 2.09–2.00 (m, 1H), 1.99–1.83 (m, 2H), 1.52 (d, *J* = 12.0 Hz, 1H), 1.45–1.34 (m, 12H), 1.34–1.25 (m, 2H), 1.22 (dd, *J* = 6.9, 1.1 Hz, 6H), 0.96 (s, 6H). HPLC: *t*_R = 7.023 min, 96.6%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)piperidin-4-amine (**30**): Intermediate **28** (0.2 g, 0.3 mmol) was dissolved in a trifluoroacetic acid–dichloromethane (1:1) solution (20 mL) and stirred at room temperature overnight. After concentration, the residue was dissolved in EtOAc (10 mL), washed with H₂O (10 mL × 3) and brine (10 mL × 3), dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether:EtOAc = 2:1) to give **30** (172.1 mg, 96.1%) as a colourless oil. ¹H-NMR (400 MHz, DMSO-*d*₆) δ 8.81 (s, 1H), 8.10–7.88 (m, 3H), 7.11 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 6.83 (d, *J* = 2.0 Hz, 1H), 3.66 (s, 3H), 3.57 (d, *J* = 2.4 Hz, 2H), 3.29 (d, *J* = 11.8 Hz, 2H), 2.89–2.68 (m, 6H), 2.26 (d, *J* = 17.6 Hz, 1H), 2.01 (s, 1H), 1.87 (d, *J* = 7.9 Hz, 2H), 1.67–1.48 (m, 4H), 1.35 (t, *J* = 6.2 Hz, 2H), 1.17 (d, *J* = 6.9 Hz, 6H), 0.92 (d, *J* = 10.5 Hz, 6H). HPLC: *t*_R = 13.003 min, 95.9%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)piperidin-4-amine-1-ethyl alcohol (**32**): Intermediate **30** (0.7 g, 1.2 mmol) was dissolved in acetonitrile (10 mL) and methyl 2-bromoacetate (0.4 g, 2.4 mmol) and triethylamine (0.6 mL, 4.7 mmol) were added. The reaction mixture was stirred at room temperature for 16 h and then poured into H₂O (10 mL) and extracted with EtOAc (10 mL × 3). The combined organic layers were washed with H₂O (10 mL × 3) and brine (10 mL × 3), dried over Na₂SO₄, and concentrated in vacuo. The residue was dissolved in THF (10 mL), and sodium borohydride (0.1 g, 2.6 mmol) and 2 drops of MeOH were added. The mixture was stirred at 70 °C for 2 h and then cooled to room temperature. After concentration, the residue was dissolved in EtOAc (10 mL), washed with H₂O (10 mL × 3) and brine (10 mL × 3), dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether:EtOAc = 1:1) to give **32** (0.4 g, 52.0%) as a colourless oil. ¹H-NMR (400 MHz, DMSO-*d*₆) δ 8.01 (s, 2H), 7.93 (s, 1H), 7.08 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 1H), 6.74 (d, *J* = 2.0 Hz, 1H), 4.39 (s, 1H), 3.64 (s, 3H), 3.55 (d, *J* = 18.4 Hz, 2H), 3.44 (d, *J* = 5.9 Hz, 2H), 2.84 (d, *J* = 13.3 Hz, 2H), 2.78 (dd, *J* = 13.7, 6.8 Hz, 1H), 2.69 (s, 2H), 2.43–2.37 (m, 3H), 2.26 (d, *J* = 17.8 Hz, 1H), 1.96–1.77 (m, 4H), 1.44–1.42 (m, 7H), 1.16 (d, *J* = 6.9 Hz, 6H), 0.91 (d, *J* = 7.1 Hz, 6H). ¹³C-NMR (150 MHz, CDCl₃) δ: 154.49, 144.61, 140.59, 132.83, 131.67, 131.25(×2), 129.57, 128.19, 128.00, 125.01, 124.38, 122.57, 120.40, 110.56(×2), 59.29, 57.73, 55.43, 55.13, 53.47, 53.39, 52.55, 52.25, 40.99, 35.60,

33.21(×2), 29.30, 28.93, 28.28, 27.72, 27.25, 24.23, 24.12. HRMS calcd for C₃₅H₄₇F₆N₂O₂, [M + H]⁺, 641.3463; found 641.3525. HPLC: t_R = 16.772 min, 97.9%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)piperidin-4-amine-1-acetic acid (**33**): Intermediate **30** (0.5 g, 0.9 mmol) was dissolved in acetonitrile (10 mL), and methyl 2-bromoacetate (0.3 g, 1.8 mmol) and triethylamine (0.4 mL, 3.4 mmol) were added. The reaction mixture was stirred at room temperature for 16 h and then poured into H₂O (10 mL) and extracted with EtOAc (10 mL × 3). The combined organic layers were washed with H₂O (10 mL × 3) and brine (10 mL × 3), dried over Na₂SO₄, and concentrated in vacuo. The residue was dissolved in MeOH (5 mL) and 1 mol/L NaOH (5 mL) was added and the mixture was stirred at room temperature for 2 h. After concentration, the residue was dissolved in H₂O (10 mL). Then, 1 mol/L HCl (5 mL) was added to the mixture, the mixture was extracted with EtOAc (10 mL × 3), and the combined organic layers were washed with H₂O (10 mL × 3) and brine (10 mL × 3), dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by chromatography on silica gel (DCM:MeOH = 5:1) to give **33** (0.4 g, 67.8%) as a colourless oil. ¹H-NMR (400 MHz, DMSO-*d*₆) δ 8.02 (s, 2H), 7.95 (s, 1H), 7.09 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.89 (d, *J* = 8.5 Hz, 1H), 6.77 (d, *J* = 2.1 Hz, 1H), 3.65 (s, 3H), 3.63–3.51 (m, 2H), 3.20 (s, 2H), 3.14 (d, *J* = 10.4 Hz, 2H), 2.79 (dt, *J* = 13.7, 6.9 Hz, 1H), 2.70 (s, 2H), 2.62–2.51 (m, 3H), 2.26 (d, *J* = 17.7 Hz, 1H), 1.96 (s, 1H), 1.91–1.75 (m, 2H), 1.70–1.51 (m, 2H), 1.40 (s, 2H), 1.35 (t, *J* = 6.3 Hz, 2H), 1.16 (d, *J* = 6.8 Hz, 6H), 0.91 (d, *J* = 8.7 Hz, 6H). ¹³C-NMR (150 MHz, DMSO-*d*₆) δ: 168.60, 154.62(×2), 145.76, 140.43, 132.53, 131.21, 130.22(×2), 129.42, 128.93, 127.74, 125.54, 124.78, 122.97, 111.48(×2), 59.03, 55.81, 54.09, 52.70, 52.43, 52.37, 51.58, 41.02, 35.50, 32.94, 29.40, 29.00, 28.53, 27.95, 24.55(×2), 24.44(×2). HRMS calcd for C₃₅H₄₅F₆N₂O₃, [M + H]⁺, 655.3256; found 655.3345. HPLC: t_R = 19.973 min, 98.1%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)piperidin-4-amine-1-methanoic acid tetrahydro-2H-pyran-4-yl ester (**34**): Intermediate **30** (0.2 g, 0.3 mmol) was dissolved in CH₂Cl₂ (5 mL), and tetrahydro-2H-pyran-4-yl carbonochloridate (0.1 g, 0.5 mmol) and triethylamine (0.2 mL, 1.4 mmol) were added. The reaction mixture was stirred at room temperature for 30 min and then poured into H₂O (10 mL) and extracted with CH₂Cl₂ (10 mL × 3). The combined organic layers were washed with H₂O (10 mL × 3) and brine (10 mL × 3), dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether:EtOAc = 4:1) to give **34** (0.1 g, 45.9%) as a colourless oil. ¹H-NMR (600 MHz, CDCl₃) δ 7.82 (s, 2H), 7.73 (s, 1H), 7.08 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.79 (d, *J* = 8.4 Hz, 1H), 6.73 (d, *J* = 2.0 Hz, 1H), 4.86–4.80 (m, 1H), 4.13 (d, *J* = 7.1 Hz, 2H), 3.90–3.85 (m, 2H), 3.71 (s, 3H), 3.56–3.53 (m, 3H), 3.49–3.41 (m, 1H), 2.84–2.81 (m, 2H), 2.75–2.53 (m, 4H), 2.37 (d, *J* = 17.7 Hz, 1H), 2.02 (s, 1H), 1.93–1.90 (m, 3H), 1.87 (s, 1H), 1.65–1.63 (m, 3H), 1.55 (d, *J* = 12.5 Hz, 1H), 1.46–1.37 (m, 3H), 1.35–1.29 (m, 1H), 1.22 (dd, *J* = 6.9, 2.4 Hz, 6H), 0.96 (s, 6H). ¹³C-NMR (150 MHz, CDCl₃) δ: 154.47(×2), 144.35, 140.64, 131.57, 131.32, 131.10, 129.35, 128.18(×2), 125.09(×2), 124.35, 122.54, 120.51, 110.61(×2), 69.63, 65.30(×2), 55.44(×2), 55.35, 52.46, 52.23, 43.82, 43.66, 41.07, 35.57, 33.19, 32.08(×2), 29.24, 28.94, 28.33, 27.64, 24.24, 24.11(×2). HRMS calcd for C₃₉H₅₁F₆N₂O₄, [M + H]⁺, 725.3675; found 725.3751. HPLC: t_R = 15.553 min, 96.0%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((5'-isopropyl-2'-methoxy-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)azetid-3-amine-1-methanoic acid ethyl ester (**35**): Intermediate **31** (0.2 g, 0.3 mmol) was dissolved in CH₂Cl₂ (5 mL), and ethyl chloroformate (0.1 g, 0.7 mmol) and triethylamine (0.2 mL, 1.4 mmol) were added. The reaction mixture was stirred at room temperature for 30 min and then poured into H₂O (10 mL) and extracted with CH₂Cl₂ (10 mL × 3). The combined organic layers were washed with H₂O (10 mL × 3) and brine (10 mL × 3), dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether:EtOAc = 4:1) to give **35** (0.2 g, 89.2%) as a colourless oil. ¹H-NMR (400 MHz, CDCl₃) δ 7.78 (s, 2H), 7.76 (s, 1H), 7.09 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 1H), 6.74 (d, *J* = 2.1 Hz, 1H), 4.07 (q, *J* = 7.1 Hz, 2H), 3.88–3.81 (m, 1H), 3.80–3.72 (m, 3H), 3.71 (s, 3H), 3.68–3.56 (m, 2H), 3.55–3.47 (m, 1H), 2.90–2.77 (m, 2H), 2.77–2.65

(m, 1H), 2.43–2.29 (m, 1H), 2.10–2.04 (m, 1H), 2.01–1.82 (m, 2H), 1.45–1.35 (m, 2H), 1.24–1.19 (m, 9H), 0.96 (s, 6H). ^{13}C -NMR (150 MHz, CDCl_3) δ : 156.60($\times 2$), 154.30($\times 2$), 140.75, 131.29($\times 2$), 131.14, 128.37, 127.94($\times 2$), 125.39, 124.21, 122.41, 120.87, 110.55($\times 2$), 60.92, 55.32, 54.44, 53.43, 41.59, 35.49, 33.14($\times 2$), 29.38, 28.97, 28.22, 27.80, 24.18, 24.12($\times 2$), 14.60($\times 2$). HRMS calcd for $\text{C}_{34}\text{H}_{43}\text{F}_6\text{N}_2\text{O}_3$, $[\text{M} + \text{H}]^+$, 641.3100; found 641.3180. HPLC: $t_{\text{R}} = 11.763$ min, 96.7%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((5'-isopropyl-2'-methoxy-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)azetidino-3-amine-1-methanoic acid isopropyl ester (**36**): Colourless oil; yield 69.3%; ^1H -NMR (400 MHz, CDCl_3) δ 7.77 (s, 2H), 7.76 (s, 1H), 7.09 (dd, $J = 8.4, 2.2$ Hz, 1H), 6.80 (d, $J = 8.4$ Hz, 1H), 6.74 (d, $J = 2.2$ Hz, 1H), 4.84 (dt, $J = 12.5, 6.2$ Hz, 1H), 3.83 (t, $J = 8.4$ Hz, 1H), 3.77 (d, $J = 7.9$ Hz, 1H), 3.73 (d, $J = 5.4$ Hz, 2H), 3.70 (s, 3H), 3.67–3.56 (m, 2H), 3.55–3.47 (m, 1H), 2.89–2.77 (m, 2H), 2.76–2.64 (m, 1H), 2.40–2.28 (m, 1H), 2.09–2.04 (m, 1H), 2.00–1.83 (m, 2H), 1.44–1.35 (m, 2H), 1.22 (d, $J = 6.9$ Hz, 6H), 1.19 (d, $J = 6.2$ Hz, 6H), 0.96 (d, $J = 2.8$ Hz, 6H). ^{13}C -NMR (150 MHz, CDCl_3) δ : 156.38($\times 2$), 154.32($\times 2$), 140.72, 131.48, 131.26, 131.19, 128.34, 127.93($\times 2$), 125.35, 124.23, 122.43, 120.83, 110.53($\times 2$), 68.17, 55.31($\times 2$), 54.44, 53.43, 51.10, 41.56, 35.50, 33.14, 29.38, 28.96($\times 2$), 28.23, 27.79, 24.19, 24.13, 22.11($\times 2$). HRMS calcd for $\text{C}_{35}\text{H}_{45}\text{F}_6\text{N}_2\text{O}_3$, $[\text{M} + \text{H}]^+$, 655.3256; found 655.3344. HPLC: $t_{\text{R}} = 10.757$ min, 96.8%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((5'-isopropyl-2'-methoxy-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)azetidino-3-amine-1-methanoic acid tetrahydro-2H-pyran-4-ol ester (**37**): Colourless oil; yield 73.6%; ^1H NMR (600 MHz, CDCl_3) δ 7.78 (s, 2H), 7.76 (s, 1H), 7.09 (dd, $J = 8.4, 2.1$ Hz, 1H), 6.80 (d, $J = 8.4$ Hz, 1H), 6.75 (d, $J = 2.2$ Hz, 1H), 4.79 (tt, $J = 8.4, 4.0$ Hz, 1H), 3.89–3.83 (m, 3H), 3.82–3.78 (m, 1H), 3.75 (s, 2H), 3.71 (s, 3H), 3.62 (s, 2H), 3.55–3.49 (m, 3H), 2.90–2.77 (m, 2H), 2.73 (s, 1H), 2.39–2.30 (m, 1H), 2.09–2.02 (m, 1H), 2.00–1.94 (m, 1H), 1.92–1.85 (m, 3H), 1.64–1.59 (m, 2H), 1.45–1.34 (m, 2H), 1.22 (d, $J = 6.9$ Hz, 6H), 0.97 (d, $J = 4.2$ Hz, 6H). ^{13}C -NMR (150 MHz, CDCl_3) δ : 155.77($\times 2$), 154.30, 140.76, 131.53, 131.31, 131.14, 128.36, 127.90($\times 3$), 125.40, 124.20, 122.40, 120.92, 110.57($\times 2$), 69.44, 65.26($\times 2$), 55.33($\times 2$), 54.49, 53.46, 41.59, 35.48, 33.14($\times 2$), 32.07($\times 2$), 29.40, 28.98($\times 2$), 28.23, 27.82, 24.20, 24.13. HRMS calcd for $\text{C}_{37}\text{H}_{47}\text{F}_6\text{N}_2\text{O}_4$, $[\text{M} + \text{H}]^+$, 697.3362; found 697.3459. HPLC: $t_{\text{R}} = 16.003$ min, 95.8%.

N-(3,5-Bis(trifluoromethyl)benzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)-2-methyl-2H-tetrazol-5-amine (**40**): The title compound was obtained in a manner similar to that described for the preparation of intermediate **28**. Colourless oil; yield 70.5%; ^1H -NMR (600 MHz, $\text{DMSO}-d_6$) δ 7.92 (s, 1H), 7.64 (s, 2H), 6.98 (dd, $J = 8.4, 2.2$ Hz, 1H), 6.77–6.73 (m, 2H), 4.50 (s, 2H), 4.10 (s, 3H), 3.99–3.93 (m, 2H), 3.60 (s, 3H), 2.65 (dt, $J = 13.7, 6.9$ Hz, 1H), 2.32 (d, $J = 18.0$ Hz, 1H), 2.01 (d, $J = 18.0$ Hz, 1H), 1.79–1.72 (m, 2H), 1.35 (t, $J = 6.4$ Hz, 2H), 1.02 (d, $J = 6.9$ Hz, 6H), 0.89 (d, $J = 13.2$ Hz, 6H). ^{13}C -NMR (150 MHz, CDCl_3) δ : 169.46, 154.12, 141.34, 140.83, 135.26, 131.26($\times 2$), 130.54, 128.05, 127.70, 127.65, 125.61, 124.16, 122.35, 120.60, 110.59($\times 2$), 55.21, 51.59, 49.22, 40.56, 39.20, 35.44, 33.02, 29.09, 29.02, 28.08, 27.99, 23.98($\times 2$). HRMS calcd for $\text{C}_{30}\text{H}_{36}\text{F}_6\text{N}_5\text{O}$, $[\text{M} + \text{H}]^+$, 596.2746; found 596.2847. HPLC: $t_{\text{R}} = 8.776$ min, 96.1%.

N-((2-(5-Isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)methyl)-2-methyl-*N*-(3-(trifluoromethyl)benzyl)-2H-tetrazol-5-amine (**41**): Colourless oil; yield 78.4%; ^1H -NMR (600 MHz, $\text{DMSO}-d_6$) δ 7.52 (d, $J = 7.7$ Hz, 1H), 7.41 (t, $J = 7.7$ Hz, 1H), 7.34 (s, 1H), 7.22 (d, $J = 7.8$ Hz, 1H), 7.02 (dd, $J = 8.5, 2.3$ Hz, 1H), 6.80 (d, $J = 8.5$ Hz, 1H), 6.77 (d, $J = 2.3$ Hz, 1H), 4.41 (s, 2H), 4.10 (s, 3H), 3.92 (q, $J = 14.7$ Hz, 2H), 3.63 (s, 3H), 2.68 (dt, $J = 13.8, 6.9$ Hz, 1H), 2.32 (d, $J = 18.0$ Hz, 1H), 2.04 (d, $J = 18.1$ Hz, 1H), 1.76 (s, 2H), 1.38 (t, $J = 6.5$ Hz, 2H), 1.05 (dd, $J = 6.9, 0.8$ Hz, 6H), 0.91 (d, $J = 5.1$ Hz, 6H). ^{13}C -NMR (150 MHz, CDCl_3) δ : 169.66, 154.26, 140.64, 139.40, 134.35, 130.80, 130.73, 128.32, 128.03, 125.36, 124.31, 124.29, 123.43, 123.40, 110.45($\times 2$), 55.17, 51.16, 49.31, 40.42, 39.15, 35.54, 33.05, 29.19, 29.03, 28.20, 27.98, 24.09, 24.01. HRMS calcd for $\text{C}_{29}\text{H}_{37}\text{F}_3\text{N}_5\text{O}$, $[\text{M} + \text{H}]^+$, 528.2872; found 528.2960. HPLC: $t_{\text{R}} = 8.335$ min, 96.2%.

N-(4-Fluorobenzyl)-*N*-((2-(5-isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)methyl)-2-methyl-2H-tetrazol-5-amine (**42**): Colourless oil; yield 80.1%; ^1H -NMR (600 MHz, $\text{DMSO}-d_6$) δ 7.07–7.05 (m, 1H),

6.97–6.93 (m, 4H), 6.86–6.85 (m, 1H), 6.80 (d, $J = 2.3$ Hz, 1H), 4.30 (q, $J = 15.8$ Hz, 2H), 4.10 (s, 3H), 3.85 (dd, $J = 44.8, 14.8$ Hz, 2H), 3.66 (s, 3H), 2.72 (dt, $J = 13.8, 6.9$ Hz, 1H), 2.32 (d, $J = 18.0$ Hz, 1H), 2.08 (d, $J = 18.0$ Hz, 1H), 1.76 (s, 2H), 1.40 (t, $J = 6.5$ Hz, 2H), 1.09 (dd, $J = 6.9, 0.6$ Hz, 6H), 0.93 (s, 6H). ^{13}C -NMR (150 MHz, CDCl_3) δ : 169.67, 154.42, 140.66, 133.58, 130.93, 129.47, 129.41, 128.30, 128.14, 127.90, 125.25, 114.72, 114.58, 110.44($\times 2$), 55.23, 50.53, 48.79, 40.35, 39.12, 35.64, 33.12, 29.28, 29.04, 28.25, 28.01, 24.20, 24.06. HRMS calcd for $\text{C}_{28}\text{H}_{37}\text{FN}_5\text{O}$, $[\text{M} + \text{H}]^+$, 478.2904; found 478.2999. HPLC: $t_{\text{R}} = 7.750$ min, 96.7%.

N-((2-(5-Isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)methyl)-2-methyl-*N*-(4-(trifluoromethyl)benzyl)-2H-tetrazol-5-amine (**43**): Colourless oil; yield 67.5%; ^1H -NMR (600 MHz, CDCl_3) δ 7.37 (d, $J = 8.1$ Hz, 2H), 7.10 (d, $J = 8.0$ Hz, 2H), 7.02 (dd, $J = 8.4, 2.3$ Hz, 1H), 6.76 (d, $J = 2.3$ Hz, 1H), 6.70 (d, $J = 8.4$ Hz, 1H), 4.45 (s, 2H), 4.11 (s, 3H), 4.02 (s, 2H), 3.65 (s, 3H), 2.73 (dt, $J = 13.8, 6.9$ Hz, 1H), 2.44 (d, $J = 18.2$ Hz, 1H), 2.12 (d, $J = 18.1$ Hz, 1H), 1.84 (d, $J = 1.6$ Hz, 2H), 1.48–1.42 (m, 2H), 1.14 (dd, $J = 6.9, 1.8$ Hz, 6H), 0.96 (s, 6H). ^{13}C -NMR (150 MHz, CDCl_3) δ : 169.62, 154.32, 142.35, 140.67, 134.24, 130.74, 128.03, 127.98, 127.70($\times 3$), 125.30, 124.87, 124.85, 110.43($\times 2$), 55.20, 50.82, 49.03, 40.41, 39.16, 35.60, 33.06, 29.22, 29.05, 28.30, 27.93, 24.15, 23.98. HRMS calcd for $\text{C}_{29}\text{H}_{37}\text{F}_3\text{N}_5\text{O}$, $[\text{M} + \text{H}]^+$, 528.2872; found 528.2953. HPLC: $t_{\text{R}} = 8.985$ min, 96.7%.

N-((2-(5-Isopropyl-2-methoxyphenyl)-5,5-dimethylcyclohex-1-enyl)methyl)-2-methyl-*N*-(4-(trifluoro-methoxy)benzyl)-2H-tetrazol-5-amine (**44**): Colourless oil; yield 66.3%; ^1H -NMR (600 MHz, $\text{DMSO-}d_6$) δ 7.14 (d, $J = 8.1$ Hz, 2H), 7.06–7.01 (m, 3H), 6.82 (d, $J = 8.5$ Hz, 1H), 6.78 (d, $J = 2.2$ Hz, 1H), 4.35 (s, 2H), 4.11 (s, 3H), 3.88 (m, 2H), 3.66 (s, 3H), 2.70 (dt, $J = 13.8, 6.9$ Hz, 1H), 2.33 (d, $J = 18.4$ Hz, 1H), 2.06 (d, $J = 18.0$ Hz, 1H), 1.76 (s, 2H), 1.39 (t, $J = 6.4$ Hz, 2H), 1.06 (dd, $J = 6.9, 1.0$ Hz, 6H), 0.92 (d, $J = 1.6$ Hz, 6H). ^{13}C -NMR (150 MHz, CDCl_3) δ : 169.66, 154.37, 140.66, 136.94, 133.96, 130.81, 129.00($\times 3$), 128.15, 128.11, 125.29, 120.46($\times 2$), 110.43($\times 2$), 55.19, 50.66, 48.76, 40.39, 39.13, 35.61, 33.10, 29.21, 29.03, 28.25, 27.97, 24.14, 24.01. HRMS calcd for $\text{C}_{29}\text{H}_{37}\text{F}_3\text{N}_5\text{O}_2$, $[\text{M} + \text{H}]^+$, 544.2821; found 544.2898. HPLC: $t_{\text{R}} = 8.302$ min, 97.3%.

3.2. In Vitro Test for CETP Inhibitory Activity

All tested compounds were dissolved in 100% DMSO, making sure the compound was dissolved totally. The solution was vibrated hard on an oscillator for more than 30 s and then stored in a nitrogen cabinet. The stock solutions (10 mM) were diluted with DMSO for an 8-point titration (1:5 serial dilutions) in a 96-well dilution plate. The activity was estimated in accordance with the instruction for the CETP inhibitor screening kit and recombinant CETP. Compounds were tested at eight concentrations, and the fluorescence intensity were measured using a fluorometer (ExEm = 465/535 nm). The IC_{50} was determined from a curve fit of the data with each concentration tested three times.

3.3. Cytochrome P450 Inhibition Assay

Five specific probe substrates (CYP3A4, 2 μM midazolam; CYP2D6, 5 μM dextromethorphan; CYP2C9, 5 μM diclofenac; CYP1A2, 10 μM phenacetin; CYP2C19, 30 μM S-mephenytoin) were used to evaluate cytochrome P450 inhibition in human liver microsomes (0.25 mg/mL). A mixture of 20 μL specific probe substrate solution and 20 μL buffer solution (100 mM K_3PO_4 , 33 mM MgCl_2 , pH = 7.4) was added compound (2 μL) and 158 μL human liver microsomes solution (0.25 mg/mL). The mixture were incubated at 37 $^\circ\text{C}$ for 10 min and another 10 min underwent after 20 μL NADPH solution added. Consecutively, 400 μL cold stop solution (200 ng/mL tolbutamide and 200 ng/mL labetalol in acetonitrile) was added to terminate the reaction. After the reactions were terminated, The plates were centrifuged (4000 rpm) at room temperature for 20 min, and the supernatants were analysed by LC/MS/MS.

3.4. Metabolic Stability Study

Ten μL (100 $\mu\text{M}/\text{L}$) of compound and 80 μL liver microsomes were mixture and incubated at 37 °C for 10 min, and then 10 μL NADPH regenerating system was added. Samples were obtained at 0 min, 5 min, 10 min, 20 min, 30 min and 60 min respectively, and 300 μL stop solution (cold in 4 °C, including 100 ng/mL tolbutamide and 100 ng/mL labetalol) was added to terminate the reaction. After oscillating for 10 min, the plates were centrifuged (4000 rpm) at room temperature for 20 min, and the supernatants were used for analyzation.

4. Conclusions

A series of novel *N,N*-substituted amine derivatives were designed by utilizing bioisosterism. New compounds were synthesized and evaluated for their inhibitory activity against CETP by a BODIPY-CE fluorescence assay. Compound 17 was identified as a promising CETP inhibitor with good inhibitory activity ($\text{IC}_{50} = 0.38 \pm 0.08 \mu\text{M}$), and compound 17 demonstrated weak human/rat liver microsomes stability and low CYP inhibition.

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Sample Availability: Samples of the compounds **12–22**, **32–37**, **40–44** are available from the authors.



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