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RESEARCH ARTICLE

Design, synthesis, and biological evaluation of pyrimidine-2-carboxamide analogs: investigation for novel RAGE inhibitors with reduced hydrophobicity and toxicity

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Abstract This paper describes an investigation of novel RAGE inhibitors with improved drug-like properties. To identify the improved drug-like RAGE inhibitor, we designed and synthesized pyrimidine-2-carboxamide analogs based on our previous work. Several potent analogs with improved hydrophilicity were identified by evaluation of RAGE inhibitory activity. In particular, one of the potent (diethylamino)ethoxymethoxy analogs did not exhibit undesired cytotoxicity in contrast with the parent RAGE inhibitors.

Keywords RAGE · RAGE inhibitor · Pyrimidine-2-carboxamide · Drug-like property

Introduction

Receptor for advanced glycation end product (RAGE) is a multi-ligand binding transmembrane receptor of the immunoglobulin superfamily. In chronic inflammatory disease conditions, such as diabetes, atherosclerosis and Alzheimer's disease (AD), RAGE interacts with various pathogenic ligands and activates nuclear factor-κB (NF-κB), a transcription factor that plays a crucial role in various inflammatory responses (Bierhaus et al. 2005). Usually, RAGE is over-expressed in chronic inflammatory diseases through positive feedback loops (Li and Schmidt 1997). Therefore, RAGE has been regarded as a therapeutic target for chronic inflammatory diseases (Alexiou et al. 2010; Barlovic et al. 2011). In the brain of AD patients, RAGE interacts with cytotoxic amyloid-β (Aβ), a major component of pathological plaques. It is a prime suspect of AD, and subsequently induces the inflammatory events. In addition, RAGE at the blood–brain barrier (BBB) is predominantly responsible for the plasma-derived Aβ influx into the brain (Schmidt et al. 2009). In this context, inhibition of the RAGE-Aβ interaction can reduce the accumulation rate of Aβ into the brain, neurotoxic inflammation, and consequent neuronal cell death. For this reason, RAGE inhibitor has been regarded as one of promising "disease-modifying agents" for AD treatment (Deane et al. 2012).

Recently, we worked on the development of small-molecule RAGE inhibitors, and reported a novel series of RAGE inhibitors. A series of 2-aminopyrimidines including bis(4-chlorophenyl)pyrimidine **1**, which exhibited significant therapeutic effects in an AD model study, as well as potent in vitro RAGE inhibitory activity (IC_{50} = 16.5 μM), were designed on the structural basis of one of the monomeric AGEs (Han et al. 2012). Based on this previous study, we identified a series of pyrazole-5-carboxamide analogs through heterocycle modification and a subsequent structure–activity relationship (SAR) study. Moreover, pyrazole-5-carboxamide **2** exhibited more potent RAGE inhibitory activity (IC_{50} = 1.9 μM), with improved aqueous solubility, than 2-aminopyrimidine **1** (Han et al. 2014). Although such significant anti-AD activities were observed, the hit compounds **1** and **2** seemed to have considerable drawbacks as a drug candidate, such as unsatisfactory pharmacokinetic profiles and undesired cytotoxicity (cell survival: 37.3 and 10.3 %, respectively, HT22 cells). We supposed that their excessive hydrophobicities (ClogP = 9.21 and 7.95, respectively. Calculated by ChemBioDraw Ultra 12.0) were strongly related to the problems by inducing poor aqueous

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solubility and immoderate non-specific protein binding (Kerns and Di 2008). For these reasons, we turned our attention to more hydrophilic analogs to identify novel RAGE inhibitors with improved drug-like properties.

To design the more hydrophilic analogs, we planned to make use of our previous studies on 2-aminopyrimidines and pyrazole-5-carboxamides, including information from SAR analysis and molecular docking studies (Han et al. 2012, 2014). As shown in Fig. 1, we designed pyrimidine-2-carboxamide analogs **3** by replacing the chloride and 2-amino moieties of the 2-aminopyrimidine **1** with the more polar fluoride and carboxamide moieties of the **2**, respectively. By considering a molecular docking study of the more active analog **2**, we anticipated that these two moieties would provide an improvement in RAGE inhibitory activities as well as hydrophilic properties. We introduced a polar methoxy moiety to the pyrimidine-2-carboxamide analogs, with the expectation of additional interaction with RAGE and increasing hydrophilicity. In addition, the positional effect of the (diethylamino)ethoxy side chain and methoxy moiety was intensively examined based on the previous observation that RAGE inhibitory activities of the 2-aminopyrimidine analogs were strongly influenced by the position of the alkoxy side chain. Herein, we report design, synthesis and biological evaluation of the pyrimidine-2-carboxamide analogs, an exploration for novel RAGE inhibitors with improved drug-like properties.

Material and method

General procedure

Unless noted otherwise, all starting materials and reagents were obtained commercially and were used without further purification. Tetrahydrofuran was distilled from sodium benzophenone ketyl. Dichloromethane was freshly distilled from calcium hydride. All solvents used for routine product isolation and chromatography were of reagent grade and glass distilled. Reaction flasks were dried at 100 °C before use, and air and moisture sensitive reactions were performed under argon. Flash column chromatography was performed using silica gel 60 (230–400 mesh, Merck) with the indicated solvents. Thin-layer chromatography was performed using 0.25 mm silica gel plates (Merck). Mass spectra were obtained using a VG Trio-2 GC–MS instrument, and high resolution mass spectra were obtained using a JEOL JMS-AX 505WA unit. ^{1}H and ^{13}C spectra were recorded on a JEOL JNM-LA 300, Brucker Analytik ADVANCE digital 400, ADVANCE digital 500 or JEOL ECA-600 spectrometer in deuteriochloroform ($CDCl_3$), deuterioacetone (aetone-d_6), deuteriodimethylsulfoxide (DMSO-d_6) or deuteriomethanol (CD_3OD). Chemical shifts are expressed in parts per million (ppm, δ) downfield from tetramethylsilane and are referenced to the deuterated solvent. ^{1}H-NMR data are reported in the order; chemical shift, multiplicity (s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet, and/or multiple resonance, numbers of protons, and coupling constants in hertz (Hz).

General procedure A: synthesis of the carboxamide analogs

To a solution of carboxylic acid (1 equiv) in CH_2Cl_2 were added catalytic amount of DMF and oxalyl chloride (5 equiv) at 0 °C. After being stirred for 1 h at same temperature, the reaction mixture was concentrated in vacuo and diluted with THF. To the solution of the acid chloride were added corresponding aniline (1 equiv) and Et_3N (5 equiv), and stirred for 1 h at ambient temperature. The reaction mixture was

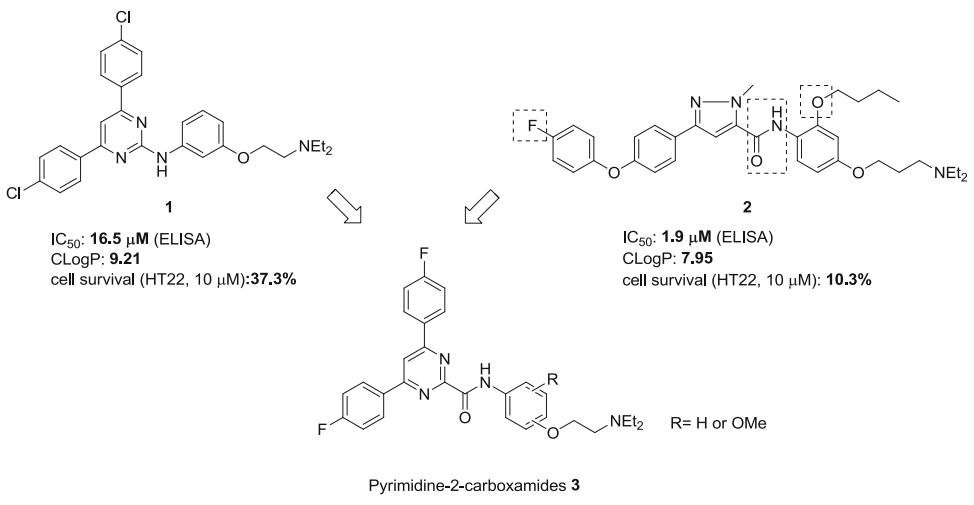


Fig. 1 Design strategy for pyrimidine-2-carboxamide analogs

quenched with H_2O, and diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (MeOH/CH_2Cl_2 = 1:10–20) afforded the titled analog.

General procedure B: Mitsunobu reaction of the phenols

To a solution of phenol (1 equiv), triphenylphosphine (1.3 equiv) and 2-(diethylamino)ethanol (1.3 equiv) in THF was added diisopropyl azodicarboxylate (DIAD, 1.3 equiv) at ambient temperature. After being stirred at ambient temperature until no starting material could be observed by TLC, the reaction mixture was quenched with H_2O and diluted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel afforded the titled compound.

General procedure C: reduction of nitrobenzenes

To a solution of nitrobenzene (1 equiv) in EtOH was added $SnCl_2{\cdot}2H_2O$ (5 equiv) at ambient temperature. The reaction mixture was refluxed for 3 h and concentrated in vacuo. The residue was diluted with EtOAc and saturated $NaHCO_3$ solution was added. The mixture was filtered using a Celite pad. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel afforded titled aniline as a brown oil.

*2-Chloro-4,6-bis(4-fluorophenyl)pyrimidine (**5**)*

To a solution of 2,4,6-trichloropyrimidine **4** (2.09 g, 11.4 mmol), 4-fluorophenylboronic acid (3.82 g, 27.3 mmol) and Na_2CO_3 (4.79 g, 57 mmol) in 50 % *aq.* THF (110 mL) were added $Pd(OAc)_2$ (128 mg, 0.57 mmol) and PPh_3 (598 mg, 2.28 mmol) at ambient temperature. After being stirred under reflux condition until no starting material could be observed by TLC, the reaction mixture was cooled to ambient temperature and diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-hexane/CH_2Cl_2 = 1:40:5) afforded 1.95 g (57 %) of the chloropyrimidine **5**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.17 − 8.12 (m, 4H), 7.91 (s, 1H), 7.20−7.18 (m, 4H); LRMS (FAB) *m/z* 303 ($M + H^+$).

*4,6-bis(4-fluorophenyl)pyrimidine-2-carbonitrile (**6**)*

To a solution of the 2-chloropyrimidine **5** (1.95 g, 6.45 mmol) and NaCN (464 mg, 9.68 mmol) in DMSO (45 mL) was added 1,4-diazabicyclo[2.2.2]octane (DABCO, 446 mg, 9.68 mmol) at ambient temperature. After being stirred for 5 h at same temperature, the reaction mixture was diluted with EtOAc and water. The organic phase was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-hexane = 1:3) afforded 1.67 g (88 %) of the carbonitrile **6**: ^{1}H-NMR (300 MHz, CD_3OD) δ 8.61 (s, 1H), 8.40−8.37 (m, 4H), 7.34−7.30 (m, 4H); LRMS (FAB) *m/z* 294 ($M + H^+$).

*4,6-bis(4-fluorophenyl)pyrimidine-2-carboxylic acid (**7**)*

To a solution of the pyrimidine-2-carbonitrile **6** (1.67 g, 5.69 mmol) in 50 % *aq*. EtOH (80 mL) was added KOH (3.19 g, 56.9 mmol) at ambient temperature. After being stirred under reflux condition for 8 h, the reaction mixture was concentrated in vacuo and diluted with EtOAc. The organic phase was acidified with 1 N HCl, and washed with water and brine. The organic phase was dried over $MgSO_4$, and concentrated in vacuo. The crude pyrimidine-2-carboxylic acid **7** (1.56 g, 88 %) was used for further reaction without purification: ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.75 (s, 1H), 8.52−8.49 (m, 4H), 7.47−7.43 (m, 4H); LRMS (FAB) *m/z* 313 ($M + H^+$).

*N-(2-(2-(Diethylamino)ethoxy)phenyl)-4,6-bis(4-fluorophenyl)pyrimidine-2-carboxamide (**9a**)*

The acid **7** (17 mg, 0.054 mmol) afforded the carboxamide **9a** (6.8 mg, 25 %) via general procedure **A**: ^{1}H-NMR (400 MHz, CD_3OD) δ 8.46 (d, 1H, J = 7.8 Hz), 8.38−8.35 (m, 5H), 7.25 (t, 4H, J = 8.6 Hz), 7.10 (t, 1H, J = 7.7 Hz), 7.02 (d, 1H, J = 8.0 Hz), 6.96 (t, 1H, J = 7.6 Hz), 4.19 (t, 2H, J = 6.3 Hz), 2.98 (t, 2H, J = 6.3 Hz), 2.58 (q, 4H, J = 7.1 Hz), 0.95 (t, 6H, J = 7.1 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.7, 164.5, 164.5, 163.4, 159.8, 158.4, 147.7, 132.4, 132.3, 129.7, 129.7, 129.6, 129.6, 127.6, 124.3, 121.5, 119.9, 116.3, 116.3, 116.1, 116.1, 112.7, 111.2, 67.5, 52.2, 48.1, 48.1, 12.1, 12.1; LRMS (FAB) *m/z* 503 ($M + H^+$); HRMS (FAB) calcd for $C_{29}H_{29}F_2N_4O_2$ ($M + H^+$): 503.2259; found 503.2266.

*N-(3-(2-(Diethylamino)ethoxy)phenyl)-4,6-bis(4-fluorophenyl)pyrimidine-2-carboxamide (**9b**)*

The acid **7** (15 mg, 0.047 mmol) afforded the carboxamide **9b** (12 mg, 51 %) via general procedure **A**: ^{1}H-NMR (300 MHz, CD_3OD) δ 8.37−8.33 (m, 4H), 8.31 (s, 1H), 7.53 (t, 1H, J = 2.1 Hz), 7.33 (m, 1H), 7.26−7.16 (m, 5H), 6.72 (ddd, J = 1.1, 2.6, 8.3 Hz), 4.09 (t, 2H, J = 5.7 Hz), 2.92 (t, 2H, J = 5.7 Hz), 2.70 (q, 4H, J = 7.2 Hz), 1.11 (t,

6H, $J = 7.2$ Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.7, 164.5, 164.5, 163.3, 159.9, 159.5, 157.9, 138.6, 132.1, 132.1, 129.8, 129.7, 129.7, 129.6, 129.6, 116.4, 116.4, 116.1, 116.1, 113.0, 112.0, 111.3, 106.0, 66.4, 51.6, 47.8, 47.8, 11.8, 11.8; LRMS (FAB) *m/z* 503 ($M + H^+$); HRMS (FAB) calcd for $C_{29}H_{29}F_2N_4O_2$ ($M + H^+$): 503.2259; found 503.2266.

*N-(4-(2-(Diethylamino)ethoxy)phenyl)-4,6-bis(4-fluorophenyl)pyrimidine-2-carboxamide (**9c**)*

The acid **7** (10 mg, 0.032 mmol) afforded the carboxamide **9c** (8 mg, 47 %) via general procedure **A**: ^{1}H-NMR (300 MHz, CD_3OD) δ 8.43−8.38 (m, 4H), 8.37 (s, 1H), 7.72 (d, 2H, $J = 9.2$ Hz), 7.26−7.20 (m, 4H), 6.93 (d, 2H, $J = 9.2$ Hz), 4.09 (t, 2H, $J = 5.7$ Hz), 2.94 (q, 4H, $J = 7.1$ Hz), 2.72 (q, 4H, $J = 7.1$ Hz), 1.12 (t, 6H, $J = 7.1$ Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 166.3, 164.5, 164.5, 163.8, 159.7, 158.1, 155.9, 132.2, 132.2, 130.7, 129.7, 129.7, 129.6, 129.6, 121.3, 121.3, 116.4, 116.4, 116.2., 116.2, 115.0, 115.0, 112.9, 66.6, 51.7, 47.8, 47.8, 11.7, 11.7; LRMS (FAB) *m/z* 503 ($M + H^+$); HRMS (FAB) calcd for $C_{29}H_{29}F_2N_4O_2$ ($M + H^+$): 503.2259; found 503.2266.

*N,N-Diethyl-2-(3-methoxy-2-nitrophenoxy)ethanamine (**11a**)*

Nitrophenol **10a** (254 mg, 1.50 mmol) afforded the ethanamine **11a** (262 mg, 65 %) via general procedure **B**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.28 (t, 1H, $J = 8.5$ Hz), 6.61 (d, 1H, $J = 3.5$ Hz), 6.58 (d, 1H, $J = 3.3$ Hz), 4.09 (t, 2H, $J = 6.2$ Hz), 3.85 (s, 3H), 2.83 (t, 2H, $J = 6.3$ Hz), 2.57 (q, 4H, $J = 7.1$ Hz), 1.01 (t, 6H, $J = 7.1$ Hz); LRMS (FAB) *m/z* 269 ($M + H^+$).

*N,N-Diethyl-2-(4-methoxy-2-nitrophenoxy)ethanamine (**11b**)*

Nitrophenol **10b** (254 mg, 1.50 mmol) afforded the ethanamine **11b** (298 mg, 74 %) via general procedure **B**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.35 (d, 1 h, $J = 2.8$ Hz), 7.09−7.00 (m, 2H), 4.10 (t, 2H, $J = 6.1$ Hz), 2.88 (t, 2H, $J = 6.1$ Hz), 2.61 (q, 4H, $J = 7.1$ Hz), 1.04 (t, 6H, $J = 7.1$ Hz); LRMS (FAB) *m/z* 269 ($M + H^+$).

*N,N-Diethyl-2-(5-methoxy-2-nitrophenoxy)ethanamine (**11c**)*

Nitrophenol **10c** (255 mg, 1.50 mmol) afforded the ethanamine **11c** (310 mg, 77 %) via general procedure **B**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.95 (d, 1H, $J = 9.2$ Hz), 6.52−6.45 (m, 2H), 4.11 (t, 2H, $J = 6.2$ Hz), 3.85 (s, 3H), 2.92 (t, 2H, $J = 6.1$ Hz), 2.63 (q, 4H, $J = 7.1$ Hz), 1.05 (t, 6H, $J = 7.1$ Hz); LRMS (FAB) *m/z* 269 ($M + H^+$).

*N,N-Diethyl-2-(2-methoxy-6-nitrophenoxy)ethanamine (**11d**)*

Nitrophenol **10d** (254 mg, 1.50 mmol) afforded the ethanamine **11d** (282 mg, 70 %) via general procedure **B**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.86 (dd, 1H, $J = 2.8$, 9.0 Hz), 7.70 (d, 1H, $J = 2.6$ Hz), 6.89 (d, 1H, $J = 8.8$ Hz), 4.14 (t, 2H, $J = 6.6$ Hz), 3.90 (s, 3H), 2.92 (t, 2H, $J = 6.6$ Hz), 2.61 (q, 4H, $J = 7.1$ Hz), 1.04 (t, 6H, $J = 7.1$ Hz); LRMS (FAB) *m/z* 269 ($M + H^+$).

*2-(2-(Diethylamino)ethoxy)-6-methoxyaniline (**12a**)*

Ethanamine **11a** (262 mg, 0.98 mmol) afforded the aniline **12a** (189, 87 %) via general procedure **C**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.66−6.61 (m, 1H), 6.53−6.52 (m, 1H), 6.50−6.49 (m, 1H), 4.07 (t, 2H, $J = 6.1$ Hz), 3.83 (s, 3H), 2.89 (t, 2H, $J = 6.1$ Hz), 2.65 (q, 4H, $J = 7.1$ Hz), 1.07 (t, 6H, $J = 7.1$ Hz); LRMS (FAB) *m/z* 239 ($M + H^+$).

*2-(2-(Diethylamino)ethoxy)-5-methoxyaniline (**12b**)*

Ethanamine **11b** (298 mg, 1.11 mmol) afforded the aniline **12b** (172 mg, 65 %) via general procedure **C**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.71 (d, 1H, $J = 8.6$ Hz), 6.29 (d, 1H, $J = 2.9$ Hz), 6.19 (dd, 1H, $J = 2.9$, 8.6 Hz), 3.97 (t, 2H, $J = 6.0$ Hz), 3.70 (s, 3H), 2.81 (t, 2H, $J = 6.0$ Hz), 2.61 (q, 4H, $J = 7.1$ Hz), 1.04 (t, 6H, $J = 7.1$ Hz); LRMS (FAB) *m/z* 239 ($M + H^+$).

*2-(2-(Diethylamino)ethoxy)-4-methoxyaniline (**12c**)*

Ethanamine **11c** (310 mg, 1.16 mmol) afforded the aniline **12c** (171 mg, 62 %) via general procedure **C**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.63 (d, 1H, J = 8.4 Hz), 6.44 (d, 1H, J = 2.6 Hz), 6.34 (dd, 1H, J = 2.7, 8.4 Hz), 4.03 (t, 2H, J = 6.4 Hz), 3.72 (s, 3H), 2.88 (t, 2H, J = 6.4 Hz), 2.63 (q, 4H, J = 7.1 Hz), 1.06 (t, 6H, J = 7.1 Hz); LRMS (FAB) *m/z* 239 ($M + H^+$).

*2-(2-(Diethylamino)ethoxy)-3-methoxyaniline (**12d**)*

Ethanamine **11d** (282 mg, 1.05 mmol) afforded the aniline **12d** (225 mg, 90 %) via general procedure **C**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.72 (d, 1H, $J = 8.2$ Hz), 6.27 (d, 1H, $J = 2.6$ Hz), 6.18 (dd, 1H, $J = 2.6$, 8.3 Hz), 4.00 (t, 2H, $J = 6.8$ Hz), 3.78 (s, 3H), 2.87 (t, 2H, $J = 6.8$ Hz), 2.62 (q, 4H, $J = 7.1$ Hz), 1.04 (t, 6H, $J = 7.1$ Hz); LRMS (FAB) *m/z* 239 ($M + H^+$).

*N,N-Diethyl-2-(2-methoxy-3-nitrophenoxy)ethanamine (**14a**)*

To a solution of NaH (105 mg, 2.63 mmol) in DMF (5 mL) was added 2-(diethylamino)ethanol (0.35 mL, 2.63 mmol) at 0 °C for 10 min, and the reaction mixture was stirred at same temperature for 30 min. To the reaction mixture was added solution of 1-fluoro-2-methoxy-3-nitrobenzene **13a** (317 mg, 1.75 mmol) in DMF (2 mL) at ambient temperature. After being stirred at ambient temperature until no starting material could be observed by TLC, the reaction mixture was quenched with H_2O and diluted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:50) afforded the ethanamine **14a** (89 mg, 19 %). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.31−7.28 (m, 1H), 7.09−7.07 (m, 2H), 4.21 (t, 2H, J = 6.4 Hz), 3.88 (s, 3H), 2.88 (t, 2H, J = 6.6 Hz), 2.62 (q, 4H, J = 7.2 Hz), 1.04 (t, 6H, J = 7.1 Hz); LRMS (FAB) *m/z* 269 ($M + H^+$).

*N,N-Diethyl-2-(4-methoxy-3-nitrophenoxy)ethanamine (**14b**)*

Nitrophenol **13c** (257 mg, 1.52 mmol) afforded the ethanamine **14b** (314 mg, 77 %) via general procedure **B**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.40 (d, 1H, J = 3.1 Hz), 7.10 (dd, 1H, J = 3.1, 9.2 Hz), 6.99 (d, 1H, J = 9.2 Hz), 4.00 (t, 2H, J = 6.1 Hz), 3.90 (s, 3H), 2.84 (t, 2H, J = 6.0 Hz), 2.61 (q, 4H, J = 7.1 Hz), 1.04 (t, 6H, J = 7.1 Hz); LRMS (FAB) *m/z* 269 ($M + H^+$).

*N,N-Diethyl-2-(3-methoxy-5-nitrophenoxy)ethanamine (**14c**)*

Ethanamine **14c** was prepared by the procedure for **14a**, using **13b** (300 mg, 1.75 mmol) instead of **13a** (122 mg, 26 %). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.36−7.33 (m, 2H), 6.74 (t, 1H, J = 2.2 Hz), 4.07 (t, 2H, J = 6.0 Hz), 3.83 (s, 3H), 2.87 (t, 2H, J = 6.0 Hz), 2.63 (q, 4H, J = 7.1 Hz), 1.06 (t, 6H, J = 7.1 Hz); LRMS (FAB) *m/z* 269 ($M + H^+$).

*N,N-Diethyl-2-(2-methoxy-5-nitrophenoxy)ethanamine (**14d**)*

5-Nitroguaiacol **13d** (256 mg, 1.51 mmol) afforded the ethanamine **14d** (361 mg, 89 %) via general procedure **B**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.88 (dd, 1H, J = 2.6, 9.0 Hz), 7.77 (d, 1H, J = 2.6 Hz), 6.87 (d, 1H, J = 9.0 Hz), 4.13 (t, 2H, J = 6.5 Hz), 3.93 (s, 3H), 2.93 (t, 2H, J = 6.5 Hz), 2.63 (q, 4H, J = 7.1 Hz), 1.06 (t, 6H, J = 7.1 Hz); LRMS (FAB) *m/z* 269 ($M + H^+$).

*3-(2-(Diethylamino)ethoxy)-2-methoxyaniline (**15a**)*

Ethanamine **14a** (89 mg, 0.33 mmol) afforded the aniline **15a** (36 mg, 52 %) via general procedure **C**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.80 (t, 1H, J = 8.1 Hz), 6.34−6.31 (m, 1H), 6.28−6.25 (m, 1H), 3.99 (t, 2H, J = 5.3 Hz), 3.80 (s, 3H), 2.74−2.72 (m, 2H), 2.61 (q, 4H, J = 7.1 Hz), 1.04 (t, 6H, J = 7.1 Hz); LRMS (FAB) *m/z* 239 ($M + H^+$).

*5-(2-(Diethylamino)ethoxy)-2-methoxyaniline (**15b**)*

Ethanamine **14b** (314 mg, 1.17 mmol) afforded the aniline **15b** (184 mg, 66 %) via general procedure **C**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.66 (d, 1H, J = 8.6 Hz), 6.31 (d, 1H, J = 2.9 Hz), 6.22 (dd, 1H, J = 2.8, 8.6 Hz), 3.95 (t, 2H, J = 6.4 Hz), 3.78 (s, 3H), 2.82 (t, 2H, J = 6.4 Hz), 2.61 (q, 4H, J = 7.1 Hz), 1.04 (t, 6H, J = 7.1 Hz); LRMS (FAB) *m/z* 239 ($M + H^+$).

*3-(2-(Diethylamino)ethoxy)-5-methoxyaniline (**15c**)*

Ethanamine **14c** (122 mg, 0.45 mmol) afforded the aniline **15c** (73 mg, 78 %) via general procedure **C**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.91 (t, 1H, J = 2.1 Hz), 5.86−5.84 (m, 2H), 3.97 (t, 2H, J = 6.4 Hz), 3.71 (s, 3H), 3.62 (bs, 2H), 2.83 (t, 2H, J = 6.3 Hz), 2.61 (q, 4H, J = 7.1 Hz), 1.05 (t, 6H, J = 7.1 Hz); LRMS (FAB) *m/z* 239 ($M + H^+$).

*3-(2-(Diethylamino)ethoxy)-4-methoxyaniline (**15d**)*

Ethanamine **14d** (362 mg, 1.35 mmol) afforded the aniline **15d** (244 mg, 76 %) via general procedure **C**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.68 (d, 1H, J = 8.4 Hz), 6.31 (d, 1H, J = 2.6 Hz), 6.21 (dd, 1H, J = 2.6, 8.4 Hz), 4.02 (t, 2H, J = 6.9 Hz), 3.76 (s, 3H), 3.41 (bs, 2H), 2.90 (t, 2H, J = 6.9 Hz), 2.61 (q, 4H, J = 7.1 Hz), 1.04 (t, 6H, J = 7.1 Hz); LRMS (FAB) *m/z* 239 ($M + H^+$).

*N,N-Diethyl-2-(2-methoxy-4-nitrophenoxy)ethanamine (**17a**)*

4-Nitroguaiacol **16a** (257 mg, 1.52 mmol) afforded the ethanamine **17a** (285 mg, 70 %) via general procedure **B**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.88 (dd, 1H, J = 2.6, 8.8 Hz), 7.72 (d, 1H, J = 2.4 Hz), 6.91 (d, 1H, J = 8.8 Hz), 4.16 (t, 2H, J = 6.6 Hz), 3.92 (s, 3H), 2.93 (t, 2H, J = 6.6 Hz), 2.63 (q, 4H, J = 7.1 Hz), 1.05 (t, 6H, J = 7.1 Hz); LRMS (FAB) *m/z* 269 ($M + H^+$).

*N,N-Diethyl-2-(3-methoxy-4-nitrophenoxy)ethanamine (**17b**)*

Nitrophenol **16b** (257 mg, 1.52 mmol) afforded the ethanamine **17b** (330 mg, 81 %) via general procedure **B**: ^{1}H-

NMR (300 MHz, $CDCl_3$) δ 7.97 (d, 1H, $J = 9.0$ Hz), 6.54 (d, 1H, $J = 2.4$ Hz), 6.48 (dd, 1H, $J = 2.6$, 9.1 Hz), 4.08 (t, 2H, $J = 6.1$ Hz), 3.92 (s, 3H), 2.86 (t, 2H, $J = 6.2$ Hz), 2.62 (q, 4H, $J = 7.1$ Hz), 1.05 (t, 6H, $J = 7.1$ Hz); LRMS (FAB) *m/z* 269 ($M + H^+$).

*4-(2-(Diethylamino)ethoxy)-3-methoxyaniline (**18a**)*

Ethanamine **17a** (285 mg, 1.06 mmol) afforded the aniline **18a** (166 mg, 66 %) via general procedure **C**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.72 (d, 1H, $J = 8.4$ Hz), 6.27 (d, 1H, $J = 2.6$ Hz), 6.18 (dd, 1H, $J = 2.6$, 8.4 Hz), 3.99 (t, 2H, $J = 6.9$ Hz), 3,78 (s, 3H), 3.41 (bs, 2H), 2.86 (t, 2H, $J = 6.9$ Hz), 2.61 (q, 4H, $J = 7.1$ Hz), 1.04 (t, 6H, $J = 7.1$ Hz); LRMS (FAB) *m/z* 239 ($M + H^+$).

*4-(2-(Diethylamino)ethoxy)-2-methoxyaniline (**18b**)*

Ethanamine **17b** (330 mg, 1.23 mmol) afforded the aniline **18b** (240 mg, 73 %) via general procedure **C**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.61 (d, 1H, $J = 8.4$ Hz), 6.45 (d, 1H, $J = 2.6$ Hz), 6.33 (dd, 1H, $J = 2.6$, 8.4 Hz), 3.97 (t, 2H, $J = 6.5$ Hz), 3.80 (s, 3H), 2.83 (t, 2H, $J = 6.4$ Hz), 2.62 (q, 4H, $J = 7.1$ Hz), 1.05 (t, 6H, $J = 7.1$ Hz); LRMS (FAB) *m/z* 239 ($M + H^+$).

*N-(2-(2-(Diethylamino)ethoxy)-3-methoxyphenyl)-4,6-bis(4-fluorophenyl)pyramidine-2-carboxamide (**19**)*

The acid **7** (8 mg, 0.026 mmol) and aniline **12d** (6.2 mg, 0.026 mmol) afforded the carboxamide **19** (12 mg, 47 %) via general procedure **A**: ^{1}H-NMR (300 MHz, CD_3OD) δ 8.42−8.37 (m, 4H), 8.36 (s, 1H), 7.59 (d, 1H, $J = 2.4$ Hz), 7.31 (dd, 1H, $J = 2.4$, 8.6 Hz), 7.26−7.20 (m, 4H), 6.93 (d, 1H, $J = 8.8$ Hz), 4.11 (t, 2H, $J = 5.9$ Hz), 2.77 (q, 4H, $J = 7.2$ Hz), 1.13 (t, 6H, $J = 7.1$ Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.7, 164.6, 164.6, 163.4, 159.8, 158.1, 149.8, 145.4, 132.2, 132.2, 131.7, 129.7, 129.7, 129.6, 129.6, 116.4, 116.4, 116.2, 116.2, 113.7, 113.0, 111.7, 105.0, 67.6, 56.0, 51.7, 47.8, 47.8, 11.6, 11.6; LRMS (FAB) *m/z* 533 ($M + H^+$); HRMS (FAB) calcd for $C_{30}H_{31}F_2N_4O_3$ ($M + H^+$): 533.2364; found 533.2376.

*N-(2-(2-(Diethylamino)ethoxy)-4-methoxyphenyl)-4,6-bis(4-fluorophenyl)pyrimidine-2-carboxamide (**20**)*

The acid **7** (8 mg, 0.026 mmol) and aniline **12c** (6.2 mg, 0.026 mmol) afforded the carboxamide **20** (9 mg, 41 %) via general procedure **A**: ^{1}H-NMR (300 MHz, CD_3OD) δ 8.41−8.33 (m, 6H), 7.30−7.24 (m, 4H), 6.59 (d, 1H, $J = 2.6$ Hz), 6.52 (dd, 1H, $J = 2.6$, 8.8 Hz), 4.18 (t, 2H, $J = 6.2$ Hz), 3.78 (s, 3H), 3.01 (t, 2H, $J = 6.2$ Hz), 2.61 (q, 4H, $J = 7.1$ Hz), 0.97 (t, 6H, $J = 7.1$ Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.7, 164.4, 164.4, 163.3, 159.4, 158.6, 156.8, 148.9, 132.4, 132.4, 129.7, 129.7, 129.6, 129.6, 121.3, 120.6, 116.3, 116.3, 116.0, 116.0, 112.6, 104.4, 99.6, 67.4, 55.6, 52.1, 48.1, 48.1, 12.0, 12.0; LRMS (FAB) *m/z* 533 ($M + H^+$); HRMS (FAB) calcd for $C_{30}H_{31}F_2N_4O_3$ ($M + H^+$): 533.2364; found 533.2361.

*N-(2-(2-(Diethylamino)ethoxy)-5-methoxyphenyl)-4,6-bis(4-fluorophenyl)pyrimidine-2-carboxamide (**21**)*

The acid **7** (8 mg, 0.026 mmol) and aniline **12b** (6.2 mg, 0.026 mmol) afforded the carboxamide **21** (13 mg, 58 %) via general procedure **A**: ^{1}H-NMR (300 MHz, CD_3OD) δ 8.52 (s, 1H), 8.48−8.43 (m, 4H), 8.22 (d, 1H, $J = 2.9$ Hz), 7.36−7.30 (m, 4H), 7.02 (d, 1H, $J = 9.2$ Hz), 6.70 (dd, 1H, $J = 2.9$, 9.0 Hz), 4.20 (t, 2H, $J = 6.2$ Hz), 3.79 (s, 3H), 2.99 (t, 2H, $J = 6.3$ Hz), 2.58 (q, 4H, $J = 7.2$ Hz), 0.94 (t, 6H, $J = 7.1$ Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.7, 164.4, 164.4, 163.3, 159.9, 158.2, 154.1, 141.8, 132.2, 132.2, 129.7, 129.7, 129.6, 129.6, 128.2, 116.3, 116.3, 116.0, 116.0, 112.7, 112.3, 109.8, 105.5, 68.0, 55.8, 52.2, 48.0, 48.0, 12.0, 12.0; LRMS (FAB) *m/z* 533 ($M + H^+$); HRMS (FAB) calcd for $C_{30}H_{31}F_2N_4O_3$ ($M + H^+$): 533.2364; found 533.2376.

*N-(2-(2-(Diethylamino)ethoxy)-6-methoxyphenyl)-4,6-bis(4-fluorophenyl)pyrimidine-2-carboxamide (**22**)*

The acid **7** (8 mg, 0.026 mmol) and aniline **12a** (6.2 mg, 0.026 mmol) afforded the carboxamide **22** (16 mg, 66 %) via general procedure **A**: ^{1}H-NMR (300 MHz, CD_3OD) δ 8.58 (s, 1H), 8.54−8.50 (m, 4H), 7.36−7.28 (m, 5H), 6.82−6.78 (m, 2H), 4.27 (t, 2H, $J = 4.9$ Hz), 3.89 (s, 3H), 3.13−3.11 (m, 2H), 2.84−2.80 (m, 4H), 1.01 (t, 6H, $J = 7.2$ Hz); ^{13}C-NMR (acetone-d_6, 150 MHz) δ 166.9, 165.4, 165.4, 165.3, 161.6, 159.8, 157.4, 156.4, 134.0, 134.0, 131.4, 131.4, 131.4, 131.4, 128.9, 117.1, 117.1, 116.9, 116.9, 116.3, 114.1, 106.7, 105.8, 68.5, 56.7, 52.7, 48.7, 48.7, 12.4, 12.4; LRMS (FAB) *m/z* 533 ($M + H^+$); HRMS (FAB) calcd for $C_{30}H_{31}F_2N_4O_3$ ($M + H^+$): 533.2364; found 533.2371.

*N-(3-(2-(Diethylamino)ethoxy)-2-methoxyphenyl)-4,6-bis(4-fluorophenyl)pyrimidine-2-carboxamide (**23**)*

The acid **7** (8 mg, 0.026 mmol) and aniline **15a** (6.2 mg, 0.026 mmol) afforded the carboxamide **23** (15 mg, 76 %) via general procedure **A**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.87 (s, 1H), 8.31−8.25 (m, 5H), 8.10 (s, 1H), 7.25−7.21 (m, 4H), 7.11 (t, 1H, $J = 8.3$ Hz), 6.73 (d, 1H, $J = 8.0$ Hz), 4.19 (t, 2H, $J = 6.7$ Hz), 3.88 (s, 3H), 2.84 (t, 2H, $J = 6.6$ Hz), 2.44 (q, 4H, $J = 6.9$ Hz), 0.82 (t, 6H, $J = 7.1$ Hz); ^{13}C-NMR (acetone-d_6, 125 MHz) δ 167.2,

165.6, 165.6, 165.2, 161.4, 161.3, 153.6, 138.2, 134.0, 134.0, 133.8, 131.5, 131.5, 131.4, 131.4, 125.1, 117.3, 117.3, 117.1, 117.1, 114.5, 113.3, 109.5, 72.2, 56.7, 53.7, 48.1, 48.1, 12.0, 12.0; LRMS (FAB) *m/z* 533 ($M + H^+$); HRMS (FAB) calcd for $C_{30}H_{31}F_2N_4O_3$ ($M + H^+$): 533.2364; found 533.2367.

*N-(3-(2-(Diethylamino)ethoxy)-4-methoxyphenyl)-4,6-bis(4-fluorophenyl)pyrimidine-2-carboxamide (**24**)*

The acid **7** (8 mg, 0.026 mmol) and aniline **15b** (6.2 mg, 0.026 mmol) afforded the carboxamide **24** (17 mg, 79 %) via general procedure **A**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.91 (s, 1H), 8.25−8.19 (m, 4H), 8.08 (s, 1H), 7.65−7.64 (m, 1H), 7.25−7.19 (m, 5H), 6.86 (d, 1H, $J = 8.6$ Hz), 4.15 (t, 2H, $J = 6.7$ Hz), 3.84 (s, 3H), 2.97 (t, 2H, $J = 6.6$ Hz), 2.66 (q, 4H, $J = 7.1$ Hz), 1.08 (t, 6H, $J = 7.1$ Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.7, 164.5, 164.5, 163.3, 159.7, 158.0, 148.4, 146.4, 132.1, 132.1, 131.1, 129.7, 129.7, 129.6, 129.6, 116.4, 116.4, 116.1, 116.1, 112.9, 112.1, 111.9, 106.0, 67.0, 56.2, 51.4, 47.7, 47.7, 11.7, 11.7; LRMS (FAB) *m/z* 533 ($M + H^+$); HRMS (FAB) calcd for $C_{30}H_{31}F_2N_4O_3$ ($M + H^+$): 533.2364; found 533.2367.

*N-(3-(2-(Diethylamino)ethoxy)-5-methoxyphenyl)-4,6-bis(4-fluorophenyl)pyrimidine-2-carboxamide (**25**)*

The acid **7** (8 mg, 0.026 mmol) and aniline **15c** (6.2 mg, 0.026 mmol) afforded the carboxamide **25** (18 mg, 80 %) via general procedure **A**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.97 (s, 1H), 8.25−8.20 (m, 4H), 8.09 (s, 1H), 7.26−7.20 (m, 4H), 7.13−7.11 (m, 1H), 7.03−7.01 (m, 1H), 6.30 (t, 1H, $J = 2.0$ Hz), 4.08 (t, 2H, $J = 6.2$ Hz), 3.80 (s, 3H), 2.89 (t, 2H, $J = 6.1$ Hz), 2.66 (q, 4H, $J = 7.1$ Hz), 1.08 (t, 6H, $J = 7.1$ Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.7, 164.6, 164.6, 163.4, 161.2, 160.2, 160.0, 157.8, 139.2, 132.1, 132.1, 129.7, 129.7, 129.6, 129.6, 116.4, 116.4, 116.2, 116.2, 113.0, 98.7, 98.3, 97.3, 66.2, 55.5, 51.5, 47.7, 47.7, 11.5, 11.5; LRMS (FAB) *m/z* 533 ($M + H^+$); HRMS (FAB) calcd for $C_{30}H_{31}F_2N_4O_3$ ($M + H^+$): 533.2364; found 533.2357.

*N-(5-(2-(Diethylamino)ethoxy)-2-methoxyphenyl)-4,6-bis(4-fluorophenyl)pyrimidine-2-carboxamide (**26**)*

The acid **7** (8 mg, 0.026 mmol) and aniline **15d** (6.2 mg, 0.026 mmol) afforded the carboxamide **26** (9 mg, 41 %) via general procedure **A**: ^{1}H-NMR (300 MHz, CD_3OD) δ 8.12−8.08 (m, 4H), 8.03 (s, 1H), 7.85 (d, 1H, $J = 2.9$ Hz), 7.07−7.01 (m, 4H), 6.67 (d, 1H, $J = 9.0$ Hz), 6.45 (dd, 1H, $J = 2.9$, 8.8 Hz), 3.91 (t, 2H, $J = 5.7$ Hz), 3.73 (s, 3H), 2.91 (t, 2H, $J = 5.6$ Hz), 2.74 (q, 4H, $J = 7.1$ Hz), 1.13 (t, 6H, $J = 7.1$ Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.7, 164.3, 164.3, 163.4, 159.8, 158.1, 153.2, 142.8, 132.2, 132.1, 129.6, 129.6, 129.5, 129.5, 128.1, 116.4, 116.4, 116.1, 116.1, 112.4, 111.0, 110.0, 106.6, 66.5, 56.6, 51.5, 47.7, 47.7, 11.5, 11.5; LRMS (FAB) *m/z* 533 ($M + H^+$); HRMS (FAB) calcd for $C_{30}H_{31}F_2N_4O_3$ ($M + H^+$): 533.2364; found 533.2359.

*N-(4-(2-(Diethylamino)ethoxy)-2-methoxyphenyl)-4,6-bis(4-fluorophenyl)pyramidine-2-carboxamide (**27**)*

The acid **7** (8 mg, 0.026 mmol) and aniline **18a** (6.2 mg, 0.026 mmol) afforded the carboxamide **27** (18 mg, 75 %) via general procedure **A**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.56 (d, 1H, $J = 8.3$ Hz), 8.28−8.24 (m, 4H), 8.08 (s, 1H), 7.24−7.19 (m, 4H), 6.56−6.53 (m, 2H), 4.07 (t, 2H, $J = 6.1$ Hz), 3.95 (s, 3H), 2.89 (t, 2H, $J = 6.1$ Hz), 2.66 (q, 4H, $J = 7.1$ Hz), 1.08 (t, 6H, $J = 7.1$ Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.6, 164.2, 164.2, 163.3, 159.3, 158.3, 156.0, 149.6, 132.2, 132.2, 129.6, 129.6, 129.5, 129.5, 121.1, 120.3, 116.3, 116.3, 116.0, 116.0, 112.3, 104.8, 99.2, 66.6, 56.0, 51.7, 47.8, 47.8, 11.8, 11.8; LRMS (FAB) *m/z* 533 ($M + H^+$); HRMS (FAB) calcd for $C_{30}H_{31}F_2N_4O_3$ ($M + H^+$): 533.2364; found 533.2361.

*N-(4-(2-(Diethylamino)ethoxy)-3-methoxyphenyl)-4,6-bis(4-fluorophenyl)pyrimi-dine-2-carboxamide (**28**)*

The acid **7** (8 mg, 0.026 mmol) and aniline **18b** (6.2 mg, 0.026 mmol) afforded the carboxamide **28** (10 mg, 46 %) via general procedure **A**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.94 (s, 1H), 8.26−8.21 (m, 4H), 8.09 (s, 1H), 7.82 (d, 1H, $J = 2.4$ Hz), 7.26−7.21 (m, 4H), 7.06 (dd, 1H, $J = 2.4$, 8.6 Hz), 6.90 (d, 1H, $J = 8.6$ Hz), 4.09 (t, 2H, $J = 6.9$ Hz), 3.91 (s, 3H), 2.92 (t, 2H, $J = 6.8$ Hz), 2.64 (q, 4H, $J = 7.1$ Hz), 1.06 (t, 6H, $J = 7.1$ Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.7, 164.5, 164.5, 163.3, 159.8, 157.8, 149.6, 145.3, 132.2, 132.2, 131.5, 129.7, 129.7, 129.6, 129.6, 116.4, 116.4, 116.1, 116.1, 113.3, 113.0, 111.6, 104.8, 67.5, 56.0, 51.6, 47.8, 47.8, 11.7, 11.7; LRMS (FAB) *m/z* 533 ($M + H^+$); HRMS (FAB) calcd for $C_{30}H_{31}F_2N_4O_3$ ($M + H^+$): 533.2364; found 533.2376.

ELISA test

One microgram of purified biotinylated-human-RAGE, 1 μL of 10 μM Aβ solution and 20 μM of the compound in 100 μL of TBS-T with 2.5 % BSA were incubated on a streptavidin-coated plate for 60 min at ambient temperature. After washing the plate with TBS-T, the horseradish-peroxidase conjugated 4G8 antibody (4G8-HRP, 1:1000 dilution) in 100 μL of TBS-T with 2.5 % BSA was added into each well to detect the bound Aβ. The plate was

incubated for 60 min at ambient temperature. After washing with TBS-T, the plate was developed with TMB substrate: the reaction was stopped with sulfuric acid. The absorbance was read on a Sunrise plate reader (TECHAN) at 450 nm.

Cell viability test

For determining cell viability, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide (MTT) assay was used. Hippocampal HT22 cells were seeded in 96-well plates with a density of 5000 cells per well, and cultured in DMEM with 10 % FBS for 24 h. The medium was replaced with serum free DMEM before treatment. Compound or DMSO were diluted in DMEM and added in each wells. After 18 h of treatment, the cells were incubated with MTT at a final concentration of 500 μg/mL for 4 h. After incubation, the medium was removed and MTT formazan was dissolved in 100 μL of DMSO. The absorbance was read with a microplate reader (Sunrise, TECHAN, Australia) at 570 nm. Cell viability was expressed as a percentage of the control.

Results and discussion

Chemistry

The pyrimidine-2-carboxamide analogs were readily prepared from commercially available 2,4,6-trichloro-pyrimidine **4** as shown in Scheme 1. Regioselective Suzuki reaction (Delia et al. 2006) between trichloropyrimidine **4** and 4-fluorophenylboronic acid provided 4,6-bis(4-fluorophenyl)pyrimidine **5**, which was treated with NaCN and DABCO to afford carbonitrile **6** (Irie et al. 2008). Hydrolysis of the carbonitrile **6** yielded an acid intermediate **7** (Suárez-Varela et al. 2008). And the carboxamide analogs **9a–c** were synthesized by an amidation reaction between the acid intermediate **7** and known aniline counterparts **8a–c** (Han et al. 2012), respectively.

To synthesize various methoxy-substituted pyrimidine-2-carboxamide analogs, methoxy-substituted aniline counterparts were prepared from commercially available nitrobenzenes as described in Schemes 2, 3 and 4. Aniline counterparts possessing a (diethylamino)ethoxy moiety at *ortho*-position **12a–d** were afforded from methoxy-2-nitrophenols as shown in Scheme 2. The Mitsunobu reaction of nitrophenols **10a–d** with 2-(diethylamino)ethanol gave phenyl ethers **11a–d**, which were subjected to nitro-reduction to afford *ortho*-(diethylamino)ethoxy anilines **12a–d**, respectively.

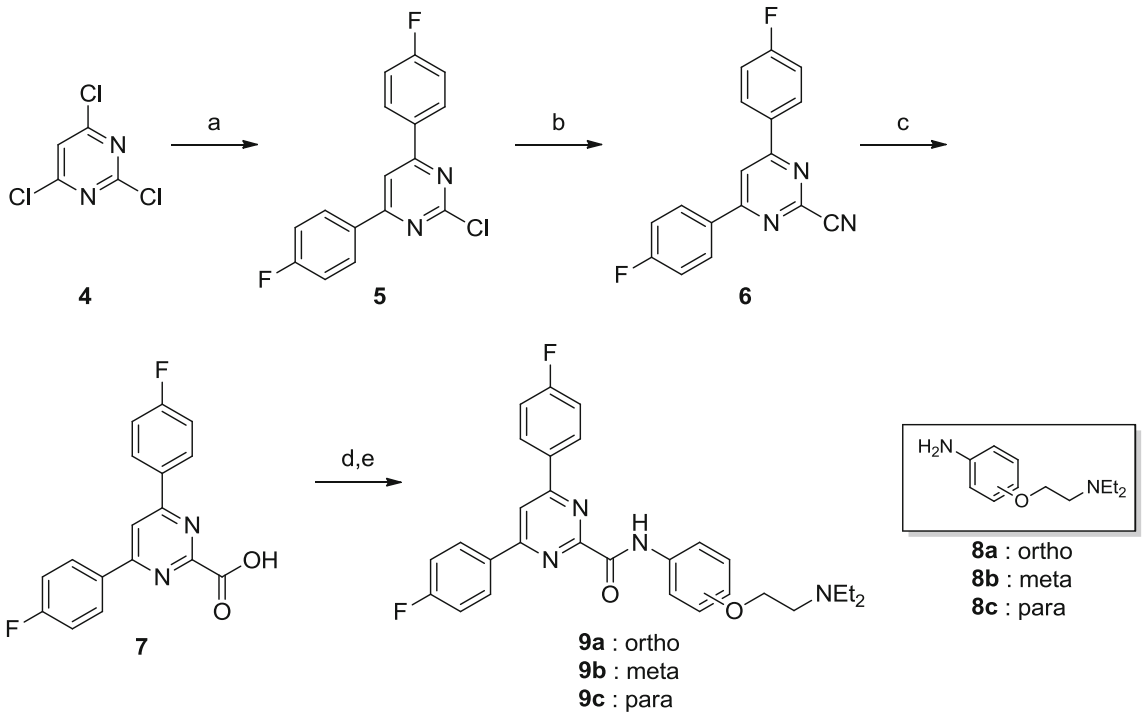
As shown in Scheme 3, nitrobenzyl ethers **14c**, **d** were also afforded from methoxy-3-nitrophenols **13c**,**d** by the Mitsunobu reaction, respectively. Nitrobenzyl ethers **14a**, **b** were synthesized from 3-fluoro-nitrobenzenes **13a**, **b** by the aromatic substitution reaction, respectively, because the corresponding methoxy-3-nitrophenols were not commercially available. The *meta*-(diethylamino)ethoxy anilines **15a–d** were also readily prepared by nitro-reduction of nitrobenzenes **14a–d**, respectively. As shown in Scheme 4, the sequential reaction of the Mitsunobu reaction and nitro-reduction afforded *para*-(diethylamino)ethoxy anilines **18a**, **b** with moderate yields from 4-nitrophenols **16a**, **b**, respectively.

In the same manner as analogs **9a–c**, methoxy-substituted analogs **19–28** were prepared by amidation reaction between the acid intermediate **7** and the corresponding anilines, as shown in Scheme 5.

Biological study

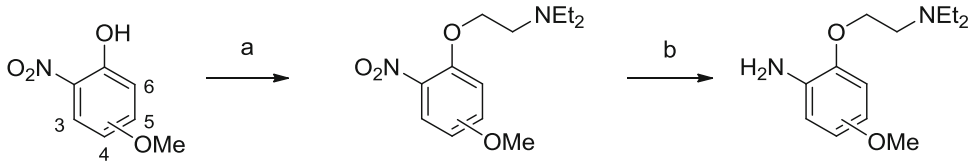
RAGE inhibitory activities of the synthesized analogs were evaluated through ELISAs (enzyme-linked immunosorbent assays) using 20 μM of each analog (Han et al. 2012, 2014). The assay results are summarized in Fig. 2. Compared to the positive control **1**, analogs **9a–9c** (ClogP = 7.04) were regarded as more hydrophilic analogs. However, these analogs, except *meta* analog **9b** (29 %), did not exhibit any RAGE inhibitory activity. Considering methoxy derivatives (**23–26**) of the *meta* analog **9b**, introduction of a methoxy moiety seemed to contribute to the improvement of hydrophilicity, as well as RAGE inhibitory activity to some extent. For example, compared with the 2-aminopyrimidine **1** and *meta* analog **9b**, 5-methoxy analog **25** and 6-methoxy analog **26** exhibited significant improvement in not only RAGE inhibitory activity (159 % and 167 %, respectively), but also hydrophilicity (ClogP = 7.02 and 6.43, respectively). Introduction of the methoxy moiety at the 2-position and the 4-position also improved the *meta* analog **9b**, although the activities of **23** (76 %) and **24** (64 %) were not greater than 2-aminopyrimidine **1**. We could not observe any RAGE inhibitory activity in the *ortho* analog **9a** and *para* analog **9c**, but their methoxy derivatives, **21** and **27**, exhibited potent RAGE inhibitory activity (151 and 152 %, respectively) with improved hydrophilic property (ClogP = 6.43).

Through this investigation, we identified several more active and hydrophilic pyrimidine-2-carboxamide analogs, such as **21**, **25**, **26**, and **27**. With the expectation that improved hydrophilicity may reduce cytotoxicity, we evaluated cytotoxicities of the identified pyrimidine-2-carboxamide analogs by MTT assay, using hippocampal HT22 cells. As shown in Table 1, analog **21** did not exhibit any cytotoxic effects as we had expected. Analogs **25–27** displayed severe cytotoxicity, which was even higher than the parent analogs, **1** and **2**.



Scheme 1 Reagents and conditions: **a** 4-fluorophenylboronic acid, $Pd(OAc)_2$, PPh_3, Na_2CO_3, THF/H_2O (1:1), reflux, 57 %, **b** NaCN, DABCO, DMSO, rt, 88 %, **c** *aq.* KOH, EtOH/H_2O (1:1), reflux, 88 %, **d** oxalyl chloride, DMF, CH_2Cl_2, 0 °C, **e** anilines (**8a**–**c**), Et_3N, THF, rt, 25 % (**9a**), 51 % (**9b**), 47 % (**9c**)

Scheme 2 Reagents and conditions: **a** 2-(diethylamino)ethanol, DIAD, PPh_3, THF, rt, 65–77 % **b** $SnCl_2{\cdot}2H_2O$, EtOH, reflux, 62–90 %

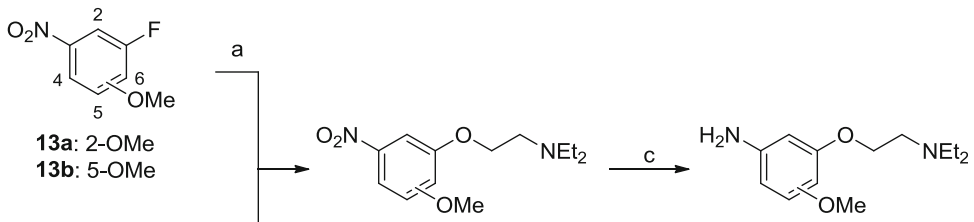


10a: 3-OMe
10b: 4-OMe
10c: 5-OMe
10d: 6-OMe

11a: 3-OMe
11b: 4-OMe
11c: 5-OMe
11d: 6-OMe

12a: 3-OMe
12b: 4-OMe
12c: 5-OMe
12d: 6-OMe

Scheme 3 Reagents and conditions: **a** 2-(diethylamino)ethanol, NaH, DMF, rt, 19 % (**14a**), 26 % (**14c**), **b** 2-(diethylamino)ethanol, DIAD, PPh_3, THF, rt, 77 % (**14b**), 89 % (**14d**), **c** $SnCl_2{\cdot}2H_2O$, EtOH, reflux, 52–78 %

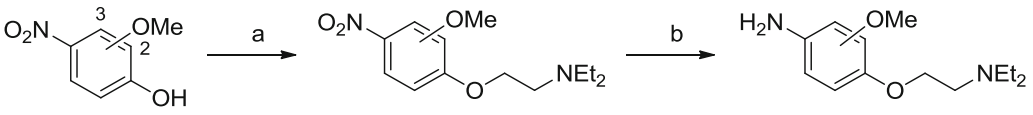


13a: 2-OMe
13b: 5-OMe

13c: 4-OMe
13d: 6-OMe

14a: 2-OMe
14b: 4-OMe
14c: 5-OMe
14d: 6-OMe

15a: 2-OMe
15b: 4-OMe
15c: 5-OMe
15d: 6-OMe



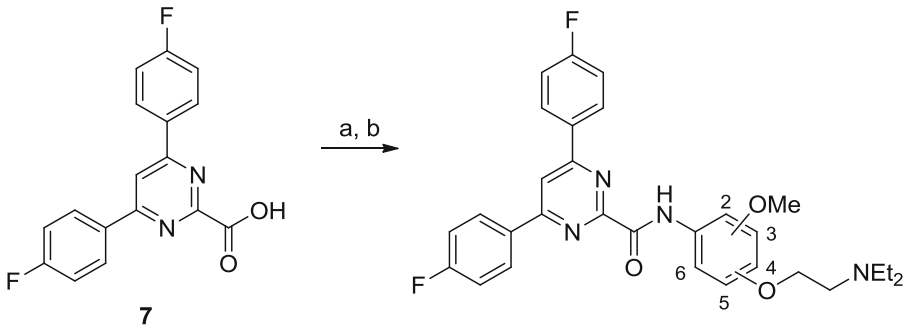
16a: 2-OMe
16b: 3-OMe

17a: 2-OMe
17b: 3-OMe

18a: 2-OMe
18b: 3-OMe

Scheme 4 Reagents and conditions: **a** 2-(diethylamino)ethanol, DIAD, PPh_3, THF, rt, 70 % (**17a**), 81 % (**17b**), **b** $SnCl_2{\cdot}2H_2O$, EtOH, reflux, 66 % (**18a**), 73 % (**18b**)

Scheme 5 Reagents and conditions: **a** oxalyl chloride, DMF, CH_2Cl_2, 0 °C, **b** anilines (**12a–d**, **15a–d**, and **18a**, **b**), Et_3N, THF, rt, 41–80 % (in 2 steps)



2-[O(CH$_2$)$_2$NEt$_2$]	3-[O(CH$_2$)$_2$NEt$_2$]	4-[O(CH$_2$)$_2$NEt$_2$]
19: 3-OMe	**23**: 2-OMe	**27**: 2-OMe
20: 4-OMe	**24**: 4-OMe	**28**: 3-OMe
21: 5-OMe	**25**: 5-OMe	
22: 6-OMe	**26**: 6-OMe	

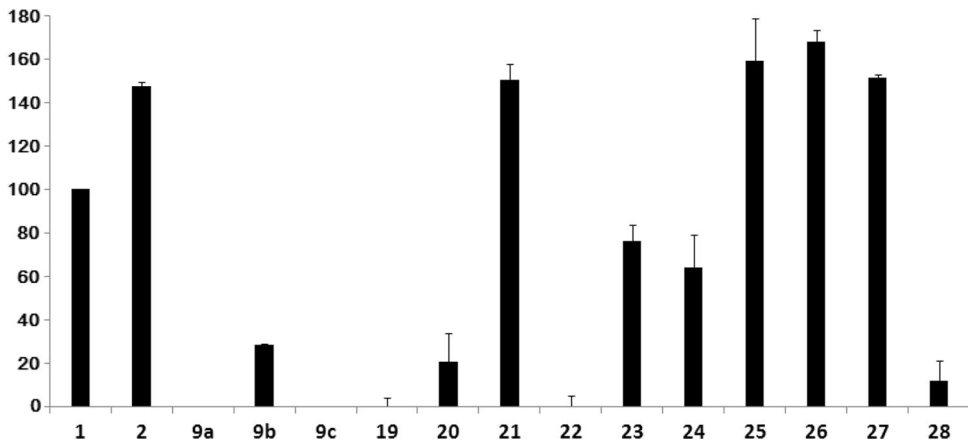


Fig. 2 Relative RAGE inhibitory activity of the analogs. Inhibitory activities were determined by duplicate experiments using 20 μM of the synthesized analogs and expressed in comparison with the activity of **1**

Table 1 Evaluation of cytotoxicity of the analogs

Analog (10 μM)	1	2	21	25	26	27
Cell viability (%)	37.3 ± 1.7	10.3 ± 7.5	100.4 ± 3.2	5.4 ± 0.2	4.5 ± 0.2	5.3 ± 0.3

Cell viabilities were determined by duplicate MTT assay in hippocampal HT22 cells using 10 μM of the synthesized analogs

In summary, to identify novel RAGE inhibitors that have favorable drug-like properties, we designed and synthesized a series of pyrimidine-2-carboxamides based on our preceding studies. Evaluation of their RAGE inhibitory activities led us to identify several potent pyrimidine-2-carboxamide analogs, with improved hydrophilicity. In particular, by contrast with the parent analogs **1** and **2**, the 2-(2-(diethylamino)ethoxy)-5-methoxyphenyl analog **21**

did not exhibit undesired cytotoxic effect. Further research on the identified pyrimidine-2-carboxamide RAGE inhibitor is currently in progress.

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