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Synthesis and Bioactivity of Substituted Benzoylguanidine Derivatives as Potent Na^+/H^+ Exchanger Inhibitors

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A novel series of substituted benzoylguanidine derivatives were designed and synthesized in order to evaluate their NHE1 inhibitory activity. Most of them were found to inhibit NHE1-mediated platelet swelling in a concentration-dependent manner, and eight compounds showed more potent NHE1 inhibitory activity than Cariporide. Compound **6f** with an IC_{50} value of 1.08×10^{-10} mol•L^{-1}, was 39 times more potent than lead compound CPU-X-050420 *in vitro* tests.

Keywords NHE1 inhibitor, benzoylguanidine derivatives, ischemia-reperfusion injury

Introduction

Intracellular Ca^{2+} overload and massive free radical produced during ischemia and reperfusion are the major pathophysiological factors.[1] It has been demonstrated that activated Na^+/H^+ exchanger (NHE) led to the excessive Ca^{2+} influx during ischemia and reperfusion.[2] NHE is a kind of integral protein membrane family expressed in various types of mammalian cells, which regulates intracellular pH (pHi) by exchanging one intracellular H^+ for an extracellular Na^+. From the data, at least nine NHE isoforms have been identified and characterized (NHE1—NHE9).[3] The most predominant isoform is NHE-1 which is involved in intracellular pH, cell-volume control, cytoskeletal organization, heart disease and cancer.[4]

NHE inhibitors have been shown to provide significant myocardial protection in reducing reperfusion Ca^{2+} overload in myocardial cells, reducing the mitochondrial ultrastructural injury in cardiac cell and leakage of enzymes, and reducing cell edema, cardiac stunned and arrhythmia incidence, which contribute to reduced post-ischemic recovery of mechanical function.[5] The structure-activity relationship study on various types of NHE1 inhibitors shows that the acylguanidine moiety is the pharmacophore of NHE1 inhibitors, although there are several exceptions. During the course of our efforts to discover novel inhibitors of NHE1, our laboratory found that CPU-X-050420 (Figure 1) showed a similar potency against NHE1 activity compared to cariporide on rat platelet swelling assay, and significantly improvement on myocardial ischemia and reperfusion injury compared with trimetazidine.[6] With CPU-X-050420 as lead compounds, we chose the benzoylguanidine as core structure, using combination principles, introduced [4-(2,3,4-trimethoxy-benzyl)-piperazin-1-yl]-acetyl group to the 5 position and alkoxy group to the 2 position of benzoylguanidine, and then synthesized target compounds **6a**—**6i**. NHE1 inhibitory activity of these compounds was tested.

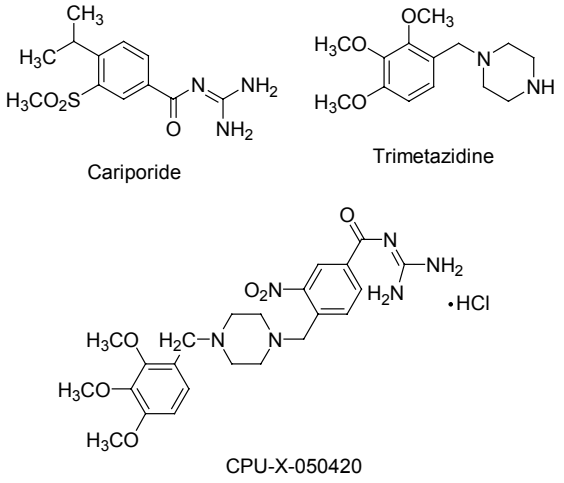

Figure 1 The structures of cariporide, trimetazidine and CPU-X-050420.

Experimental

Apparatuses and materials

Melting points were obtained with an RDCSY-I capillary apparatus and were uncorrected. The IR spectra

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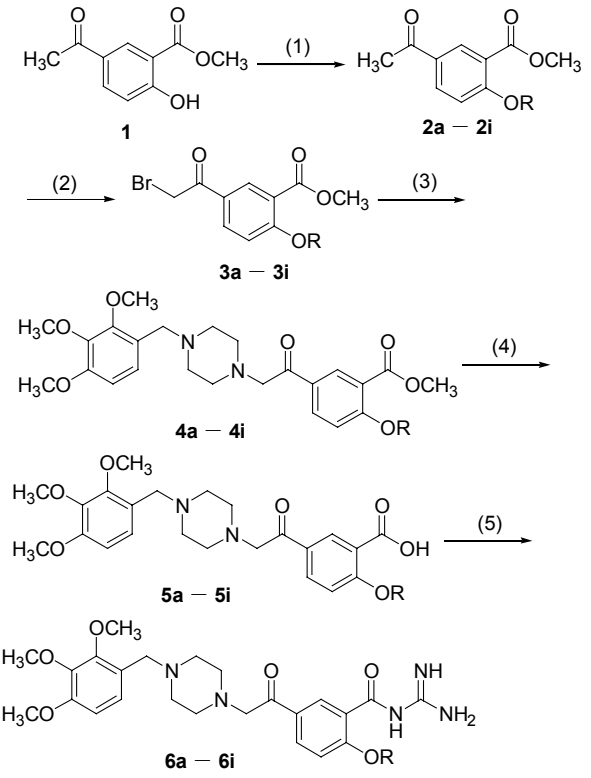
(in KBr pellets) were recorded on a Nicolet Impact 410 spectrophotometer. The ^{1}H NMR spectra were recorded on a Brucker AV-300 or AV-500 NMR spectrometer using TMS as an internal standard and chemical shifts were given relative to tetramethylsilane (TMS). Mass spectra were taken in an Agilent 1100 series LC/MSD Tarp (SL).

Preparation and analysis data

The synthetic route for target compounds **6a**—**6i** is depicted in Scheme 1. The key intermediates **4a**—**4i** were obtained from methyl 5-acetyl-2-hydroxybenzoate (**1**) by *O*-alkylation with alkyl halides to form **2a**—**2i** which were subjected to *α*-bromination with bromine, followed by reaction with trimetazidine. Hydrolysis of **4a**—**4i** with potassium carbonate in methanol-water (6∶4, *w*/*w*) afforded **5a**—**5i**. Treatment of **5a**—**5i** with excessive guanidine, 1-hydroxybenzotrizole (HOBt) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) in anhydrous *N*,*N*-dimethylformamide (DMF) afforded the target compounds **6a**—**6i** which were purified by silica gel column chromatography (acetic ether/methanol/triethylamine = 15∶1∶0.1, *V*/*V*/*V*).

Scheme 1 Syntheses of compounds **6a**—**6i**



a R = CH_3; **b** R = CH_2CH_3; **c** R = $CH_2CH_2CH_3$; **d** R = $(CH_2)_3CH_3$; **e** R = $CH_2CH(CH_3)_2$; **f** R = $(CH_2)_4CH_3$; **g** R = $(CH_2)_5CH_3$; **h** R = $(CH_2)_6CH_3$; **i** R = $(CH_2)_7CH_3$

Reagents and conditions: (1) RX, K_2CO_3, KI, anhydrous DMF, 50 ℃; (2) Br_2, $CHCl_3$, room temperature; (3) trimetazidine, K_2CO_3, anhydrous CH_3CN, room temperature; (4) K_2CO_3, 60% methanol, reflux; (5) a) HOBt, EDCI, DMF; b) guanidine, room temperature.

General procedure for the synthesis of compounds 2

Methyl 5-acetyl-2-hydroxybenzoate (10 mmol) was dissolved in anhydrous DMF (20 mL) with stirring, then KI (1.8 mmol) and the powder of K_2CO_3 (20 mmol) were added and the mixture was stirred for 1 h at room temperature. After slowly dropping a mixture of alkyl halide (10 mmol) in DMF (5 mL), the mixture was heated to 50 ℃ for 5 h. After cooling, the resultant mixture was poured into water (100 mL), then the aqueous phase was extracted with acetic ether (50 mL×3), and the combined organic layers were washed with saturated sodium chloride solution and dried over anhydrous $MgSO_4$. The filtrate was concentrated to dryness under reduced pressure to give compounds **2**.

Methyl 5-acetyl-2-methoxy-benzoate (**2a**): Yield 97.0%, white solid, m.p. 95—97 ℃ (Lit.[7]: 95—96 ℃).

Methyl 5-acetyl-2-ethoxy-benzoate (**2b**): Yield 87.8%, white solid, m.p. 56—58 ℃ (Lit.[7]: 47—50 ℃).

Methyl 5-acetyl-2-propoxy-benzoate (**2c**): Yield 89.7%, white solid, m.p. 49—51 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*: 1.09 (t, *J*=7.5 Hz, 3H, CH_3), 1.85—1.92 (m, 2H, CH_2), 2.58 (s, 3H, $COCH_3$), 3.92 (s, 3H, OCH_3), 4.08 (t, *J*=6.4 Hz, 2H, OCH_2), 7.00 (d, *J*=8.8 Hz, 1H, ArH), 8.07 (dd, J_1=8.8 Hz, J_2=2.4 Hz, 1H, ArH), 8.37 (d, *J*=2.4 Hz, 1H, ArH).

Methyl 5-acetyl-2-butoxy-benzoate (**2d**): Yield 92.0%, white solid, m.p. 53—56 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*: 0.99 (t, *J*=7.4 Hz, 3H, CH_3), 1.50—1.57 (m, 2H, CH_2), 1.81—1.86 (m, 2H, CH_2), 2.58 (s, 3H, $COCH_3$), 3.91 (s, 3H, OCH_3), 4.11 (t, *J*=6.4 Hz, 2H, OCH_2), 7.00 (d, *J*=8.8 Hz, 1H, ArH), 8.08 (dd, J_1=8.8 Hz, J_2=2.4 Hz, 1H, ArH), 8.39 (d, *J*=2.4 Hz, 1H, ArH).

Methyl 5-acetyl-2-isobutoxy-benzoate (**2e**): Yield 89.3%, pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*: 1.08 (d, *J*=2.6 Hz, 6H, CH_3), 2.12—2.21 (m, 1H, CH), 2.58 (s, 3H, $COCH_3$), 3.87 (d, *J*=6.4 Hz, 2H, OCH_2), 3.92 (s, 3H, OCH_3), 6.99 (d, *J*=8.8 Hz, 1H, ArH), 8.08 (dd, J_1=8.8 Hz, J_2=2.4 Hz, 1H, ArH), 8.40 (d, *J*=2.4 Hz, 1H, ArH).

Methyl 5-acetyl-2-pentyloxy-benzoate (**2f**): Yield 89.3%, white solid, m.p. 27—30 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*: 0.94 (t, *J*=7.2 Hz, 3H, CH_3), 1.36—1.52 (m, 4H, CH_2CH_2), 1.82—1.91 (m, 2H, CH_2), 2.58 (s, 3H, $COCH_3$), 3.91 (s, 3H, OCH_3), 4.11 (t, *J*=6.6 Hz, 2H, OCH_2), 7.01 (d, *J*=8.7 Hz, 1H, ArH), 8.08 (dd, J_1=8.7 Hz, J_2=2.4 Hz, 1H, ArH), 8.39 (d, *J*=2.4 Hz, 1H, ArH).

Methyl 5-acetyl-2-hexyloxy-benzoate (**2g**): Yield 95.9%, yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz) *δ*: 0.94 (t, *J*=4.1 Hz, 3H, CH_3), 1.37—1.41 (m, 4H, CH_2CH_2), 1.50—1.56 (m, 2H, CH_2), 1.85—1.91 (m, 2H, CH_2), 2.60 (s, 3H, $COCH_3$), 3.93 (s, 3H, OCH_3), 4.14 (t, *J*=3.9 Hz, 2H, OCH_2), 7.03 (d, *J*=8.8 Hz, 1H, ArH), 8.11 (dd, J_1=8.8 Hz, J_2=2.4 Hz, 1H, ArH), 8.41 (d, *J*=2.4 Hz, 1H, ArH).

Methyl 5-acetyl-2-heptyloxy-benzoate (**2h**): Yield 97.6%, white solid, m.p. 34—35 ℃; ^{1}H NMR ($CDCl_3$,

300 MHz) δ: 0.90 (t, J=6.6 Hz, 3H, CH_3), 1.26—1.41 (m, 6H, $CH_2CH_2CH_2$), 1.44—1.51 (m, 2H, CH_2), 1.81—1.90 (m, 2H, CH_2), 2.58 (s, 3H, $COCH_3$), 3.91 (s, 3H, OCH_3), 4.10 (t, J=6.6 Hz, 2H, OCH_2), 7.00 (d, J=8.7 Hz, 1H, ArH), 8.08 (dd, J_1=8.7 Hz, J_2=2.4 Hz, 1H, ArH), 8.41 (d, J=2.4 Hz, 1H, ArH).

Methyl 5-acetyl-2-octyloxy-benzoate (**2i**): Yield 93.5%, white solid, m.p. 35—36 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.89 (t, J=6.9 Hz, 3H, CH_3), 1.29—1.34 (m, 8H, $CH_2CH_2CH_2CH_2$), 1.47—1.52 (m, 2H, CH_2), 1.81—1.88 (m, 2H, CH_2), 2.58 (s, 3H, $COCH_3$), 3.91 (s, 3H, OCH_3), 4.11 (t, J=6.6 Hz, 2H, OCH_2), 7.00 (d, J=8.7 Hz, 1H, ArH), 8.08 (dd, J_1=8.7 Hz, J_2=2.4 Hz, 1H, ArH), 8.39 (d, J=2.4 Hz, 1H, ArH).

General procedure for synthesis of compounds 3

2 (12.7 mmol) was dissolved in chloroform (20 mL) with stirring, then added 2 drops of a mixture of bromine (19.0 mmol) and chloroform (10 mL). Added the remaining mixture dropwise after color fading, and finished in 4 h. The mixture was concentrated to dryness under reduced pressure to give yellow solid. The crude product was recrystallised from methanol to give compounds **3**.

Methyl 5-(2-bromo-acetyl)-2-methoxy-benzoate (**3a**): Yield 72.2%, white crystal, m.p. 150—153 ℃ (Lit.[7]: 149—153 ℃).

Methyl 5-(2-bromo-acetyl)-2-ethoxy-benzoate (**3b**): Yield 44.3%, white crystal, m.p. 148—150 ℃ (Lit.[7]: 147—149 ℃).

Methyl 5-(2-bromo-acetyl)-2-propoxy-benzoate (**3c**): Yield 65.3%, white crystal, m.p. 102—105 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.09 (t, J=7.4 Hz, 3H, CH_3), 1.86—1.93 (m, 2H, CH_2), 3.92 (s, 3H, OCH_3), 4.10 (t, J=6.4 Hz, 2H, OCH_2), 4.42 (s, 2H, $COCH_2$), 7.03 (d, J=8.8 Hz, 1H, ArH), 8.11 (dd, J_1=8.8 Hz, J_2=2.4 Hz, 1H, ArH), 8.42 (d, J=2.4 Hz, 1H, ArH).

Methyl 5-(2-bromo-acetyl)-2-butoxy-benzoate (**3d**): Yield 52.1%, white crystal, m.p. 93—95 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.99 (t, J=7.4 Hz, 3H, CH_3), 1.51—1.58 (m, 2H, CH_2), 1.82—1.87 (m, 2H, CH_2), 3.92 (s, 3H, OCH_3), 4.14 (t, J=6.4 Hz, 2H, OCH_2), 4.42 (s, 2H, $COCH_2$), 7.03 (d, J=8.8 Hz, 1H, ArH), 8.11 (dd, J_1=8.8 Hz, J_2=2.4 Hz, 1H, ArH), 8.42 (d, J=2.4 Hz, 1H, ArH).

Methyl 5-(2-bromo-acetyl)-2-isobutoxy-benzoate (**3e**): Yield 53.7%, white crystal, m.p. 98—100 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.08 (d, J=6.7 Hz, 6H, CH_3), 2.14—2.23 (m, 1H, CH), 3.90 (d, J=6.4 Hz, 2H, OCH_2), 3.93 (s, 3H, OCH_3), 4.42 (s, 2H, $COCH_2$), 7.02 (d, J=8.8 Hz, 1H, ArH), 8.12 (dd, J_1=8.8 Hz, J_2=2.4 Hz, 1H, ArH), 8.43 (d, J=2.4 Hz, 1H, ArH).

Methyl 5-(2-bromo-acetyl)-2-pentyloxy-benzoate (**3f**): Yield 67.2%, white crystal, m.p. 91—93 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.94 (t, J=7.2 Hz, 6H, CH_3), 1.36—1.52 (m, 4H, CH_2CH_2), 1.82—1.90 (m, 2H, CH_2), 3.92 (s, 3H, OCH_3), 4.13 (t, J=6.6 Hz, 2H, OCH_2), 4.42 (s, 2H, $COCH_2$), 7.03 (d, J=9 Hz, 1H, ArH), 8.12 (dd, J_1=9 Hz, J_2=2.4 Hz, 1H, ArH), 8.42 (d, J=2.4 Hz, 1H, ArH).

Methyl 5-(2-bromo-acetyl)-2-hexyloxy-benzoate (**3g**): Yield 64.4%, white crystal, m.p. 89—91 ℃; ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 0.94 (t, J=6.7 Hz, 3H, CH_3), 1.36—1.38 (m, 4H, CH_2CH_2), 1.51—1.60 (m, 2H, CH_2), 1.85—1.91 (m, 2H, CH_2), 3.94 (s, 3H, OCH_3), 4.15 (t, J=6.5 Hz, 2H, OCH_2), 4.44 (s, 2H, $COCH_2$), 7.05 (d, J=8.8 Hz, 1H, ArH), 8.14 (dd, J_1=8.8 Hz, J_2=2.1 Hz, 1H, ArH), 8.44 (d, J=2.1 Hz, 1H, ArH).

Methyl 5-(2-bromo-acetyl)-2-heptyloxy-benzoic acid methyl ester (**3h**): Yield 47.8%, white crystal, m.p. 96—98 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.90 (t, J=6.9 Hz, 3H, CH_3), 1.25—1.39 (m, 6H, $CH_2CH_2CH_2$), 1.45—1.52 (m, 2H, CH_2), 1.82—1.91 (m, 2H, CH_2), 3.92 (s, 3H, OCH_3), 4.13 (t, J=6.6 Hz, 2H, OCH_2), 4.42 (s, 2H, $COCH_2$), 7.03 (d, J=9 Hz, 1H, ArH), 8.14 (dd, J_1=9 Hz, J_2=2.4 Hz, 1H, ArH), 8.44 (d, J=2.4 Hz, 1H, ArH).

5-(2-Bromo-acetyl)-2-octyloxy-benzoate (**3i**): Yield 50.1%, white crystal, m.p. 87—88 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.89 (t, J=6.9 Hz, 3H, CH_3), 1.29—1.34 (m, 8H, $CH_2CH_2CH_2CH_2$), 1.45—1.52 (m, 2H, CH_2), 1.81—1.91 (m, 2H, CH_2), 3.92 (s, 3H, OCH_3), 4.12 (t, J=6.3 Hz, 2H, OCH_2), 4.42 (s, 2H, $COCH_2$), 7.03 (d, J=9 Hz, 1H, ArH), 8.12 (dd, J_1=9 Hz, J_2=2.4 Hz, 1H, ArH), 8.42 (d, J=2.4 Hz, 1H, ArH).

General procedure for synthesis of compounds 4

K_2CO_3 powder (17.5 mmol) was added to a solution of **3** (3.5 mmol) in anhydrous acetonitrile (20 mL). Trimetazidine (1-(2,3,4-trimethoxybenzyl)piperazine) (3.5 mmol) in anhydrous acetonitrile (5 mL) was added dropwise. TLC showed the completion of the reaction in a flash, and then the mixture was filtered, concentrated, and submitted to silica gel column chromatography (petroleum/EtOAc=4∶1, V/V).

Methyl 5-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)acetyl)-2-(methyl)benzoate (**4a**): Yield 74.5%, yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.58 (s, 8H, piperazine H), 3.52 (s, 2H, CH_2), 3.76 (s, 2H, CH_2), 3.85—3.92 (m, 12H, OCH_3), 3.98 (s, 3H, OCH_3), 6.64 (d, J=8.4 Hz, 1H, ArH), 6.99 (d, J=8.4 Hz, 1H, ArH), 7.03 (d, J=9 Hz, 1H, ArH), 8.19 (dd, J_1=9 Hz, J_2=2.1 Hz, 1H, ArH), 8.49 (d, J=2.1 Hz, 1H, ArH).

Methyl 5-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)acetyl)-2-(ethyl)benzoate (**4b**): Yield 77.8%, yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.50 (t, J=7.2 Hz, 3H, CH_3), 2.59 (s, 8H, piperazine H), 3.52 (s, 2H, CH_2), 3.77 (s, 2H, CH_2), 3.85—3.92 (m, 12H, OCH_3), 4.20 (q, J=6.9 Hz, 2H, OCH_2), 6.64 (d, J=8.4 Hz, 1H, ArH), 7.03 (d, J=9 Hz, 2H, ArH), 8.15 (dd, J_1=9 Hz, J_2=2.4 Hz, 1H, ArH), 8.46 (d, J=2.4 Hz, 1H, ArH).

Methyl 5-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)acetyl)-2-(propoxy)benzoate (**4c**): Yield 63.0%, yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.8 (t, J=7.4 Hz, 3H, CH_3), 1.86—1.90 (m, 2H, CH_2), 2.62 (s, 8H, piperazine H), 3.55 (s, 2H, CH_2), 3.76 (s, 2H, CH_2),

3.85—3.91 (m, 12H, OCH_3), 4.07 (t, J=6.4 Hz, 2H, OCH_2), 6.64 (d, J=8.4 Hz, 1H, ArH), 6.98 (d, J=8.4 Hz, 1H, ArH), 7.00 (d, J=8.8 Hz, 1H, ArH), 8.14 (dd, J_1=8.8 Hz, J_2=2.3 Hz, 1H, ArH), 8.46 (d, J=2.3 Hz, 1H, ArH).

Methyl 5-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)acetyl)-2-(butoxy)benzoate (**4d**): Yield 79.2%, pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.99 (t, J=7.4 Hz, 3H, CH_3), 1.50—1.60 (m, 2H, CH_2), 1.81—1.88 (m, 2H, CH_2), 2.63 (s, 8H, piperazine H), 3.56 (s, 2H, CH_2), 3.76 (s, 2H, CH_2), 3.85—3.92 (m, 12H, OCH_3), 4.11 (t, J=6.4 Hz, 2H, OCH_2), 6.64 (d, J=8.5 Hz, 1H, ArH), 6.98 (d, J=8.5 Hz, 1H, ArH), 7.01 (d, J=8.8 Hz, 1H, ArH), 8.14 (dd, J_1=8.8 Hz, J_2=2.3 Hz, 1H, ArH), 8.45 (d, J=2.3 Hz, 1H, ArH).

Methyl 5-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)acetyl)-2-(isobutoxy)benzoate (**4e**): Yield 59.6%, pale yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.07 (d, J=6.7 Hz, 6H, CH_3), 2.13—2.19 (m, 1H, CH), 2.63 (s, 8H, piperazine H), 3.55 (s, 2H, CH_2), 3.75 (s, 2H, CH_2), 3.85—3.91 (m, 12H, OCH_3), 3.92 (d, J=12.5 Hz, 2H, OCH_2), 6.64 (d, J=8.5 Hz, 1H, ArH), 6.97 (d, J=8.8 Hz, 1H, ArH), 7.01 (d, J=8.5 Hz, 1H, ArH), 8.14 (dd, J_1=8.8 Hz, J_2=2.0 Hz, 1H, ArH), 8.46 (d, J=2.0 Hz, 1H, ArH).

Methyl 5-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)acetyl)-2-(pentyloxy)benzoate (**4f**): Yield 73.9%, pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.94 (t, J=6.9 Hz, 3H, CH_3), 1.36—1.51 (m, 4H, CH_2CH_2), 1.84—1.88 (m, 2H, CH_2), 2.60 (s, 8H, piperazine H), 3.52 (s, 2H, CH_2), 3.76 (s, 2H, CH_2), 3.85—3.91 (m, 12H, OCH_3), 4.11 (t, J=6.6 Hz, 2H, OCH_2), 6.64 (d, J=8.4 Hz, 1H, ArH), 6.99 (d, J=8.7 Hz, 2H, ArH), 8.15 (dd, J_1=8.7 Hz, J_2=2.1 Hz, 1H, ArH), 8.47 (d, J=2.1 Hz, 1H, ArH).

Methyl 5-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)acetyl)-2-(hexyloxy)benzoate (**4g**): Yield 83.6%, pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.91 (t, J=6.6 Hz, 3H, CH_3), 1.34—1.36 (m, 4H, CH_2CH_2), 1.50—1.52 (m, 2H, CH_2), 1.80—1.87 (m, 2H, CH_2), 2.65 (s, 8H, piperazine H), 3.63 (s, 2H, CH_2), 3.78 (s, 2H, CH_2), 3.85—3.94 (m, 12H, OCH_3), 4.10 (t, J=6.3 Hz, 2H, OCH_2), 6.64 (d, J=8.4 Hz, 1H, ArH), 6.98 (d, J=8.4 Hz, 1H, ArH), 7.05 (d, J=8.7 Hz, 1H, ArH), 8.13 (dd, J_1=8.7 Hz, J_2=2.1 Hz, 1H, ArH), 8.46 (d, J=2.1 Hz, 1H, ArH).

Methyl 5-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)acetyl)-2-(heptyloxy)benzoate (**4h**): Yield 87.3%, pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.90 (t, J=6.6 Hz, 3H, CH_3), 1.26—1.52 (m, 8H, CH_2CH_2-CH_2CH_2), 1.81—1.87 (m, 2H, CH_2), 2.60 (s, 8H, piperazine H), 3.52 (s, 2H, CH_2), 3.76 (s, 2H, CH_2), 3.85—3.91 (m, 12H, OCH_3), 4.10 (t, J=6.6 Hz, 2H, OCH_2), 6.64 (d, J=8.4 Hz, 1H, ArH), 6.99 (d, J=8.7 Hz, 1H, ArH), 8.15 (dd, J_1=8.7 Hz, J_2=2.1 Hz, 1H, ArH), 8.46 (d, J=2.1 Hz, 1H, ArH).

Methyl 5-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)acetyl)-2-(octyloxy)benzoate (**4i**): Yield 62.5%, pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.86 (t, J=6.9 Hz, 3H, CH_3), 1.29—1.32 (m, 8H, CH_2CH_2-CH_2CH_2), 1.47—1.51 (m, 2H, CH_2), 1.83—1.87 (m, 2H, CH_2), 2.60 (s, 8H, piperazine H), 3.52 (s, 2H, CH_2), 3.76 (s, 2H, CH_2), 3.85—3.91 (m, 12H, OCH_3), 4.10 (t, J=6.6 Hz, 2H, OCH_2), 6.64 (d, J=8.4 Hz, 1H, ArH), 6.99 (d, J=8.7 Hz, 1H, ArH), 8.15 (dd, J_1=8.7 Hz, J_2=2.1 Hz, 1H, ArH), 8.46 (d, J=2.1 Hz, 1H, ArH).

General procedure for synthesis of compounds 5a—5g

K_2CO_3 (4.8 mmol) was added to a solution of **4** (1.6 mmol) in 60% methanol solution (35 mL), and the mixture was stirred and refluxed for 1 h, then methanol was removed under vacuum. Acidified to pH 7 with 2% HCl, then extracted with dichlormethane (25 mL×3). The combined organic layers were dried over anhydrous $MgSO_4$. Then, the filtrate was concentrated to dryness under reduced pressure, and the resultant solid was used without further purification.

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(methoxy)benzoic acid (**5a**): Yield 81.6%, yellow solid, m.p. 110—114 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.89—3.24 (m, 8H, piperazine H), 3.48 (s, 2H, CH_2), 3.86—4.02 (m, 12H, OCH_3), 4.22 (s, 2H, CH_2), 6.70 (d, J=8.7 Hz, 1H, ArH), 7.04 (d, J=9 Hz, 1H, ArH), 7.33 (d, J=8.7 Hz, 1H, ArH), 8.07 (d, J=8.1 Hz, 1H, ArH), 8.43 (s, 1H, ArH).

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(ethoxy)benzoic acid (**5b**): Yield 77.3%, yellow solid, m.p. 78—80 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.51 (t, J=6.9 Hz, 3H, CH_3), 2.85—3.15 (m, 8H, piperazine H), 3.86 (s, 9H, OCH_3), 3.95 (s, 2H, CH_2), 4.14 (s, 2H, CH_2), 4.26 (q, J=6.9 Hz, 2H, OCH_2), 6.68 (d, J=8.4 Hz, 1H, ArH), 7.00 (d, J=8.7 Hz, 1H, ArH), 7.25 (d, J=8.4 Hz, 1H, ArH), 8.02 (dd, J_1=8.7 Hz, J_2=2.1 Hz, 1H, ArH), 8.30 (d, J=2.1 Hz, 1H, ArH).

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(propoxy)benzoic acid (**5c**): Yield 79.5%, pale yellow solid, m.p. 74—78 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.06 (t, J=7.3 Hz, 3H, CH_3), 1.89—1.92 (m, 2H, CH_2), 2.85—3.04 (m, 8H, piperazine H), 3.86 (s, 2H, CH_2), 3.92 (s, 9H, OCH_3), 4.05 (s, 2H, CH_2), 4.13 (t, J=6.5 Hz, 2H, OCH_2), 6.64 (d, J=8.6 Hz, 1H, ArH), 7.00 (d, J=8.8 Hz, 1H, ArH), 7.23 (d, J=8.6 Hz, 1H, ArH), 8.02 (d, J=8.0 Hz, 1H, ArH), 8.31 (s, 1H, ArH).

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(butoxy)benzoic acid (**5d**): Yield 80.2%, pale yellow solid, m.p. 81—82 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.99 (t, J=7.4 Hz, 3H, CH_3), 1.49—1.56 (m, 2H, CH_2), 1.87—1.92 (m, 2H, CH_2), 3.00—3.15 (m, 8H, piperazine H), 3.86—3.93 (m, 9H, OCH_3), 3.94 (s, 2H, CH_2), 4.13 (s, 2H, CH_2), 4.26 (t, J=6.5 Hz, 2H, OCH_2), 6.71 (d, J=8.6 Hz, 1H, ArH), 7.07 (d, J=8.8 Hz, 1H, ArH), 7.35 (d, J=8.6 Hz, 1H, ArH), 8.09 (dd, J_1=8.8 Hz, J_2=2.3 Hz, 1H, ArH), 8.55 (d, J=2.3 Hz, 1H, ArH).

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-

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acetyl)-2-(isobutoxy)benzoic acid (**5e**): Yield 74.6%, pale yellow solid, m.p. 88—91℃; ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.06 (d, J=6.7 Hz, 6H, CH_3), 2.18—2.20 (m, 1H, CH), 2.94—3.10 (m, 8H, piperazine H), 3.85 (s, 2H, CH_2), 3.92 (s, 9H, OCH_3), 3.96 (d, J=6.4 Hz, 2H, OCH_2), 4.07 (s, 2H, CH_2), 6.68 (d, J=8.6 Hz, 1H, ArH), 7.01 (d, J=8.9 Hz, 1H, ArH), 7.30 (d, J=8.6 Hz, 1H, ArH), 8.04 (d, J=8.5 Hz, 1H, ArH), 8.45 (s, 1H, ArH).

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(pentyloxy)benzoic acid (**5f**): Yield 83.7%, pale yellow solid, m.p. 81—84 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.93 (t, J=6.9 Hz, 3H, CH_3), 1.35—1.50 (m, 4H, CH_2CH_2), 1.87—1.94 (m, 2H, CH_2), 3.03—3.20 (m, 8H, piperazine H), 3.85—3.93 (m, 9H, OCH_3), 3.96 (s, 2H, CH_2), 4.17 (s, 2H, CH_2), 4.25 (t, J=6.6 Hz, 2H, OCH_2), 6.72 (d, J=8.4 Hz, 1H, ArH), 7.07 (d, J=8.7 Hz, 1H, ArH), 7.37 (d, J=8.4 Hz, 1H, ArH), 8.09 (dd, J_1=8.7 Hz, J_2=1.8 Hz, 1H, ArH), 8.56 (d, J=1.8 Hz, 1H, ArH).

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(hexyloxy)benzoic acid (**5g**): Yield 83.6%, pale yellow solid, m.p. 59—62 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.88 (t, J=6.9 Hz, 3H, CH_3), 1.31—1.33 (m, 4H, CH_2CH_2), 1.46—1.48 (m, 2H, CH_2), 1.85—1.92 (m, 2H, CH_2), 2.86—3.06 (m, 8H, piperazine H), 3.86 (s, 9H, OCH_3), 3.92 (s, 2H, CH_2), 4.07 (s, 2H, CH_2), 4.18 (t, J=6.6 Hz, 2H, OCH_2), 6.67 (d, J=8.4 Hz, 1H, ArH), 7.01 (d, J=8.7 Hz, 1H, ArH), 7.26 (d, J=8.4 Hz, 1H, ArH), 8.05 (dd, J_1=8.7 Hz, J_2=2.1 Hz, 1H, ArH), 8.38 (d, J=2.1 Hz, 1H, ArH).

General procedure for synthesis of compounds 5h and 5i

K_2CO_3 (1.8 mmol) was added to a solution of compounds **4** (0.58 mmol) in 60% methanol solution (15 mL), and the mixture was stirred and refluxed for 1 h, then methanol was removed under vacuum. Acidified to pH 7 with 2% HCl, then filtered, followed by drying under IR lamp.

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(heptyloxy)benzoic acid (**5h**): Yield 77.3%, white solid, m.p. 58—60 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.86 (t, J=6.3 Hz, 3H, CH_3), 1.29—1.48 (m, 8H, $CH_2CH_2CH_2CH_2$), 1.85—1.94 (m, 2H, CH_2), 2.85—3.07 (m, 8H, piperazine H), 3.86 (s, 9H, OCH_3), 3.93 (s, 2H, CH_2), 4.08 (s, 2H, CH_2), 4.17 (t, J=6.9 Hz, 2H, OCH_2), 6.67 (d, J=8.4 Hz, 1H, ArH), 7.00 (d, J=8.7 Hz, 1H, ArH), 7.04 (d, J=8.4 Hz, 1H, ArH), 8.03 (d, J=8.7 Hz, 1H, ArH), 8.32 (s, 1H, ArH).

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(octyloxy) benzoic acid (**5i**): Yield 75.5%, white solid, m.p. 71—74 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.86 (t, J=6.9 Hz, 3H, CH_3), 1.25—1.30 (m, 8H, $CH_2CH_2CH_2CH_2$), 1.45—1.47 (m, 2H, CH_2), 1.85—1.92 (m, 2H, CH_2), 2.89—3.08 (m, 8H, piperazine H), 3.86 (s, 9H, OCH_3), 3.92 (s, 2H, CH_2), 4.08 (s, 2H, CH_2), 4.20 (t, J=6.6 Hz, 2H, OCH_2), 6.68 (d, J=8.7 Hz, 1H, ArH), 7.02 (d, J=9 Hz, 1H, ArH), 7.06 (d, J=4.2 Hz, 1H, ArH), 8.06 (d, J=8.4 Hz, 1H, ArH), 8.41 (s, 1H, ArH).

General procedure for synthesis of compounds 6

1-Hydroxybenzotriazole (0.53 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (0.53 mmol) were added to a solution of compounds **5** (0.41 mmol) in DMF (3 mL), and the mixture was stirred for 0.5 h at room temperature, and then guanidine (1.65mmol) was quickly added. The resultant mixture was stirred for 10 min at room temperature and poured into 50 mL of EtOAc/H_2O (3∶2, *V/V*), the aqueous phase was extracted with ethyl acetate (30 mL×3), and the combined organic layers were washed with saturated sodium chloride solution (50 mL×3) and dried over anhydrous $MgSO_4$. The filtrate was concentrated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (acetic ether/methanol/triethylamine=15∶1∶0.1, *V/V/V*).

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(methoxyl)benzoyl guanidine (**6a**): Yield 30.5%, yellow solid, m.p. 69—71 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.57 (s, 8H, piperazine H), 3.49 (s, 2H, CH_2), 3.79 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 6.64 (d, J=8.4 Hz, 1H, ArH), 6.97—7.00 (m, 2H, ArH), 8.08 (d, J=9 Hz, 1H, ArH), 8.34 (d, J=2.1 Hz, 1H, ArH); IR (KBr) ν: 3405, 2925, 2849, 1688 (C=O), 1601 (C=O), 1495, 1463, 1355, 1264, 1095 (OCH_3), 1004, 818 cm^{-1}; MS (ESI(+)70 V) *m/z*: 500.3 $[M+H]^+$. Anal. calcd for $C_{25}H_{33}N_5O_6$•1.8H_2O: C 56.44, H 6.93, N 13.16; found C 56.74, H 7.32, N 12.71.

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(ethoxyl)benzoyl guanidine (**6b**): Yield 29.8%, yellow solid, m.p. 146—148 ℃; ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.46 (t, J=7.0 Hz, 3H, CH_3), 2.55—2.60 (m, 8H, piperazine H), 3.49 (s, 2H, CH_2), 3.76 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.22 (q, J=7.1 Hz, 2H, OCH_2), 6.63 (d, J=8.5 Hz, 1H, ArH), 6.93—7.00 (m, 2H, ArH), 8.04 (dd, J_1=8.8 Hz, J_2=2.0 Hz, 1H, ArH), 8.33 (d, J=2.0 Hz, 1H, ArH); IR (KBr) ν: 3372, 2938, 2805, 1662 (C=O), 1601 (C=O), 1495, 1468, 1363, 1266, 1096 (OCH_3), 1009, 698 cm^{-1}; MS (ESI(+)70 V) *m/z*: 514.3 $[M+H]^+$. Anal. calcd for $C_{26}H_{35}N_5O_6$: C 60.80, H 6.87, N 13.64; found C 60.59, H 7.08, N 13.65.

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(propoxy)benzoyl guanidine (**6c**): Yield 28.5%, yellow solid, m.p. 83—85 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.02 (t, J=7.4 Hz, 3H, CH_3), 1.80—1.87 (m, 2H, CH_2), 2.53—2.56 (m, 8H, piperazine H), 3.48 (s, 2H, CH_2), 3.78 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.04 (t, J=6.7 Hz, 2H, OCH_2), 6.63 (d, J=8.6 Hz, 1H, ArH), 6.92 (d, J=8.8 Hz, 1H, ArH), 6.97 (d, J=8.6 Hz, 1H, ArH), 7.97 (dd, J_1=8.8 Hz, J_2=2.3 Hz, 1H, ArH), 8.19 (d, J=2.3 Hz, 1H, ArH); IR (KBr) ν: 3405, 2938, 2822, 1602 (C=O), 1527, 1495, 1465, 1353, 1265, 1166, 1095 (OCH_3),

1003, 819 cm^{-1}; MS (ESI(+)70 V) *m/z*: 527.3 $[M+H]^+$. Anal. calcd for $C_{27}H_{37}N_5O_6$: C 61.46, H 7.07, N 13.27; found C 61.15, H 7.40, N 12.78.

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(butoxy)benzoyl guanidine (**6d**): Yield 36.1%, yellow solid, m.p. 88—92 ℃; ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.94 (t, J=7.4 Hz, 3H, CH_3), 1.44—1.51 (m, 2H, CH_2), 1.75—1.85 (m, 2H, CH_2), 2.56 (s, 8H, piperazine H), 3.48 (s, 2H, CH_2), 3.78 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.09 (t, J=6.6 Hz, 2H, OCH_2), 6.63 (d, J=8.5 Hz, 1H, ArH), 6.93 (d, J=8.8 Hz, 1H, ArH), 6.97 (d, J=8.5 Hz, 1H, ArH), 7.98 (dd, J_1=8.8 Hz, J_2=2.1 Hz, 1H, ArH), 8.20 (s, 1H, ArH); IR (KBr) *v*: 3406, 2936, 2821, 1604 (C=O), 1527, 1495, 1464, 1352, 1271, 1166, 1095 (OCH_3), 1004, 801 cm^{-1}; MS (ESI(+)70 V) *m/z*: 542.3 $[M+H]^+$. Anal. calcd for $C_{28}H_{39}N_5O_6 \cdot 0.5H_2O$: C 61.07, H 7.32, N 12.71; found C 60.91, H 7.45, N 12.32.

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(isobutoxy)benzoyl guanidine (**6e**): Yield 37.6%, yellow solid, m.p. 140—144 ℃; ^1H NMR ($CDCl_3$, 500 MHz) δ: 1.01 (d, J=6.7 Hz, 6H, CH_3), 2.11—2.14 (m, 1H, CH), 2.55—2.59 (m, 8H, piperazine H), 3.47 (s, 2H, CH_2), 3.77 (s, 2H, CH_2), 3.83 (d, J=6.6 Hz, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.63 (d, J=8.5 Hz, 1H, ArH), 6.91 (d, J=8.8 Hz, 1H, ArH), 6.97 (d, J=8.5 Hz, 1H, ArH), 7.96 (dd, J_1=8.8 Hz, J_2=2.3 Hz, 1H, ArH), 8.16 (d, J=2.3 Hz, 1H, ArH); IR (KBr) *v*: 3415, 2937, 2821, 1603 (C=O), 1528, 1495, 1465, 1351, 1274, 1167, 1096 (OCH_3), 1002, 819 cm^{-1}; MS (ESI(+)70 V) *m/z*: 542.3 $[M+H]^+$. Anal. calcd for $C_{28}H_{39}N_5O_6 \cdot 0.5H_2O$: C 61.07, H 7.32, N 12.71; found C 61.08, H 7.79, N 12.48.

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(pentyloxy)benzoyl guanidine (**6f**): Yield 26.4%, yellow solid, m.p. 80—83 ℃; ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.90 (t, J=6.3 Hz, 3H, CH_3), 1.26—1.39 (m, 4H, CH_2CH_2), 1.82—1.84 (m, 2H, CH_2), 2.57—2.62 (m, 8H, piperazine H), 3.49 (s, 2H, CH_2), 3.63 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.11 (t, J=6.3 Hz, 2H, OCH_2), 6.63 (d, J=8.1 Hz, 1H, ArH), 6.94 (d, J=8.4 Hz, 1H, ArH), 6.98 (d, J=8.4 Hz, 1H, ArH), 8.02 (d, J=8.1 Hz, 1H, ArH), 8.27 (s, 1H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 13.9, 22.3, 28.0, 28.6, 29.7, 52.7 (piperazine C), 53.7 (piperazine C), 56.0, 60.7, 61.2, 64.2, 69.2, 107.2, 112.4, 124.2, 125.1, 128.4, 129.4, 130.3, 131.3, 142.4, 152.6, 152.9, 160.7, 161.8, 177.4, 195.5 (C=O); IR (KBr) *v*: 3420, 2353, 2310, 1632 (C=O), 1458, 1096 (OCH_3), 668 cm^{-1}; MS (ESI(+)70 V) *m/z*: 556.3 $[M+H]^+$. Anal. calcd for $C_{29}H_{41}N_5O_6$: C 62.68, H 7.43, N 12.60; found C 62.47, H 7.48, N 12.22.

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(hexyloxy)benzoyl guanidine (**6g**): Yield 38.2%, white solid, m.p. 153—155 ℃; ^1H NMR ($CDCl_3$, 500 MHz) δ: 0.87 (t, J=7.1 Hz, 3H, CH_3), 1.30—1.33 (m, 4H, CH_2CH_2), 1.43—1.46 (m, 2H, CH_2), 1.79—1.85 (m, 2H, CH_2), 2.55—2.59 (m, 8H, piperazine H), 3.48 (s, 2H, CH_2), 3.77 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.09 (t, J=6.8 Hz, 2H, OCH_2), 6.63 (d, J=8.5 Hz, 1H, ArH), 6.93 (d, J=8.8 Hz, 1H, ArH), 6.98 (d, J=8.5 Hz, 1H, ArH), 7.99 (dd, J_1=8.8 Hz, J_2=2.1 Hz, 1H, ArH), 8.46 (s, 1H, ArH); IR (KBr) *v*: 3374, 2933, 2810, 1663 (C=O), 1601 (C=O), 1531, 1495, 1467, 1362, 1266, 1096 (OCH_3), 1008, 698 cm^{-1}; MS (ESI(+)70 V) *m/z*: 570.4 $[M+H]^+$. Anal. calcd for $C_{30}H_{43}N_5O_6$: C 63.25, H 7.61, N 12.29; found C 63.52, H 7.44, N 12.14.

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(heptyloxy)benzoyl guanidine (**6h**): Yield 31.4%, white solid, m.p. 160—162 ℃; ^1H NMR ($CDCl_3$, 500 MHz) δ: 0.86 (t, J=6.7 Hz, 3H, CH_3), 1.26—1.34 (m, 6H, $CH_2CH_2CH_2$), 1.40—1.45 (m, 2H, CH_2), 1.79—1.83 (m, 2H, CH_2), 2.57 (d, J=20.4 Hz, 8H, piperazine H), 3.48 (s, 2H, CH_2), 3.77 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.08 (t, J=6.8 Hz, 2H, OCH_2), 6.63 (d, J=8.5 Hz, 1H, ArH), 6.93 (d, J=8.8 Hz, 1H, ArH), 6.97 (d, J=8.5 Hz, 1H, ArH), 7.98 (dd, J_1=8.8 Hz, J_2=2.1 Hz, 1H, ArH), 8.46 (s, 1H, ArH); IR (KBr) *v*: 3376, 2930, 2854, 1662 (C=O), 1602 (C=O), 1529, 1495, 1466, 1363, 1266, 1096 (OCH_3), 1006, 821 cm^{-1}; MS (ESI(+)70 V) *m/z*: 584.4 $[M+H]^+$. Anal. calcd for $C_{31}H_{45}N_5O_6 \cdot 0.5H_2O$: C 62.82, H 7.82, N 11.81; found C 62.71, H 7.91, N 11.81.

5-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)-acetyl)-2-(octyloxy)benzoyl guanidine (**6i**): Yield 34.8%, white solid, m.p. 135—138 ℃; ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.86 (t, J=6.9 Hz, 3H, CH_3), 1.25—1.43 (m, 10H, $CH_2CH_2CH_2CH_2CH_2$), 1.77—1.84 (m, 2H, CH_2), 2.57 (s, 8H, piperazine H), 3.48 (s, 2H, CH_2), 3.78 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.08 (t, J=6.6 Hz, 2H, OCH_2), 6.63 (d, J=8.7 Hz, 1H, ArH), 6.92 (d, J=9 Hz, 1H, ArH), 6.98 (d, J=8.7 Hz, 1H, ArH), 7.98 (dd, J_1=9 Hz, J_2=1.8 Hz, 1H, ArH), 8.46 (d, J=1.8 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.1, 22.6, 25.8, 52.6 (piperazine C), 53.7 (piperazine C), 56.0, 56.6, 60.8, 61.2, 64.1, 69.2, 107.0, 112.3, 124.1, 125.2, 128.2, 130.3, 131.3, 142.3, 152.6, 152.9, 160.7, 161.7, 172.5, 195.5 (C=O); IR (KBr) *v*: 3373, 2929, 2855, 1663 (C=O), 1601 (C=O), 1531, 1495, 1467, 1363, 1267, 1096 (OCH_3), 1009, 844 cm^{-1}; MS (ESI(+)70 V) *m/z*: 598.4 $[M+H]^+$. Anal. calcd for $C_{32}H_{47}N_5O_6$: C 64.30, H 7.92, N 11.72; found C 63.94, H 8.14, N 11.76.

Rat platelet swelling assay

All target compounds, along with the reference compound cariporide and CPU-X-050420, were evaluated in rat platelet swelling assay (PSA) for NHE1 inhibitory activity screening. The experimental procedure was similar as in the literature,[8] with only minor modifications. Collect 5 mL blood from the eyeholes of each adult male and female Sprague-Dawley rat (160—250

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g), then mix acid-citrate-dextrose (ACD: citric acid 65 mmol/L, sodium citrate 85 mmol/L and 2% glucose) in the test tubes. After centrifugation of whole blood at 1300 g/min for 10 min at r.t., the upper two-thirds of the supernatants were used for the further measurements and performed within 4 h.

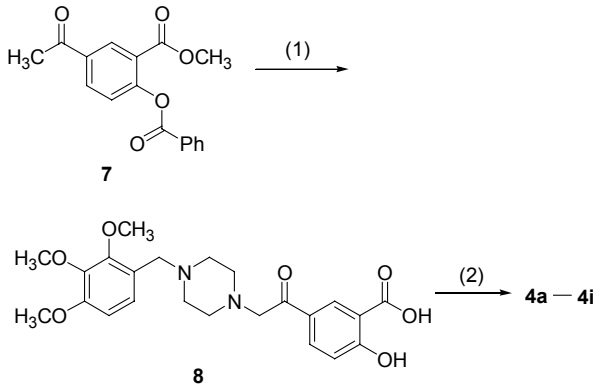
Each compound was tested for six concentrations, and the measurement of each concentration repeated three times. All compounds were dissolved in DMSO, and diluted to test concentration with standard media. Add 25 μL of the solution of the tested compound and 175 μL of propionate buffer (sodium propionate 140 mmol/L, glucose 10 mmol/L, KCl 5 mmol/L, $MgCl_2$ 1 mmol/L, $CaCl_2$ 1 mmol/L, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) 20 (quantum sufficit, pH 6.7) to a spectrophotometer cuvette. After pre-heated to 37 ℃, 50 μL of platelet-rich plasma (PRP) was added. Each 7.5 s for 2 min recorded the change in optical density (OD) due to the platelet swelling at 550 nm. The IC_{50} of the tested compounds was obtained from the linear part of the relationship between the log concentration and NHE activity using linear regression analysis. As the result, the average of IC_{50} of three samples was presented in this paper.

Results and Discussion

Key intermediates **4a**—**4i** can readily be obtained from **1** using the method described in Scheme 1, but this route is time-consuming and low-efficient. In order to improve the synthetic efficiency of **4a**—**4i**, we designed an alternative route (Scheme 2) to prepare **4a**—**4i** using **7** as starting materials by *α*-bromination, condensation with trimetazidine, *O*-deacylation, esterification and *O*-alkylation. Compound **8** could be obtained in good yield (overall yield 56%). However, a lot of impurities were formed when converting **8** to **4**. The isolated yield of **4** after column chromatography was less than 10%.

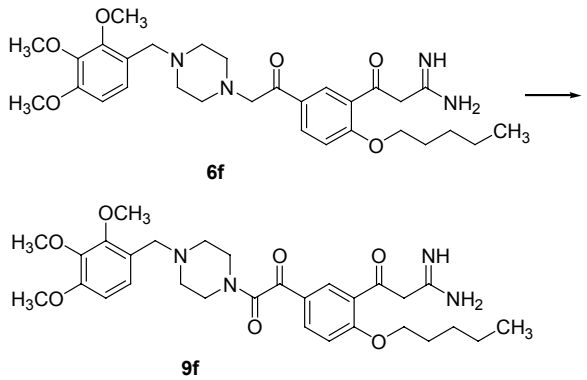
In the synthesis of target compounds **6a**—**6i**, we isolated corresponding analogue **9** from the reaction mixture of **5** and guanidine, and found that the content of **9** was increased when prolonging the reaction time. For example, compound **9f** was separated out during the preparation of **6f**, and its structure was identified by spectral data and elemental analysis.

Scheme 2



Reagents and conditions: (1) (a) $Br_2/CHCl_3$, r.t., 71.5%; (b) trimetazidine/K_2CO_3/CH_3CN, r.t., 86%; (c) K_2CO_3/60% methanol, 50 ℃, 88.7%; (2) (a) $SOCl_2/CH_3OH$; (b) $RX/K_2CO_3/CH_3CN$, reflux.

Scheme 3



The *β*-position methylene of *N*,*N*-dialkyl-*α*-oxo-*β*-phenylethylamine is susceptible to be oxided into carbonyl in the presence of oxidants such as $NaIO_4$,[9] $O_2/FeCl_3$.[10] But such oxidation conversion under our experimental conditions is not clear.

NHE1 inhibitory activities of **6a**—**6i**, along with the reference compound cariporide and CPU-X-050420, were determined in rat platelet swelling assay, and the result (Table 1) showed that all the tested compounds and positive controls inhibited rat platelet NHE1 in a concentration-dependent manner, among which compounds **6b**, **6d**, **6f**, **6g** and CPU-X-050420 were significantly more effective than cariporide in NHE1 inhibi-

Table 1 NHE1 inhibitory activities of compounds **6a**—**6i**

Compd.	IC_{50}/(mol•L^{-1})	Compd.	IC_{50}/(mol•L^{-1})
Cariporide	6.50×10^{-8}	**6e**	1.08×10^{-7}
CPU-X-050420	4.22×10^{-9}	**6f**	1.08×10^{-10}
6a	9.59×10^{-8}	**6g**	5.32×10^{-9}
6b	6.58×10^{-9}	**6h**	5.86×10^{-8}
6c	3.90×10^{-8}	**6i**	9.80×10^{-8}
6d	2.28×10^{-9}		

tion, and compound **6f** was 39 times more potent than CPU-X-050420.

Preliminary structure-activity relationship showed that the good inhibitory effects of target compounds on NHE1 can be achieved when the number of carbon atom in straight-chain alkoxy group is 4 to 6. Because of the relatively less number of tested compounds, the conclusion above need to be further confirmed.

Conclusions

In summary, a series of novel substituted benzoylguanidine derivatives on the basis of the structure of an initial lead compound CPU-X-050420, were designed, synthesized and evaluated for their NHE1 inhibitions. Most compounds showed more potent NHE1 inhibitory activities than cariporide, and the most potent one was **6f**. Further pharmacological studies are in progress.

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