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Original article

Synthesis and Na^+/H^+ exchanger inhibitory activity of benzoylguanidine derivatives

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ABSTRACT

Twenty-two compounds of substituted benzoylguanidine derivatives were designed and synthesized as potent NHE1 inhibitors. Twelve compounds showed more potent NHE1 inhibitory activity than cariporide. The activities of compounds **7e**, **7h** and **7j** ($IC_{50} = 0.073 \pm 0.021$, 0.084 ± 0.012 and 0.068 ± 0.021 nmol/L, respectively) were two orders of magnitude higher than that of cariporide (30.7 ± 2.5 nmol/L). Myocardial cells in vitro screening showed **7j** had highlighted protective effect on cardiomyocytes subjected to hypoxia/reoxygenation. Thus it is valuable for further investigation.

1. Introduction

The Na^+/H^+ exchanger (NHE), which regulates intracellular pH by removing a proton in exchange for an extracellular sodium ion, is an integral membrane protein ubiquitously expressed in mammalian cells. To date, nine isoforms of the exchanger have been identified in various organs in the human body and are designated NHE1–NHE9 [1]. NHE1 was the first isoform discovered and is ubiquitously expressed in the plasma membrane of mammalian cells [2,3]. Other isoforms have more restricted tissue distributions and some have predominantly intracellular localization [4,5]. In mammals, NHE1 is the predominant isoform in heart and is involved in numerous physiological processes, including regulation of intracellular pH, cell-volume control, cytoskeletal organization, heart disease and cancer [6,7]. The activity of NHE1 is elevated in animal models of myocardial infarcts and in left ventricular hypertrophy [8]. During ischemia and reperfusion of the myocardium, NHE activity catalyzes increased uptake of intracellular Na^+. This in turn is exchanged for extracellular calcium by the Na^+/Ca^{2+} exchanger resulting in calcium overload and damage to the myocardium, such as myocardial infarction activation, stunning and tissue necrosis [9,10].

NHE1 inhibitors, in their cation form, combine with NHE1 at the extracellular Na^+ binding site to competitively inhibit NHE1 function and reduce Na^+ and Ca^{2+} influx, and hence abolish the post-ischemia Ca^{2+} overload in myocardial cells and lower the risk of cell dysfunction and injury.

Aroylguanidine, a subunit typically present in most of the known NHE1 inhibitors, such as cariporide and sabiporide (Fig. 1) [11–14], is well-considered as the possible pharmacophore among the reported NHE1 inhibitors [15]. TG-6 (Fig. 1) was synthesized and evaluated in our laboratory before, which showed potent NHE1 inhibitory activity and could excellently improve the cardiac function and reduce infarct size against ischemia-reperfusion injury [16]. We replaced the methylene which connected the piperazine nitrogen with the para-position of benzoylguanidine with ethylene-1-oxy to afford another compound TG-7 ($IC_{50} = 1.06 \pm 0.18$ nM) which exhibited more potent NHE1 inhibitory potency than TG-6 ($IC_{50} = 1.82 \pm 0.36$ nM). We selected TG-7 as lead compound and introduced a series of substituted amide groups to replace the nitro group at the meta-position of the aroylguanidine group bound to the benzene ring of TG-7, and then synthesized our target compounds **7a**–**7v** (Table 1).

2. Results and discussion

2.1. Synthesis

The synthetic route for target compounds is depicted in Scheme 1. Intermediates **2a** and **2b**, obtained by condensation of 4-hydroxy-3-nitro-benzoic acid ethyl ester and 1,2-dibromo ethane or 1,3-dibromo propane, were coupled with 1-(2,3,4-trimethoxybenzyl)

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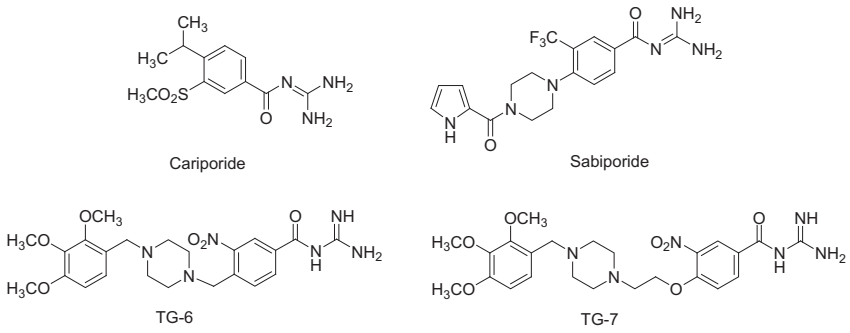


Fig. 1. Structures of cariporide, sabiporide, TG-6 and TG-7.

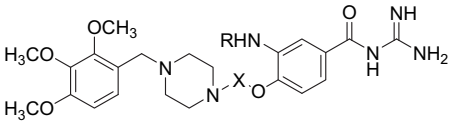
piperazine (trimetazidine) to give intermediates **3a** and **3b**. Treatment of **3a** and **3b** with Fe/HCl afforded **4a** and **4b**. Compounds **4a** and **4b** were coupled with acyl chloride to afford intermediates **5a**–**5v**. Compounds **6a**–**6v**, which are zwitterionic compounds and can be separated from the reaction mixture simply by adjusting their isoelectric points by 2% dilute hydrochloric acid, were synthesized by hydrolysis of corresponding benzoates **5a**–**5v** with K_2CO_3 in MeOH/H_2O (v/v = 2:3). Compounds **7a**–**7v** can be obtained by reaction of the acids **6a**–**6v** with 4 equiv of guanidine in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotrizole (HOBt) [17], and purified by silica gel column chromatography (EtOAc/CH_3OH/Et_3N = 15:1:0.1) one time to be free from by-products and guanidine.

2.2. Biological activity

All synthesized compounds, along with reference compound cariporide, were evaluated in rat platelet swelling assay (PSA) for NHE1 inhibitory activity screening. The experiment procedure was similar as in the literature [18], with only minor modifications. 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. The PSA results showed that most of the tested compounds did inhibit rat platelet NHE1 in a concentration-dependent manner. Twelve compounds showed more potent NHE1 inhibitory activity than cariporide. The activities of compounds **7e**, **7h** and **7j** ($IC_{50} = 0.073 \pm 0.021$, 0.084 ± 0.012 and 0.068 ± 0.021 nmol/L, respectively) were two orders of magnitude higher than that of cariporide (30.7 ± 2.5 nmol/L).

Compounds **7e**, **7h** and **7j**, which showed a good NHE1 inhibitory activity, together with TG-6, were evaluated *in vitro* for their effect on cardiomyocytes viability subjected to hypoxia/reoxygenation injury induced by $Na_2S_2O_4$ (Tables 3 and 4). The protective effect of these compounds against hypoxia/reoxygenation injury induced by $Na_2S_2O_4$ was determined in primary rat cardiomyocytes hypoxia/reoxygenation model. After 2 h of hypoxia led by $Na_2S_2O_4$,

Table 1
Structures and characterization table of target compounds **7a**–**7v**.



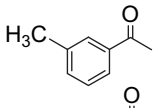
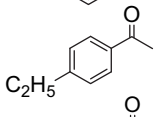
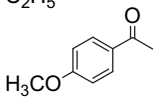
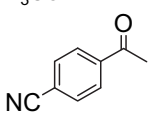
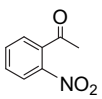
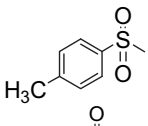
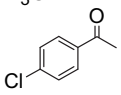
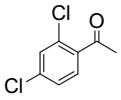
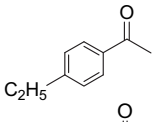
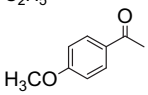
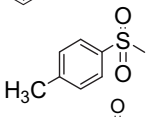
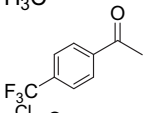
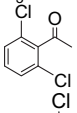
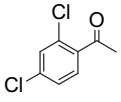
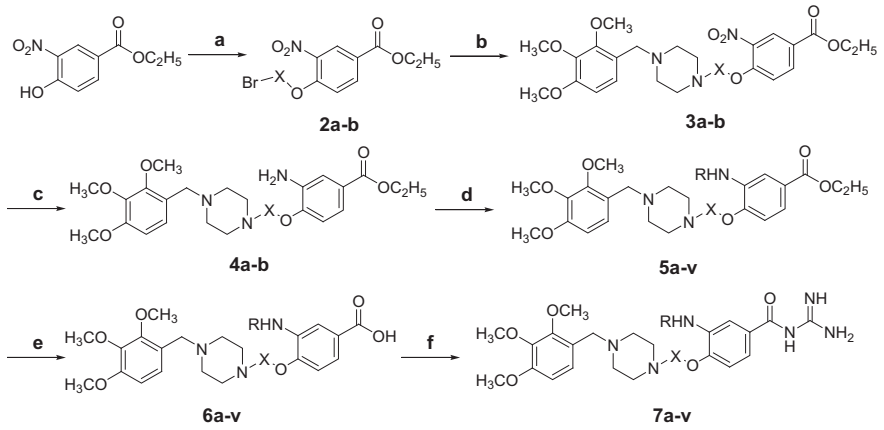
Compd. no.	X	R	Mol. formula	M.p. (°C)	Analysis (%)		
					Found (calculated)		
					C	H	N
7a	$(CH_2)_2$		$C_{32}H_{40}N_6O_6$	141–145	63.27 (63.56)	7.06 (6.66)	13.79 (13.89)
7b	$(CH_2)_2$		$C_{33}H_{42}N_6O_6$	180–185	63.97 (64.06)	6.98 (6.84)	13.74 (13.58)
7c	$(CH_2)_2$		$C_{32}H_{40}N_6O_7 \cdot 1H_2O$	184–189	60.35 (60.17)	6.72 (6.62)	13.02 (13.15)
7d	$(CH_2)_2$		$C_{32}H_{37}N_7O_6 \cdot 0.5H_2O$	149–152	61.04 (61.52)	6.04 (6.13)	15.69 (15.69)
7e	$(CH_2)_2$		$C_{31}H_{37}N_7O_8 \cdot 0.5H_2O$	192–195	58.24 (57.75)	6.20 (5.94)	14.81 (15.21)

Table 1 (*continued*)

Compd. no.	X	R	Mol. formula	M.p. (°C)	Analysis (%)		
					Found (calculated)		
					C	H	N
7f	$(CH_2)_2$		$C_{31}H_{40}N_6O_7S$	202–205	57.83 (58.11)	6.45 (6.29)	12.76 (13.11)
7g	$(CH_2)_2$		$C_{31}H_{37}N_6O_6Cl\cdot 1H_2O$	162–166	57.90 (57.89)	5.94 (6.11)	12.84 (13.06)
7h	$(CH_2)_2$		$C_{31}H_{36}N_6O_6Cl_2\cdot 1H_2O$	146–148	55.35 (54.95)	5.43 (5.65)	11.89 (12.40)
7i	$(CH_2)_2$		$C_{29}H_{36}N_6O_6S\cdot 1H_2O$	160–162	56.51 (56.66)	6.11 (6.23)	13.38 (13.67)
7j	$(CH_2)_2$		$C_{30}H_{38}N_6O_6S\cdot 1H_2O$	120–125	57.27 (57.30)	6.30 (6.41)	13.06 (13.36)
7k	$(CH_2)_2$		$C_{29}H_{36}N_6O_6S\cdot 0.5H_2O$	167–170	57.74 (57.50)	6.69 (6.15)	13.52 (13.87)
7l	$(CH_2)_3$		$C_{32}H_{40}N_6O_6.0.5H_2O$	185–187	62.68 (62.63)	6.87 (6.73)	13.89 (13.69)
7m	$(CH_2)_3$		$C_{34}H_{44}N_6O_6$	172–176	64.26 (64.54)	6.85 (7.00)	12.92 (13.28)
7n	$(CH_2)_3$		$C_{33}H_{42}N_6O_7\cdot 1H_2O$	194–196	60.66 (60.72)	6.59 (6.79)	12.88 (12.87)
7o	$(CH_2)_3$		$C_{32}H_{39}N_7O_8$	138–142	58.85 (59.15)	6.30 (6.05)	15.12 (15.09)
7p	$(CH_2)_3$		$C_{32}H_{42}N_6O_7S.0.5H_2O$	198–202	57.84 (57.90)	6.72 (6.53)	12.31 (12.66)
7q	$(CH_2)_3$		$C_{33}H_{39}N_6O_6F_3\cdot 1H_2O$	148–152	57.47 (57.38)	6.13 (5.98)	11.80 (12.16)
7r	$(CH_2)_3$		$C_{32}H_{38}N_6O_6Cl_2$	140–145	56.77 (57.06)	5.71 (5.68)	12.58 (12.47)
7s	$(CH_2)_3$		$C_{32}H_{38}N_6O_6Cl_2$	54–58	57.21 (57.06)	5.64 (5.68)	12.38 (12.47)
7t	$(CH_2)_3$		$C_{30}H_{38}N_6O_6S\cdot 1H_2O$	174–178	56.91 (57.31)	6.32 (6.41)	13.17 (13.36)
7u	$(CH_2)_3$		$C_{31}H_{40}N_6O_6S\cdot 0.5H_2O$	183–186	58.82 (58.75)	6.81 (6.52)	12.94 (13.26)
7v	$(CH_2)_3$		$C_{30}H_{38}N_6O_6S\cdot 0.5H_2O$	183–185	58.14 (58.14)	6.34 (6.34)	13.18 (13.56)



Scheme 1. Reagents and conditions: (a) 1,2-dibromo ethane/1,3-dibromo propane, K_2CO_3, CH_3CN, reflux; (b) trimetazidine, K_2CO_3, CH_3CN, 20–80 °C; (c) Fe/HCl/50% (v/v) ethanol, reflux; (d) CH_2Cl_2/Et_3N, 0–5 °C; (e) methanol/K_2CO_3, reflux; (f) guanidine/HOBt/EDCI/DMF, room temperature.

the cardiomyocytes were reoxygenated for 4 h. TG-6, **7e**, **7h** and **7j** were given 1 h before reoxygenation. The evaluation of cardiomyocyte survival in a dose-dependent manner was measured as an index of the ratio of treatment group OD to normal OD. Compared with the model group, TG-6 and **7j** could significantly improve the survival at 10^{-4} mol/L ($P < 0.01$). TG-6 and **7j** at 10^{-5} mol/L, **7e** and **7h** at 10^{-4} mol/L could improve the survival ($P < 0.05$). There was no significant difference among the compounds in the myocardial survival at 10^{-6} mol/L.

3. Conclusion

In summary, a series of novel substituted benzoylguanidine derivatives were designed, synthesized and evaluated for their NHE1 inhibitions. Most compounds showed more potent NHE1 inhibitory activity than cariporide, and **7e**, **7h** and **7j** were more potent than others. Further testing showed that, **7j** could excellently improve the cardiomyocytes survival rate against hypoxia/reoxygenation injury induced by $Na_2S_2O_4$.

Preliminary structure–activity relationship analysis indicated that, introduction of electron-withdrawing group like nitro, chloro or methylsulfonyl group to benzene ring of the amide group caused better NHE1 inhibitory potency. Moreover, compounds whose amide group has thiophene ring as core structure also exhibited a good NHE1 inhibitory activity. On the contrary, the lengthening of the connection chain between piperazine nitrogen and para-position of benzoylguanidine from two carbons to three carbons was likely to lead to decreased activity (0.073 ± 0.021 nM of **7e** vs 0.31 ± 0.07 nM of **7o**, 0.084 ± 0.012 nM of **7h** vs 55.7 ± 7.1 nM of **7s**, 0.091 ± 0.017 nM of **7i** vs 1.50 ± 0.26 nM of **7t**, 0.068 ± 0.021 nM of **7j** vs 0.92 ± 0.08 nM of **7u**).

Table 2
Activities of target compounds **7a**–**7v**.

Compd.	IC_{50}[a] (nM)	Compd.	IC_{50}[a] (nM)
Cariporide	30.7 ± 2.5	TG-6	1.82 ± 0.36
TG-7	1.06 ± 0.18	**7l**	139 ± 11.8
7a	154 ± 9.5	**7m**	49.3 ± 11.0
7b	93.5 ± 7.5	**7n**	225 ± 13.2
7c	3020 ± 245.8	**7o**	0.31 ± 0.07
7d	142 ± 12.5	**7p**	0.56 ± 0.07
7e	0.073 ± 0.021	**7q**	1.10 ± 0.36
7f	0.11 ± 0.04	**7r**	1530 ± 55.7
7g	125 ± 6.2	**7s**	55.7 ± 7.1
7h	0.084 ± 0.012	**7t**	1.50 ± 0.26
7i	0.091 ± 0.017	**7u**	0.92 ± 0.08
7j	0.068 ± 0.021	**7v**	0.67 ± 0.16
7k	0.53 ± 0.09		

[a] Drug concentration to achieve half-maximal inhibition of acid-induced swelling in rat platelets.

4. Experiment protocol

Melting points were determined on a RDCSY-I capillary apparatus and were uncorrected. The IR spectra (in KBr pellets) were recorded on a Nicolet Impact 410 spectrophotometer. The ^1H NMR and ^{13}C 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 in parts per million with tetramethylsilane (TMS). Mass spectra were recorded on an Agilent 1100 series LC/MSD Tarp (SL). All solvents were purchased from commercial sources and used as received unless otherwise stated.

4.1. General procedure for synthesis of compounds *2*

To a solution of ethyl 4-hydroxy-3-nitrobenzoate (5.1 g, 24 mmol) in anhydrous acetonitrile (70 mL), comminuted potassium carbonate powder (10.4 g, 75 mmol) was added with stirring. To the resulting mixture was then added 1,2-dibromo ethane (1,3-dibromopropane) (48 mmol) and heated to reflux for 4 h. After filtering, the filtrate was concentrated to dryness under reduced pressure to give crude **2a** and **2b**, which were used for the next step without further purification.

4.1.1. Ethyl 4-(2-bromoethoxy)-3-nitrobenzoate (*2a*)

Crude yield 40.6%, light yellow solid, m.p. 49–50 °C.

4.1.2. Ethyl 4-(3-bromopropoxy)-3-nitrobenzoate (*2b*)

Crude yield 60.0%, yellow oil.

Table 3
Effect of TG-6, **7e**, **7h** and **7j** on cardiomyocytes viability subjected to hypoxia/reoxygenation injury induced by $Na_2S_2O_4$ ($\bar{X} \pm s$, $n = 6$).

Compd.	Concn.		
	10^{-4} mol/L	10^{-5} mol/L	10^{-6} mol/L
Normal (A_{490})	0.768 ± 0.141	0.736 ± 0.095	0.735 ± 0.063
Model (%)	0.334 ± 0.045##	0.364 ± 0.040##	0.346 ± 0.059##
TG-6 (%)	0.532 ± 0.025**	0.440 ± 0.063*	0.370 ± 0.035
7e (%)	0.434 ± 0.077*	0.379 ± 0.058	0.353 ± 0.054
7h (%)	0.488 ± 0.084*	0.409 ± 0.080	0.362 ± 0.067
7j (%)	0.556 ± 0.077**	0.454 ± 0.061*	0.391 ± 0.085

##$P < 0.01$ vs normal; *$P < 0.05$, **$P < 0.01$ vs model.

Table 4
Effect of TG-6, **7e**, **7h** and **7j** on cardiomyocytes survival rate subjected to hypoxia/reoxygenation injury induced by $Na_2S_2O_4$ ($\overline{X} \pm s$, $n = 6$).

Compd.	Concn.		
	10^{-4} mol/L	10^{-5} mol/L	10^{-6} mol/L
Model (%)	43.43 ± 1.45	49.46 ± 6.20	47.10 ± 4.56
TG-6 (%)	69.24 ± 4.28**	59.78 ± 4.74**	50.41 ± 5.29
7e (%)	56.48 ± 3.60**	51.45 ± 3.86	48.05 ± 4.85
7h (%)	58.33 ± 4.46**	55.52 ± 5.70	49.27 ± 4.21
7j (%)	72.41 ± 6.94**	61.63 ± 6.16**	53.22 ± 7.58

**$P < 0.01$ vs model.

4.2. General procedure for synthesis of compounds **3**

Preparation of free trimetazidine: Trimetazidine hydrochloride (3 g, 8.8 mmol) was dissolved in water (10 mL). An aqueous solution of potassium hydroxide (40 g in 100 mL of water) was then added, the addition of alkali was continued till the pH of the solution was raised to ca. 11. The basified solution was extracted with ethyl acetate (3×10 mL). The combined organic phases were washed with saturated sodium chloride solution, dried over anhydrous sodium sulfate and concentrated under vacuum to give free trimetazidine (1.88 g, 6.7 mmol).

Free trimetazidine (1.88 g, 6.7 mmol) was dissolved in anhydrous acetonitrile (10 mL). To this solution was added a solution of ethyl 4-(2(3)-bromoethoxy)-3-nitrobenzoate (**2**) (4 mmol) in anhydrous acetonitrile (20 mL), followed by anhydrous potassium carbonate (3.1 g, 22 mmol) and the reaction mixture was refluxed for 4 h. After filtering, the filtrate was concentrated to dryness under reduced pressure. The residue was dissolved in ethyl acetate, and the resulting solution was washed with water to remove the excess free trimetazidine. The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by silica gel column chromatography (petroleum/acetone = 8:1) to give **3a** and **3b**.

4.2.1. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-nitro benzoate (**3a**)

Yield 85.0%, light yellow solid, m.p. 105–109 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.36 (t, 3H, $J = 7.2$ Hz, $-CH_3$), 2.52–2.62 (m, 8H, –N⌒N–), 2.87 (t, 2H, $J = 5.7$ Hz, $-CH_2$), 3.49 (s, 2H, $-CH_2$), 3.84 (s, 3H, $-OCH_3$), 3.86 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 4.29 (t, 2H, $J = 5.7$ Hz, $-CH_2$), 4.33 (q, 2H, $J = 7.2$ Hz, $-CH_2$), 6.62 (d, 1H, $J = 8.7$ Hz, ArH), 6.98 (d, 1H, $J = 8.4$ Hz, ArH), 7.09 (d, 1H, $J = 9.0$ Hz, ArH), 8.18 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 9$ Hz, ArH), 8.47 (d, 1H, $J = 2.1$ Hz, ArH).

4.2.2. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-nitro benzoate (**3b**)

Yield 83.4%, yellow solid, m.p. 87–92 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.39 (t, 3H, $J = 7.1$ Hz, $-CH_3$), 2.02 (q, 2H, $J = 6.7$ Hz, $-CH_2$), 2.49–2.56 (m, 10H, –N⌒N–, $-CH_2$), 3.48 (s, 2H, $-CH_2$), 3.85 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 3.88 (s, 3H, $-OCH_3$), 4.23 (t, 2H, $J = 6.2$ Hz, $-CH_2$), 4.38 (q, 2H, $J = 7.2$ Hz, $-CH_2$), 6.62 (d, 1H, $J = 8.5$ Hz, ArH), 7.00 (d, 1H, $J = 8.5$ Hz, ArH), 7.13 (d, 1H, $J = 8.9$ Hz, ArH), 8.18 (dd, 1H, $J_1 = 2.2$ Hz, $J_2 = 8.8$ Hz, ArH), 8.47 (d, 1H, $J = 2.1$ Hz, ArH).

4.3. General procedure for synthesis of compounds **4**

A mixture of ethyl 4-(2(3)-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy(propoxy))-3-nitro benzoate (**3**) (15 mmol), 50% (v/v) ethanol (105 mL), reduced iron (6.7 g, 0.12 mol) and acid alcohol (prepared by adding 0.1 mL concentrated hydrochloric acid to 1 mL of 50% (v/v) ethanol) (1 mL) was refluxed with mechanical agitation for 1 h. The reaction mixture was filtered under this temperature, and the filtrate was concentrated to dryness under reduced pressure to give compounds **4a** and **4b**.

4.3.1. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-amino benzoate (**4a**)

Yield 98%, light yellow solid, m.p. 124–126 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.36 (t, 3H, $J = 7.2$, $-CH_3$), 2.56–2.62 (m, 8H, –N⌒N–), 2.83 (t, 2H, $J = 5.1$ Hz, $-CH_2$), 3.51 (s, 2H, $-CH_2$), 3.85 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 3.88 (s, 3H, $-OCH_3$), 4.16 (t, 2H, $J = 5.1$ Hz, $-CH_2$), 4.31 (q, 2H, $J = 7.2$, $-CH_2$), 6.63 (d, 1H, $J = 8.7$ Hz, ArH), 6.77 (d, 1H, $J = 8.4$, ArH), 6.90 (d, 1H, $J = 8.4$, ArH), 7.38–7.45 (m, 2H, ArH).

4.3.2. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl) propoxy)-3-amino benzoate (**4b**)

Yield 98%, light yellow solid, m.p. 180–185 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.36 (t, 3H, $J = 7.1$ Hz, $-CH_3$), 2.01 (q, 2H, $J = 6.3$ Hz, $-CH_2$), 2.50–2.55 (m, 10H, –N⌒N–, $-CH_2$), 3.49 (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, 2H, $J = 6.3$ Hz, $-CH_2$), 4.31 (q, 2H, $J = 7.1$ Hz, $-CH_2$), 6.62 (d, 1H, $J = 8.55$ Hz, ArH), 6.78 (d, 1H, $J = 8.55$ Hz, ArH), 6.98 (d, 1H, $J = 8.4$ Hz, ArH), 7.38 (1H, d, $J = 2.05$ Hz, ArH), 7.44 (dd, 1H, $J_1 = 2.05$ Hz, $J_2 = 8.4$ Hz, ArH).

4.4. General procedure for synthesis of compounds **5**

A mixture of ethyl 4-(2(3)-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy(propoxy))-3-amino benzoate (**4**) (2 mmol) and triethylamine (0.4 mL) in dichloromethane (20 mL) was cooled to 0–5 °C. A solution of appropriate acyl chloride (3 mmol) in dichloromethane (1 mL) was then added dropwise. After stirring at room temperature for 2 h, the reaction solution was washed three times with saturated sodium bicarbonate solution and once with saturated sodium chloride solution. The organic phase was dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by silica gel column chromatography (petroleum/acetone/Et_3N = 8:1:0.1) to give compounds **5a**–**5v**.

4.4.1. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(3-methylbenzamino)benzoate (**5a**)

Yield 63.2%, yellow oil.

4.4.2. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(4-ethylbenzamino)benzoate (**5b**)

Yield 49.0%, white solid, m.p. 71–77 °C.

4.4.3. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(4-methoxybenzamino)benzoate (**5c**)

Yield 27%, yellow oil.

4.4.4. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(4-cyanobenzamino)benzoate (**5d**)

Yield 53.9%, white solid, m.p. 100–105 °C.

4.4.5. N-(2-(2-(4-(2,3,4-Trimethoxybenzyl)piperazin-1-yl)ethoxy)-5-(guanidinocarbonyl)benzyl)-2-nitrobenzamide (**5e**)

Yield 36%, yellow solid.

4.4.6. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(4-methylphenylsulfonamino)benzoate (**5f**)

Yield 37.9%, yellow oil.

4.4.7. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(4-chlorobenzamino)benzoate (**5g**)

Yield 51.8%, yellow oil.

Table 5
IR, MS and ^{1}H NMR data of compounds **7a**–**7v**.

NO.	IR (cm^{-1})	MS (m/z)[a]	^{1}H NMR (δ)
7a	3396, 2938, 2822, 1652, 1533, 1495, 1344, 1268, 1096, 1014, 789.	605.3 $[M+H]^+$	[c,d]2.40 (s, 3H, $-CH_3$), 2.39–2.54 (m, 8H, –N⟨⟩N–), 2.78 (t, 2H, $J=5.1$ Hz, $-CH_2$), 3.38 (s, 2H, $-CH_2$), 3.84 (s, 3H, $-OCH_3$), 3.85 (s, 3H, $-OCH_3$), 3.86 (s, 3H, $-OCH_3$), 4.25 (t, 2H, $J=5.1$ Hz, $-CH_2$), 6.61 (d, 1H, $J=8.4$ Hz, ArH), 6.92 (d, 1H, $J=8.4$ Hz, ArH), 6.99 (d, 1H, $J=8.7$ Hz, ArH), 7.36–7.38 (m, 2H, ArH), 7.66–7.70 (m, 2H, ArH), 7.98 (dd, 1H, $J_1=1.8$ Hz, $J_2=8.55$ Hz, ArH), 8.78 (d, 1H, $J=1.8$ Hz, ArH), 8.85 (s, 1H, NHCO)
7b	3440, 3385, 2935, 2821, 1679, 1535, 1493, 1346, 1270, 1097, 1011, 788	619.4 $[M+H]^+$	[c,d]1.28 (t, 3H, $J=7.5$ Hz, $-CH_3$), 2.41–2.54 (m, 8H, –N⟨⟩N–), 2.74 (q, 2H, $J=7.5$ Hz, $-CH_2$), 2.76 (t, 2H, $J=5.4$ Hz, $-CH_2$), 3.40 (s, 2H, $-CH_2$), 3.84 (s, 3H, $-OCH_3$), 3.85 (s, 3H, $-OCH_3$), 3.86 (s, 3H, $-OCH_3$), 4.25 (t, 2H, $J=5.4$ Hz, $-CH_2$), 6.62 (d, 1H, $J=8.7$ Hz, ArH), 6.93 (d, 1H, $J=8.7$ Hz, ArH), 6.99 (d, 1H, $J=8.7$ Hz, ArH), 7.34 (d, 2H, $J=8.1$ Hz, ArH), 7.83 (d, 2H, $J=8.1$ Hz, ArH), 7.97 (d, 1H, $J=8.4$ Hz, ArH), 8.71 (s, 1H, ArH), 8.82 (s, 1H, NHCO)
7c	3326, 2934, 2817, 1650, 1607, 575, 1534, 1511, 1493, 1347, 1252, 1093, 1011, 788	621.3 $[M-H]^+$	[c,d]2.43–2.54 (m, 8H, –N⟨⟩N–), 2.77 (t, 2H, $J=5.3$ Hz, $-CH_2$), 3.40 (s, 2H, $-CH_2$), 3.84 (s, 3H, $-OCH_3$), 3.85 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 3.89 (s, 3H, $-OCH_3$), 4.22 (t, 2H, $J=5.3$ Hz, $-CH_2$), 6.61 (d, 1H, $J=8.6$ Hz, ArH), 6.93 (d, 1H, $J=8.6$ Hz, ArH), 6.97–7.00 (m, 3H, ArH), 7.85–7.89 (m, 2H, ArH), 7.94 (dd, 1H, $J_1=2.0$ Hz, $J_2=8.5$ Hz, ArH), 8.7 (d, 1H, $J=2.0$ Hz, ArH), 8.79 (s, 1H, NHCO)
7d	3339, 2938, 2823, 1689, 1535, 1494, 1344, 1269, 1095, 1010, 787	616.2 $[M+H]^+$	[b,d]2.27–2.50 (m, 8H, –N⟨⟩N–), 2.72 (t, 2H, $J=5.1$ Hz, $-CH_2$), 3.29 (s, 2H, $-CH_2$), 3.73 (s, 3H, $-OCH_3$), 3.74 (s, 3H, $-OCH_3$), 3.78 (s, 3H, $-OCH_3$), 4.19 (t, 2H, $J=5.1$ Hz, $-CH_2$), 6.76 (d, 1H, $J=8.4$ Hz, ArH), 6.89 (d, 1H, $J=8.4$ Hz, ArH), 7.13 (d, 1H, $J=8.4$ Hz, ArH), 7.93 (dd, 1H, $J_1=1.8$ Hz, $J_2=8.4$ Hz, ArH), 8.03 (d, 2H, $J=8.1$ Hz, ArH), 8.08 (d, 2H, $J=8.1$ Hz, ArH), 8.36 (s, 1H, ArH), 9.78 (d, 1H, NHCO)
7e	3386, 2936, 2822, 1668, 1528, 1494, 1344, 1269, 1098, 1010, 783	636.3 $[M+H]^+$	[c,d]2.72–2.91 (m, 8H, –N⟨⟩N–), 3.06 (t, 2H, $-CH_2$, $J=5.1$ Hz), 3.68 (s, 2H, $-CH_2$), 3.85 (s, 3H, $-OCH_3$), 3.86 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 4.21 (t, 2H, $J=5.1$ Hz, $-CH_2$), 6.61 (d, 1H, $J=8.7$ Hz, ArH), 6.88 (d, 1H, $J=8.4$ Hz, ArH), 7.03 (d, 1H, $J=8.7$ Hz, ArH), 7.64–7.75 (m, 3H, ArH), 7.98 (d, 1H, $J=8.7$ Hz, ArH), 8.11 (d, 1H, $J=7.8$ Hz, ArH), 8.60 (s, 1H, ArH), 9.51 (s, 1H, NHCO)
7f	3348, 2928, 1695, 1499, 1466, 1320, 1269, 1095, 1012, 664	641.3 $[M+H]^+$	[c,d]2.31 (s, 3H, $-CH_3$), 2.44–2.49 (m, 8H, –N⟨⟩N–), 2.50 (t, 2H, $J=5.2$ Hz, $-CH_2$), 3.42 (s, 2H, $-CH_2$), 3.76 (s, 3H, $-OCH_3$), 3.77 (s, 3H, $-OCH_3$), 3.78 (s, 3H, $-OCH_3$), 3.87 (t, 2H, $J=5.2$ Hz, $-CH_2$), 6.76 (d, 1H, $J=8.5$ Hz, ArH), 6.93 (d, 1H, $J=8.5$ Hz, ArH), 6.98 (d, 1H, $J=8.5$ Hz, ArH), 7.27 (d, 2H, $J=8.0$ Hz, ArH), 7.52 (d, 2H, $J=8.0$ Hz, ArH), 7.83 (dd, 1H, $J_1=1.9$ Hz, $J_2=8.5$ Hz, ArH), 8.04 (d, 1H, $J=1.9$ Hz, ArH)
7g	3433, 2934, 2825, 1687, 1536, 1495, 1353, 1270, 1096, 1012, 787	625.2 $[M+H]^+$	[c,d]2.40–2.53 (m, 8H, –N⟨⟩N–), 2.76 (t, 2H, $J=5.1$ Hz, $-CH_2$), 3.40 (s, 2H, $-CH_2$), 3.84 (s, 3H, $-OCH_3$), 3.85 (s, 3H, $-OCH_3$), 3.86 (s, 3H, $-OCH_3$), 4.24 (t, 2H, $-CH_2$, $J=5.1$ Hz), 6.63 (d, 1H, $J=8.4$ Hz, ArH), 6.93 (d, 1H, $J=8.4$ Hz, ArH), 6.98 (d, 1H, $J=8.7$ Hz, ArH), 7.49 (d, 2H, $J=8.7$ Hz, ArH), 7.87 (d, 2H, $J=8.4$ Hz, ArH), 7.95 (dd, 1H, $J_1=1.8$ Hz, $J_2=8.7$ Hz, ArH), 8.67 (d, 1H, $J=1.8$ Hz, ArH), 8.98 (s, 1H, NHCO).
7h	3372, 2938, 2822, 1671, 1602, 1582, 1532, 1494, 1347, 1270, 1097, 1009, 786	659.3 $[M+H]^+$	[c,e]2.22–2.45 (m, 8H, –N⟨⟩N–), 2.67 (t, 2H, $J=5.4$ Hz, $-CH_2$), 3.33 (s, 2H, $-CH_2$), 3.84 (s, 3H, $-OCH_3$), 3.86 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 4.21 (t, 2H, $J=5.4$ Hz, $-CH_2$), 6.62 (d, 1H, $J=8.5$ Hz, ArH), 6.91 (d, 1H, $J=8.5$ Hz, ArH), 7.00 (d, 1H, $J=8.6$ Hz, ArH), 7.37 (dd, 1H, $J_1=1.9$ Hz, $J_2=8.2$ Hz, ArH), 7.50 (d, 1H, $J=1.9$ Hz, ArH), 7.71 (d, 1H, $J=8.2$ Hz, ArH), 7.98 (dd, 1H, $J_1=1.9$ Hz, $J_2=8.6$ Hz, ArH), 8.77 (d, 1H, $J=1.8$ Hz, ArH), 9.33 (s, 1H, NHCO)

Table 5 (*continued*)

NO.	IR (cm^{-1})	MS (m/z)[a]	^1H NMR (δ)
7i	3453, 3376, 2942, 1706, 1537, 1462, 1344, 1264, 1097, 1051, 799	597.3 $[M+H]^+$	[c,d]2.47–2.58 (m, 8H, –N◯N–), 2.82 (t, 2H, J = 5.1 Hz, $-CH_2$), 3.44 (s, 2H, $-CH_2$), 3.85 (s, 3H, $-OCH_3$), 3.86 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 4.24 (t, 2H, J = 5.1 Hz, $-CH_2$), 6.62 (d, 1H, J = 8.4 Hz, ArH), 6.93–6.98 (m, 2H, ArH), 7.12–7.14 (m, 1H, ArH), 7.56–7.58 (m, 1H, ArH), 7.69–7.70 (m, 1H, ArH), 7.96 (dd, 1H, J_1 = 1.8 Hz, J_2 = 8.4 Hz, ArH), 8.71 (s, 1H, ArH), 8.76 (s, 1H, NHCO)
7j	3427, 3341, 2936, 1702, 1535, 1462, 1350, 1271, 1099, 1036, 750	611.3 $[M+H]^+$	[c,e]2.41–2.53 (m, 8H, –N◯N–), 2.62 (s, 3H, $-CH_3$), 2.78 (t, 2H, J = 5.5 Hz, $-CH_2$), 3.41 (s, 2H, $-CH_2$), 3.85 (s, 3H, $-OCH_3$), 3.86 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 4.23 (t, 2H, J = 5.5 Hz, $-CH_2$), 6.62 (d, 1H, J = 8.5 Hz, ArH), 6.93–6.97 (m, 3H, ArH), 7.37 (d, 1H, J = 5.0 Hz, ArH), 7.95 (dd, 1H, J_1 = 2.0 Hz, J_2 = 8.5 Hz, ArH), 8.49 (s, 1H, ArH), 8.73 (d, 1H, J = 2.0 Hz, NHCO)
7k	3380, 2938, 1678, 1531, 1496, 1358, 1272, 1095, 1011, 790	597.3 $[M+H]^+$	[c,d]2.46–2.56 (m, 8H, –N◯N–), 2.80 (t, 2H, J = 5.1 Hz, $-CH_2$), 3.44 (s, 2H, $-CH_2$), 3.84 (s, 3H, $-OCH_3$), 3.85 (s, 3H, $-OCH_3$), 3.86 (s, 3H, $-OCH_3$), 4.23 (t, 2H, J = 5.1 Hz, $-CH_2$), 6.62 (d, 1H, J = 8.5 Hz, ArH), 6.93–6.98 (m, 2H, ArH), 7.37–7.39 (m, 1H, ArH), 7.53 (dd, 1H, J_1 = 1.2 Hz, J_2 = 5.0 Hz, ArH), 7.96 (dd, 1H, J_1 = 2.0 Hz, J_2 = 8.5 Hz, ArH), 8.05–8.06 (m, 1H, ArH), 8.75 (s, 1H, ArH), 8.77 (d, 1H, J = 2.0 Hz, NHCO)
7l	3366, 2933, 2848, 1673, 1648, 1531, 1465, 1356, 1267, 1095, 1010, 790	605.3 $[M+H]^+$	[c,d]2.02 (m, 2H, $-CH_2$), 2.50–2.55 (m, 10H, –N◯N–, $-CH_2$), 3.49 (s, 2H, $-CH_2$), 3.86 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 3.88 (s, 3H, $-OCH_3$), 4.16 (t, 2H, J = 6.3 Hz, $-CH_2$), 6.63 (d, 1H, J = 8.7 Hz, ArH), 6.97–7.00 (m, 2H, ArH), 7.49–7.61 (m, 3H, ArH), 7.87–7.89 (m, 2H, ArH), 7.98 (dd, 1H, J_1 = 2.1 Hz, J_2 = 8.5 Hz, ArH), 8.45 (s, 1H, ArH), 8.82 (s, 1H, NHCO)
7m	3425, 2935, 2822, 1673, 1602, 1532, 1494, 1350, 1269, 1095, 1011, 788	633.4 $[M+H]^+$	[c,d]1.28 (m, 3H, J = 7.5 Hz, $-CH_3$), 2.04 (m, 2H, $-CH_2$), 2.50–2.54 (m, 10H, –N◯N–, $-CH_2$), 2.73 (q, 2H, J = 7.5 Hz, $-CH_2$), 3.49 (s, 2H, $-CH_2$), 3.85 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 3.88 (s, 3H, $-OCH_3$), 4.17 (t, 2H, J = 6.3 Hz, $-CH_2$), 6.62 (d, 1H, J = 8.7 Hz, ArH), 6.96–6.99 (m, 2H, ArH), 7.33 (d, 2H, J = 8.4 Hz, ArH), 7.79 (d, 2H, J = 8.4 Hz, ArH), 7.96 (dd, 1H, J_1 = 1.8 Hz, J_2 = 8.55 Hz, ArH), 8.42 (s, 1H, ArH), 8.76 (d, 1H, J = 1.8 Hz, NHCO)
7n	3440, 2927, 2850, 1691, 1608, 1539, 1463, 1384, 1269, 1095, 1036, 743	635.4 $[M+H]^+$	[c,e]2.05 (m, 2H, $-CH_2$), 2.51–2.54 (m, 10H, –N◯N–, $-CH_2$), 3.49 (s, 2H, $-CH_2$), 3.86 (s, 3H, $-OCH_3$), 3.88 (s, 3H, $-OCH_3$), 3.89 (s, 3H, $-OCH_3$), 3.90 (s, 3H, $-OCH_3$), 4.18 (t, 2H, J = 6.2 Hz, $-CH_2$), 6.64 (d, 1H, J = 8.5 Hz, ArH), 6.98–6.99 (m, 2H, ArH), 7.01 (d, 2H, J = 8.8 Hz, ArH), 7.86 (d, 2H, J = 8.8 Hz, ArH), 7.95 (d, 1H, J = 8.62 Hz, ArH), 8.35 (s, 1H, ArH), 8.7 (s, 1H, NHCO)
7o	3376, 2939, 2821, 1673, 1601, 1528, 1494, 1346, 1267, 1096, 1010, 786	650.4 $[M+H]^+$	[c,e]1.98 (m, 2H, $-CH_2$), 2.47–2.50 (m, 10H, –N◯N–, $-CH_2$), 3.47 (s, 2H, $-CH_2$), 3.84 (s, 3H, $-OCH_3$), 3.86 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 4.13 (t, 2H, J = 6.9 Hz, $-CH_2$), 6.63 (d, 1H, J = 8.5 Hz, ArH), 6.94–6.97 (m, 2H, ArH), 7.61–7.74 (m, 3H, ArH), 7.95 (d, 1H, J = 8.5 Hz, ArH), 8.04 (d, 1H, J = 8.2 Hz, ArH), 8.73 (d, 1H, J = 1.5 Hz, ArH)
7p	3353, 2944, 2824, 1735, 1688, 1600, 1495, 1301, 1269, 1093, 1012, 767	655.3 $[M+H]^+$	[c,e]1.74 (m, 2H, $-CH_2$), 2.31––2.34 (m, 5H, $-CH_2$, $-CH_3$), 2.45–2.51 (m, 8H, –N◯N–), 3.49 (s, 2H, $-CH_2$), 3.79 (t, 2H, J = 6.4 Hz, $-CH_2$), 3.85 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 3.88 (s, 3H, $-OCH_3$), 6.63 (d, 1H, J = 8.5 Hz, ArH), 6.74 (d, 1H, J = 8.5 Hz, ArH), 6.99 (d, 1H, J = 8.5 Hz, ArH), 7.15 (d, 2H, J = 8.0 Hz, ArH), 7.56 (d, 2H, J = 8.0 Hz, ArH), 7.89 (dd, 1H, J_1 = 2.0 Hz, J_2 = 8.6 Hz, ArH), 8.22 (d, 1H, J = 2.0 Hz, ArH)
7q	3420, 2935, 2825, 1704, 1605, 1537, 1464, 1326, 1267, 1127, 1095, 1013, 758	673.4 $[M+H]^+$	[c,d]2.04 (m, 2H, $-CH_2$), 2.48–2.53 (m, 10H, –N◯N–, $-CH_2$), 3.49 (s, 2H, $-CH_2$), 3.85 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 3.88 (s, 3H, $-OCH_3$), 4.18 (t, 2H, J = 6.3 Hz, $-CH_2$), 6.63 (d, 1H, J = 8.7 Hz, ArH), 6.96–7.01 (m, 2H, ArH), 7.78 (d, 2H, J = 8.4 Hz, ArH), 7.97–8.01 (m, 3H, ArH), 8.46 (s, 1H, ArH), 8.81 (d, 1H, J = 1.8 Hz, NHCO)
7r	3435, 2937, 2817, 1667, 1601, 1527, 1496, 1362, 1273, 1096, 1014, 796	673.3 $[M+H]^+$	[c,d]1.97 (m, 2H, $-CH_2$), 2.45–2.51 (m, 10H, –N◯N–, $-CH_2$), 3.47 (s, 2H, $-CH_2$), 3.85 (s, 3H, $-OCH_3$), 3.86 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 4.14 (t, 2H, J = 6.3 Hz, $-CH_2$), 6.63 (d, 1H, J = 8.4 Hz, ArH), 6.96–7.00 (m, 2H, ArH), 7.30–7.41 (m, 3H, ArH), 7.95 (s, 1H, ArH), 8.01 (dd, 1H, J_1 = 1.5 Hz, J_2 = 8.8 Hz, ArH), 8.7 (d, 1H, J = 1.5 Hz, NHCO)

(*continued on next page*)

Table 5 (*continued*)

NO.	IR (cm^{-1})	MS (m/z)[a]	^1H NMR (δ)
7s	3396, 2937, 2822, 1656, 1601, 1533, 1464, 1348, 1266, 1097, 1009, 786	673.3 $[M+H]+$	[c,e]1.99 (m, 2H, $-CH_2$), 2.47–2.50 (m, 10H, –N⟨⟩N–, $-CH_2$), 3.48 (s, 2H, $-CH_2$), 3.85 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 3.88 (s, 3H, $-OCH_3$), 4.15 (t, 2H, $J=6.8$ Hz, $-CH_2$), 6.63 (d, 1H, $J=8.5$ Hz, ArH), 6.96–6.99 (m, 2H, ArH), 7.39 (dd, 1H, $J_1=8.3$ Hz, $J_2=1.7$ Hz, ArH), 7.49 (d, 1H, $J=1.7$ Hz, ArH), 7.77 (d, 1H, $J=8.3$ Hz, ArH), 7.99 (d, 1H, $J=8.6$ Hz, ArH), 8.57 (s, 1H, ArH), 8.87 (s, 1H, NHCO)
7t	3445, 2940, 2816, 1664, 1609, 1541, 1495, 1355, 1271, 1097, 1010, 746	611.3 $[M+H]^+$	[c,d]2.05 (m, 2H, $-CH_2$), 2.52–2.83 (m, 10H, –N⟨⟩N–, $-CH_2$), 3.49 (s, 2H, $-CH_2$), 3.85 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 3.88 (s, 3H, $-OCH_3$), 4.17 (t, 2H, $J=6.3$ Hz, $-CH_2$), 6.63 (d, 1H, $J=8.7$ Hz, ArH), 6.95–6.99 (m, 2H, ArH), 7.13–7.16 (m, 1H, ArH), 7.57–7.63 (m, 2H, ArH), 7.95 (d, 1H, $J=8.4$ Hz, ArH), 8.34 (s, 1H, ArH), 8.68 (s, 1H, NHCO)
7u	3424, 2954, 2815, 1664, 1630, 1519, 1492, 1349, 1267, 1095, 1010, 784	625.3 $[M+H]^+$	[c,e]2.03 (m, 2H, $-CH_2$), 2.49–2.54 (m, 10H, –N⟨⟩N–, $-CH_2$), 2.62 (s, 3H, $-CH_3$), 3.49 (s, 2H, $-CH_2$), 3.85 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 3.88 (s, 3H, $-OCH_3$), 4.17 (t, 2H, $J=6.4$ Hz, $-CH_2$), 6.63 (d, 1H, $J=8.5$ Hz, ArH), 6.95–6.99 (m, 3H, ArH), 7.37 (d, 1H, $J=5.0$ Hz, ArH), 7.96 (dd, 1H, $J_1=2.0$ Hz, $J_2=8.6$ Hz, ArH), 8.23 (s, 1H, ArH), 8.77 (d, 1H, $J=2.0$ Hz, NHCO)
7v	3397, 2937, 2849, 1672, 1604, 1535, 1494, 1358, 1267, 1095, 1036, 797	611.4 $[M+H]^+$	[c,d]2.05 (m, 2H, $-CH_2$), 2.50–2.54 (m, 10H, –N⟨⟩N–, $-CH_2$), 3.49 (s, 2H, $-CH_2$), 3.85 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 3.88 (s, 3H, $-OCH_3$), 4.17 (t, 2H, $J=6.3$ Hz, $-CH_2$), 6.63 (d, 1H, $J=8.4$ Hz, ArH), 6.96–6.99 (m, 2H, ArH), 7.41–7.48 (m, 2H, ArH), 7.93–7.98 (m, 2H, ArH), 8.26 (s, 1H, ArH), 8.7 (s, 1H, NHCO)

[a] ESI(+), 70 V.
[b] DMSO-d_6.
[c] $CDCl_3$.
[d] 300 MHz.
[e] 500 MHz.

*4.4.8. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(2,4-dichlorobenzamino)benzoate (**5h**)*
Yield 63.9%, yellow oil.

*4.4.9. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(thiophene-2-carboxamino)benzoate (**5i**)*
Yield 41.9%, yellow oil.

*4.4.10. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(3-methylthiophene-2-carboxamino)benzoate (**5j**)*
Yield 34.1%, white solid, m.p. 121–125 °C.

*4.4.11. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(thiophene-3-carboxamino)benzoate (**5k**)*
Yield 31.5%, yellow oil.

*4.4.12. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(benzamino)benzoate (**5l**)*
Yield 61.9%, yellow oil.

*4.4.13. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(4-ethylbenzamino)benzoate (**5m**)*
Yield 69.4%, white solid, m.p. 97–102 °C.

*4.4.14. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(4-methoxybenzamino)benzoate (**5n**)*
Yield 36.1%, white solid, m.p. 82–87 °C.

*4.4.15. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(2-nitrobenzamino)benzoate (**5o**)*
Yield 33.8%, yellow oil.

*4.4.16. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(4-methylphenylsulfonamino)benzoate (**5p**)*
Yield 61.6%, white solid, m.p. 125–127 °C.

*4.4.17. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(4-(trifluoromethyl)benzamino)benzoate (**5q**)*
Yield 51.2%, yellow oil.

*4.4.18. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(2,6-dichlorobenzamino)benzoate (**5r**)*
Yield 44.4%, yellow oil.

*4.4.19. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(2,4-dichlorobenzamino)benzoate (**5s**)*
Yield 35.5%, yellow oil.

*4.4.20. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(thiophene-2-carboxamino)benzoate (**5t**)*
Yield 38.5%, yellow oil.

*4.4.21. Ethyl4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl) propoxy)-3-(3-methylthiophene-2-carboxamino)benzoate (**5u**)*
Yield 39.1%, yellow oil.

*4.4.22. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl) propoxy)-3-(thiophene-3-carboxamino)benzoate (**5v**)*
Yield 30.6%, yellow oil.

*4.5. General procedure for synthesis of compounds **6***

The appropriate 3-amino substituted ethyl 4-(2(3)-(4-(2,3,4-trimethoxybenzyl)piperazin1-yl)ethoxy(propoxy))benzoate (**5**)

(1.5 mmol) was dissolved in a mixture of methanol (16 mL) and water (24 mL). Potassium carbonate (0.62 g, 4.5 mmol) was then added and the reaction mixture was refluxed under stirring for 2 h. After filtering, the methanol in the filtrate was removed under vacuum. To the residue was dropped 2% dilute hydrochloric acid to adjust the pH to 7 (isoelectric point), then the resulting solution was extracted with ethyl acetate (3 × 15 mL). The organic layer was washed with saturated sodium chloride solution, dried over anhydrous sodium sulfate and concentrated under vacuum to give compound **6**. If the product could be precipitated under isoelectric point directly, it was filtered off and dried to afford compound **6**.

*4.5.1. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(3-methylbenzamino)benzoic acid (**6a**)*
Yield 87.5%, white solid, m.p. 78–83 °C.

*4.5.2. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(4-ethylbenzamino)benzoic acid (**6b**)*
Yield 56.0%, white solid, m.p. 79–84 °C.

*4.5.3. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(4-methoxybenzamino)benzoic acid (**6c**)*
Yield 90%, yellow oil.

*4.5.4. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(4-cyanobenzamino)benzoic acid (**6d**)*
Yield 87.5%, white solid, m.p. 132–137 °C.

*4.5.5. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(2-nitrobenzamino)benzoic acid (**6e**)*
Yield 89.7%, white solid, m.p. 99–103 °C.

*4.5.6. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(4-methylphenylsulfonamino)benzoicacid (**6f**)*
Yield 87.0%, white solid, m.p. 50–55 °C.

*4.5.7. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(4-chlorobenzamino)benzoic acid (**6g**)*
Yield 75.5%, white solid, m.p. 89–94 °C.

*4.5.8. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(2,4-dichlorobenzamino)benzoic acid (**6h**)*
Yield 69%, white solid, m.p. 91–93 °C.

*4.5.9. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(thiophene-2-carboxamino)benzoic acid (**6i**)*
Yield 67.0%, yellow oil.

*4.5.10. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(3-methylthiophene-2-carboxamino)benzoic acid (**6j**)*
Yield 89.0%, white solid, m.p. 70–75 °C.

*4.5.11. Ethyl 4-(2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)ethoxy)-3-(thiophene-3-carboxamino)benzoic acid (**6k**)*
Yield 77.5%, yellow oil.

*4.5.12. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-benzaminobenzoic acid (**6l**)*
Yield 89.5%, white solid, m.p. 142–143 °C.

*4.5.13. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(4-ethylbenzamino)benzoic acid (**6m**)*
Yield 89.0%, white solid, m.p. 160–165 °C.

*4.5.14. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(4-methoxybenzamino)benzoic acid (**6n**)*
Yield 77.1%, white solid, m.p. 67–72 °C.

*4.5.15. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(2-nitrobenzamino)benzoic acid (**6o**)*
Yield 67.5%, white solid, m.p. 83–87 °C.

*4.5.16. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(4-methylphenylsulfonamino)benzoic acid (**6p**)*
Yield 79.0%, yellow oil.

*4.5.17. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(4-(trifluoromethyl)benzamino)benzoic acid (**6q**)*
Yield 86.5%, white solid, m.p. 61–65 °C.

*4.5.18. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(2,6-dichlorobenzamino)benzoic acid (**6r**)*
Yield 88.4%%, white solid, m.p. 50–55 °C.

*4.5.19. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(2,4-dichlorobenzamino)benzoic acid (**6s**)*
Yield 67.5%, yellow oil.

*4.5.20. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(thiophene-2-carboxamino)benzoic acid (**6t**)*
Yield 89.9%, white solid, m.p. 121–125 °C.

*4.5.21. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(3-methylthiophene-2-carboxamino)benzoic acid (**6u**)*
Yield 69.0%, white solid, m.p. 75–80 °C.

*4.5.22. Ethyl 4-(3-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)propoxy)-3-(thiophene-3-carboxamino)benzoic acid (**6v**)*
Yield 79.9%, white solid, m.p. 96–101 °C.

*4.6. General procedure for synthesis of compounds **7***

To a solution of the appropriate 3-amino substituted ethyl 4-(2(3)-(4-(2,3,4-trimethoxybenzyl)piperazin1-yl)ethoxy(propoxy))benzoic acid (**6**) (0.69 mmol), 1-hydroxybenzotriazole (HOBT) (0.09 g, 0.69 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.13 g, 0.69 mmol) in anhydrous DMF (2 mL) was added free guanidine (0.16 g, 2.79 mmol), followed by water (2 mL). After stirring at room temperature for 2 h, the reaction mixture was poured onto ethyl acetate/water (v/v = 3:2) and extracted with ethyl acetate. The combined organic layers were washed with saturated sodium chloride solution, dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by silica gel column chromatography (EtOAC/CH_3OH/Et_3N = 15:1:0.1) to give compounds **7a**–**7v**.

For the physicochemical characters and spectral data of compounds **7a**–**7v**, see Tables 2 and 5.

4.7. Rat platelet swelling assay

Sprague–Dawley rats (380–420 g) were anesthetized with ethyl ether and blood was collected from their eyeholes with 25% (v/v) acid–citrate–dextrose (ACD; sodium citrate 2.77 g, citric acid 1.07 g and glucose 3.07 g in 125 mL distilled H_2O). Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 1300 g/min for 10 min at room temperature. The upper 2/3 of the supernatants were used for the further measurements and stored at r.t. until used. All measurements were performed within 4–5 h.

All compounds were dissolved in DMSO and diluted with propionate medium (pH 7.4). A solution of the tested compound (25 mL) was added to 175 mL of propionate buffer (sodium propionate, 140 mmol/L; HEPES, 20 qs; glucose, 10 mmol/L; KCl, 5 mmol/L; $MgCl_2$, 1 mmol/L; $CaCl_2$, 1 mmol/L; pH 6.7) contained in a spectrophotometer cuvette. Then, 50 mL of PRP prewarmed to 37 °C was added. The suspension was stirred, and the change in optical density (OD) was recorded each 7.5 s for 2 min at 550 nm (ThermoMultiscan Spectrum). The IC_{50} value was calculated according to the regression analysis. For each molecule, the measurements were performed in triplicate.

4.8. Screening of primary culture of myocardial cell in vitro in neonatal rat

The primary culture of myocardial cell in neonatal rat is cultivated in vitro, and inoculated in culture plates. Regular pulsating myocardial cells in flakiness are randomly and equally divided into six experimental groups, and each groups with six parallel holes: normal control group, which used 200 μL DMEM culture solution containing 10% serum, and cultivated 6 h placed in 5% CO_2 incubator; hypoxia/reoxygenation model group, which used 200 μL serum-free DMEM culture solution containing 50 mmol/L $Na_2S_2O_4$, and discarded the supernatant after cultivated 2 h placed in incubator, and then cultivated 4 h placed in incubator after adding 200 μL DMEM culture solution containing 10% serum to reoxygenate; the groups of TG-6, **7e**, **7h** and **7j**, which are added serum-free DMEM culture solution with TG-6, **7e**, **7h** and **7j**, respectively before the step of reoxygenation to incubate 1 h, and made the groups reached the final concentration of 10^{-6}, 10^{-5}, 10^{-4} mol/L containing the drugs, and the rest operation is the same as the hypoxia/reoxygenation model group.

MTT method to determinate the viability of myocardial cells: each hole is added 20 uL MTT solution (5 mg/mL, or 0.5% MTT), and blotted the culture solution carefully after 4-h continuing cultivating. Then, each hole is added 150 uL DMSO, and shaked 10 min in low speed at the table concentrator to fully dissolve crystals. Measure the absorbance of each hole at 490 nm in the enzyme-linked immunosorbent detector.

The ratio of cell survival = OD value of treated group/OD value of control group.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2011.06.011.

References

[1] R. Zhang, J. Dong, Y.G. Xu, W.Y. Hua, N. Wen, Q.D. You, Eur. J. Med. Chem. 44 (2009) 3771–3776.
[2] R.J. Gazmuri, I.M. Ayoub, J. Kolarova, Inhibition of the sodium-hydrogen exchanger: a potential treatment for resuscitation from cardiac arrest. in: J.L. Vincent (Ed.), 2003 Yearbook of Intensive Care and Emergency Medicine. Springer-Verlag, 2003, pp. 461–471.
[3] D. Mao, Y.G. Xu, X.P. Hu, G.M. Zhang, J. Dong, G.Q. Gong, Chem. Biodivers. 6 (2009) 1727–1736.
[4] M.E. Malo, L. Fliegel, Can. J. Physiol. Pharmacol. 84 (2006) 1081–1095.
[5] G.J. Gross, R.J. Gumina, Drugs Future 26 (2001) 253–260.
[6] E.R. Slepkov, J.K. Rainey, B.D. Sykes, L. Fliegel, Biochem. J. 401 (2007) 623–633.
[7] H. Izumi, T. Torigoe, H. Ishiguchi, H. Uramoto, Y. Yoshida, M. Tanabe, T. Ise, T. Murakami, T. Yoshida, M. Nomoto, K. Kohno, Cancer Treat. Rev. 29 (2003) 541–549.
[8] K. Leineweber, G. Heusch, R. Schulz, Cardiovasc. Drug Rev. 25 (2007) 123–131.
[9] B. Masereel, L. Pochet, D. Laeckmann, Eur. J. Med. Chem. 38 (2003) 547–554.
[10] M. Karmazyn, M. Sawyer, L. Fliegel, Curr. Drug Targets Cardiovasc. Haematol. Disord. 5 (2005) 323–335.
[11] H.S. Park, B.K. Lee, S. Park, S.U. Kim, S.H. Lee, E.J. Baik, S. Lee, K.Y. Yi, S.E. Yoo, C.H. Moon, Y.S. Jung, Brain Res. 1061 (2005) 67–71.
[12] Y.G. Xu, R. Zhang, W.Y. Hua, Prog. Pharm. Sci. 27 (2003) 264–269.
[13] X.M. Jiang, P. Du, Y.G. Xu, D. Zhang, G.Q. Gong, Q.D. You, Chin. J. Org. Chem. 28 (2008) 2142–2148.
[14] M. Ren, Y.G. Xu, W. Na, D.Y. Zhang, W.Y. Hua, Chin. Chem. Lett. 18 (2007) 539–541.
[15] R. Zhang, L. Lei, Y.G. Xu, W.Y. Hua, G.Q. Gong, Bioorg. Med. Chem. Lett. 17 (2007) 2430–2433.
[16] W.T. Xu, N. Jin, J. Xu, Y.G. Xu, Q.J. Wang, Q.D. You, Bioorg. Med. Chem. Lett. 19 (2009) 3283–3287.
[17] P.C. Ting, S.P. Umland, R. Aslanian, J.H. Cao, G.C. Garlisi, Y. Huang, J. Jakway, Z.D. Liu, H. Shah, F. Tian, Y.T. Wan, N.Y. Shih, Bioorg. Med. Chem. Lett. 15 (2005) 3020–3023.
[18] D. Rosskopf, E. Morgenstern, W. Scholzt, U. Osswald, W. Siffert, J. Hypertens. 9 (1991) 231–238.