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Short communication

Synthesis and bioactivity of substituted indan-1-ylidene aminoguanidine derivatives

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ABSTRACT

In our efforts to discover more potent and lasting NHE1 inhibitors, we designed and synthesized a series of substituted indan-1-ylidene aminoguanidine derivatives (**5**). NHE1 inhibitory activity of twenty-one compounds **5** was evaluated in a rat platelet swelling assay. It is found that most of the tested compounds possess NHE1 inhibitory effects. 2-(5-methoxybenzimidazol-2-ylthio)-5-chloro-2,3-dihydroinden-1-ylidene aminoguanidine hydrobromide (**5m**) proved to be sixty-nine times more potent than cariporide. Furthermore, when tested in vivo, compound **5m** also displayed superior cardioprotective effects against SD rat myocardial ischemic–reperfusion injury over those of cariporide.

1. Introduction

The Na^+/H^+ exchangers (NHE) comprise a family of membrane proteins that are involved in the transport of H^+ in exchange for Na^+ [1–3]. To date, nine NHE isoforms (NHE1–NHE9) have been identified in various organs in the human body. NHE1 is involved in numerous physiological processes in mammals, including regulation of intracellular pH, cell-volume control, cytoskeletal organization, heart disease and cancer [4–7]. During ischemia and reperfusion of the myocardium, NHE1 activity catalyzes increased uptake of intracellular sodium. 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 [8,9]. NHE1 inhibitors, which competitively inhibit NHE1 function and reduce Na^+ and Ca^{2+} influx, would prevent damage to the myocardium in ischemia–reperfusion [3,10].

While most known NHE1 inhibitors are acylguanidines based on diverse aryl ring templates including benzene, pyrazole, quinoline, etc. [9–14], few studies have been carried out on the non-acylguanidine NHE1 inhibitors. T-162559 (Fig. 1), an aminoguanidine derivative with a more potent NHE1 inhibitory effect against ischemia and reperfusion injury than cariporide and eniporide [15,16], encouraged us to identify a novel NHE1 inhibitor with an aminoguanidine group. Previously, we found that the inhibitory effect of 2-benzimidazol-2-ylthio-1-(4-nitro phenylethylidene) aminoguanidine (CPU-X-050519, $IC_{50} = 1.17$ nM) (Fig. 2) on rat platelet NHE1 is fifty-five times more potent than that of cariporide [17]. As the extension of our efforts to increase the NHE1 inhibitory activity and improve the stability due to the imine structure of CPU-X-050519, we designed a series of substituted indan-1-ylidene aminoguanidine derivatives **5a**–**u** (Fig. 2). This paper describes the synthesis and preliminary results of biological evaluation.

2. Results and discussion

2.1. Synthesis

Synthetic routes of target compounds are depicted in Scheme 1. 2-Bromo-indan-1-ones **2**, obtained by bromination of indan-1-ones **1** with copper (II) bromide [18], were reacted with benzimidazol-(or benzothiazol)-2-thiols **3** to offer 2-(benzimidazol-(or benzothiazol)-2-ylthio)-indan-1-ones **4**, which were then treated with aminoguanidine hydrochloride to give target compounds **5** (Table 1).

When compounds **1** were brominated with copper (II) bromide according to a modified method [19], compounds **2** were obtained in good yields with only small amounts of dibrominated by-products. The reaction between compounds **2** and compounds **3** could be carried out in dimethylformamide (DMF) in the absence of an

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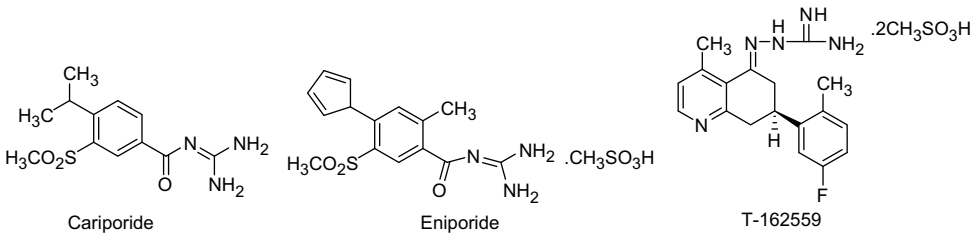


Fig. 1. The structure of cariporide, T-162559 and CPU-X-050519.

alkali metal catalyst. Using such catalysts brought about the formation of undesirable side reactions. When compounds **4** reacted with aminoguanidine hydrochloride in the presence of a methanol solution saturated with hydrogen chloride (HCl) gas, compounds **5** could be obtained as their hydrochloride salt.

2.2. Biological activity

The NHE1 inhibitory activities of twenty-one target compounds **5a**–**u** and cariporide were evaluated by rat platelet swelling assay (PSA). The experimental procedure was similar to the literature [20,21], with minor modifications. The PSA results (Table 2) showed that most of the tested compounds inhibited rat platelet NHE1 in a concentration-dependent manner. Compounds **5a**, **5g**, **5m**, **5o** and **5p** were markedly superior to cariporide in NHE1 inhibition. The IC_{50} value of compound **5m** was 0.94 nM, making it sixty-nine times more potent than cariporide ($IC_{50} = 65.0$ nM).

In comparison with the compounds possessing the same substituent in the indane ring, introduction of a benzothiazole ring instead of a benzimidazole ring led to an improvement of the in vitro activity (**5p** vs **5b**, **5q** vs **5c**, **5s** vs **5d**, and **5t** vs **5e**). This may be due to the sulfur atom in the benzimidazole ring which favors the interaction with the docking site in NHE1.

The compounds showing good NHE1 inhibitory activity were further investigated for their cardioprotective effects against ischemia–reperfusion injury in SD rat hearts [22]. The infarct size and the CK level of cariporide were $45.74 \pm 3.33\%$ and 3.53 ± 0.97 U/ml, respectively. Compounds **5a**, **5m** and **5o** exhibited a good cardioprotective efficacy both *in vitro* and *in vivo* (Table 3). Especially, compound **5m** significantly reduced the infarct size to $36.16 \pm 2.58\%$ and the CK level to 2.82 ± 0.87 U/ml at a dose of 1 mg/kg.

3. Conclusion

We investigated a series of substituted indan-1-ylidene aminoguanidine derivatives **5** on the basis of the structure of an initial lead compound CPU-X-050519. It is found that seven compounds showed more potent NHE1 inhibitory activity than cariporide among the twenty-one tested compounds in vitro. Four of the seven compounds were selected for in vivo testing, and the cardioprotective effect against SD rat myocardial ischemic–reperfusion injury of compound **5m** at high (1 mg/kg), medium (0.5 mg/kg) and low (0.25 mg/kg) dose levels were comparable with cariporide (1 mg/kg). Further pharmacological studies are in progress.

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 ^{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 in δ ppm 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 (un)substituted indan-1-ones (**1**) (26 mmol) in chloroform/ethyl acetate (1:1, 100 ml), cupric bromide (52 mmol) was added portionwise with stirring under reflux (60%, 24% and 16% each portion), the second and third parts of cupric bromide were added after black cupric bromide was transformed into white cuprous bromide. After completion of addition of cupric bromide, the reaction mixture was stirred and refluxed for 0.5–2 h. The mixture was filtered, and the filtrate was decolorized and filtered. The filtrate was concentrated to dryness under reduced pressure, and the residue was recrystallised from methanol to give (un)substituted 2-bromo-1-indanones (**2**) as crystals in 67–91% yield [19].

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

A mixture of (un)substituted 2-mercaptobenzimidazoles (**3**) (10 mmol) and (un)substituted 2-bromo-1-indanones (**2**) (10 mmol) in DMF (20 ml) was stirred at 50 °C for 4 h. After cooling, the mixture was poured into ice-water (300 ml) and filtered, washed with water. The residue was purified by silica gel column chromatography (petroleum/EtOAC = 2:1).

*2-(Benzimidazol-2-ylthio)-2,3-dihydroinden-1-one hydrobromide (**4a**)*: Yield 80.8%, gray solid, m.p. 199–202 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.10 (dd, 1H, $J_1 = 4.3$ Hz, $J_2 = 17.6$ Hz, indanone 3-H),

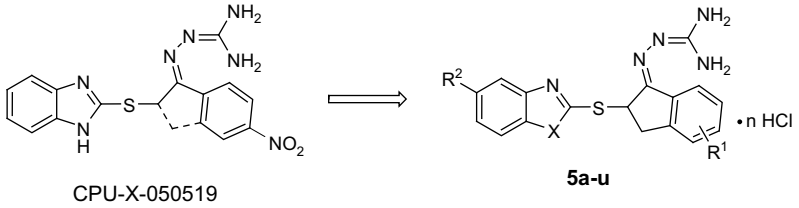
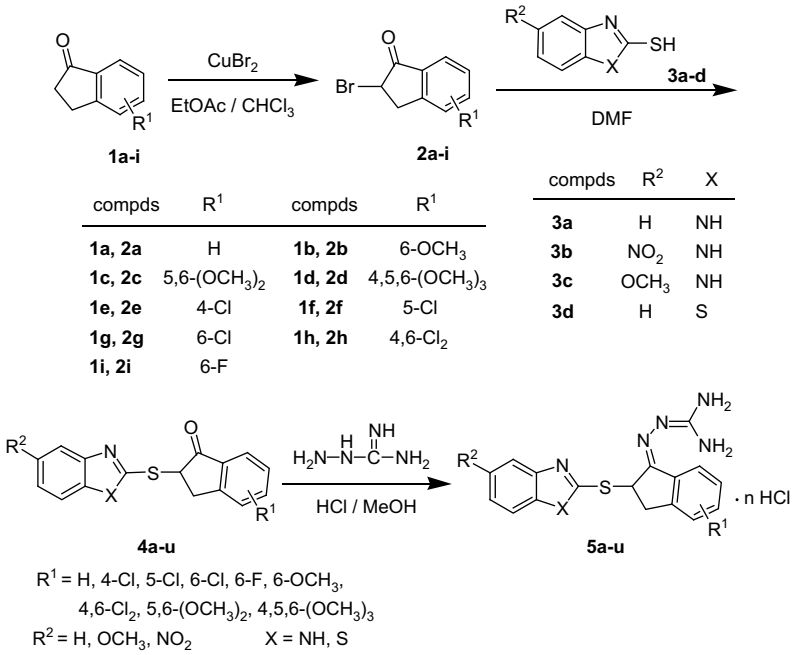


Fig. 2. Synthetic design of compounds **5a**–**u**.



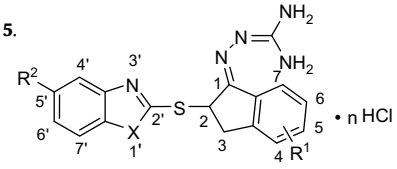
Scheme 1. Synthesis of compounds **5**.

4.03 (dd, 1H, $J_1 = 8.2$ Hz, $J_2 = 17.6$ Hz, indanone 3-H), 5.43 (dd, 1H, $J_1 = 4.3$ Hz, $J_2 = 8.2$ Hz, indanone 2-H), 7.37–7.45 (m, 5H, ArH), 7.63–7.70 (m, 3H, ArH).

*2-(Benzimidazol-2-ylthio)-5,6-dimethoxy-2,3-dihydroinden-1-one hydrobromide (**4b**)*: Yield 42.9%, white solid, m.p. 242–245 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.06 (dd, 1H, $J_1 = 4.2$ Hz, $J_2 = 17.4$ Hz, indanone 3-H), 3.89 (dd, 1H, $J_1 = 8.3$ Hz, $J_2 = 17.4$ Hz, indanone 3-H), 3.95 (s, 3H, OCH_3), 4.04 (s, 3H, OCH_3), 4.71 (dd, 1H, $J_1 = 4.2$ Hz, $J_2 = 8.3$ Hz, indanone 2-H), 6.97 (s, 1H, indanone 4-H), 7.20 (s, 1H, indanone 7-H), 7.49–7.52 (m, 2H, ArH), 7.82–7.85 (m, 2H, ArH). MS (ESI(+), 70 V) m/z: 341 $[M + H]^+$.

*2-(Benzimidazol-2-ylthio)-4,5,6-trimethoxy-2,3-dihydroinden-1-one (**4c**)*: Yield 52.1%, white solid, m.p. 70–74 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.00 (dd, 1H, $J_1 = 3.6$ Hz, $J_2 = 18.0$ Hz, indanone 3-H), 3.77 (dd, 1H, $J_1 = 8.1$ Hz, $J_2 = 18.0$ Hz, indanylidene 3-H), 3.92 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 4.40 (dd, 1H,

Table 1
Characterization table of compounds **5**.



Compd. no.	R^1	R^2	X	*n*	Mol. formula	m.p. (°C)	Analysis (%)		
							Found (calculated)		
							C	H	N
5a	H	H	NH	2	$C_{17}H_{16}N_6S \cdot 2HCl \cdot 3H_2O$	181 (dec.)	43.93 (44.06)	5.26 (5.22)	17.89 (18.14)
5b	5,6-$(OCH_3)_2$	H	NH	2	$C_{19}H_{20}N_6O_2S \cdot 2HCl \cdot H_2O$	165 (dec.)	46.82 (46.82)	4.83 (4.96)	16.96 (17.24)
5c	4,5,6-$(OCH_3)_3$	H	NH	1	$C_{20}H_{22}N_6O_3S \cdot HCl \cdot H_2O$	169–171	48.92 (49.03)	5.10 (5.35)	17.10 (17.15)
5d	5-Cl	H	NH	–	$C_{17}H_{15}ClN_6S \cdot 0.5H_2O$	178–179	53.85 (53.75)	4.37 (4.25)	21.93 (22.12)
5e	6-Cl	H	NH	1	$C_{17}H_{15}ClN_6S \cdot HCl \cdot 2H_2O$	158–160	46.36 (46.05)	4.49 (4.55)	19.07 (18.96)
5f	4,6-Cl_2	H	NH	–	$C_{17}H_{14}Cl_2N_6S \cdot 0.25H_2O$	177–178	49.88 (49.82)	3.49 (3.57)	20.46 (20.51)
5g	H	NO_2	NH	1	$C_{17}H_{15}N_7O_2S \cdot HCl \cdot H_2O$	193–195	46.68 (46.84)	4.16 (4.16)	22.34 (22.49)
5h	4,5,6-$(OCH_3)_3$	NO_2	NH	1	$C_{20}H_{21}N_7O_5S \cdot HCl \cdot H_2O$	180–182	45.65 (45.67)	4.70 (4.60)	18.35 (18.64)
5i	5-Cl	NO_2	NH	1	$C_{17}H_{14}ClN_7O_2S \cdot HCl \cdot 0.5H_2O$	177–179	44.18 (44.26)	3.54 (3.50)	20.99 (21.25)
5j	6-Cl	NO_2	NH	1	$C_{17}H_{14}ClN_7O_2S \cdot HCl \cdot H_2O$	177–180	43.18 (43.41)	3.68 (3.64)	20.89 (20.85)
5k	6-F	NO_2	NH	1	$C_{17}H_{14}FN_7O_2S \cdot HCl \cdot 0.75H_2O$	176–178	45.45 (45.44)	3.57 (3.70)	21.75 (21.82)
5l	H	OCH_3	NH	2	$C_{18}H_{18}N_6OS \cdot 2HCl \cdot 1.5H_2O$	196–198	46.18 (46.35)	4.89 (4.97)	18.17 (18.02)
5m	5-Cl	OCH_3	NH	1	$C_{18}H_{17}ClN_6OS \cdot HCl \cdot H_2O$	160–163	47.39 (47.48)	4.51 (4.43)	18.29 (18.46)
5n	6-F	OCH_3	NH	1	$C_{18}H_{17}FN_6OS \cdot HCl \cdot H_2O$	158–160	49.16 (49.26)	4.65 (4.59)	18.78 (19.15)
5o	6-OCH_3	H	S	1	$C_{18}H_{17}N_5OS_2 \cdot HCl \cdot H_2O$	138–140	49.57 (49.36)	4.42 (4.60)	15.74 (15.99)
5p	5,6-$(OCH_3)_2$	H	S	2	$C_{19}H_{19}N_5OS_2 \cdot 2HCl \cdot H_2O$	172 (dec.)	45.50 (45.24)	4.65 (4.60)	14.02 (13.88)
5q	4,5,6-$(OCH_3)_3$	H	S	1	$C_{20}H_{21}N_5OS_2 \cdot HCl \cdot 2H_2O$	153–155	46.68 (46.55)	4.90 (5.08)	13.61 (13.57)
5r	4-Cl	H	S	2	$C_{17}H_{14}ClN_5S_2 \cdot 2HCl \cdot 0.5H_2O$	133–135	43.65 (43.46)	3.52 (3.65)	14.63 (14.91)
5s	5-Cl	H	S	1	$C_{17}H_{14}ClN_5S_2 \cdot HCl \cdot 0.5H_2O$	168–170	47.17 (47.11)	3.63 (3.72)	16.49 (16.16)
5t	6-Cl	H	S	1	$C_{17}H_{14}ClN_5S_2 \cdot HCl \cdot 1.5H_2O$	149–152	45.29 (45.23)	3.85 (4.02)	15.26 (15.51)
5u	6-F	H	S	2	$C_{17}H_{14}FN_5S_2 \cdot 2HCl \cdot H_2O$	145–147	44.45 (44.16)	3.85 (3.92)	15.04 (15.15)

Table 2
NHE1 inhibitory activities of compounds **5**.

Compd.	PSA	Compd.	PSA	Compd.	PSA
	IC_{50} (nM)[a]		IC_{50} (nM)[a]		IC_{50} (nM)[a]
5a	1.73	**5h**	31.5	**5o**	1.90
5b	11.2	**5i**	12.2	**5p**	2.44
5c	10.8	**5j**	38.9	**5q**	1.98
5d	21.4	**5k**	38.0	**5r**	20.3
5e	326.2	**5l**	16.8	**5s**	2.34
5f	653.8	**5m**	0.94	**5t**	34.6
5g	1.74	**5n**	25.5	**5u**	287.3

[a] The IC_{50} value of cariporide in the same testing was 65.0 nM.

$J_1 = 3.6$ Hz, $J_2 = 8.1$ Hz, indanone 2-H), 7.08 (s, 1H, indanone 7-H), 7.26–7.29 (m, 2H, ArH), 7.59–7.62 (m, 2H, ArH).

*2-(Benzimidazol-2-ylthio)-5-chloro-2,3-dihydroinden-1-one hydrobromide (**4d**)*: Yield 59.9%, light yellow solid, m.p. 158–161 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.14 (dd, 1H, $J_1 = 4.2$ Hz, $J_2 = 18.3$ Hz, indanone 3-H), 3.83 (dd, 1H, $J_1 = 8.4$ Hz, $J_2 = 18.3$ Hz, indanone 3-H), 4.34 (dd, 1H, $J_1 = 4.2$ Hz, $J_2 = 8.4$ Hz, indanone 2-H), 7.24–7.28 (m, 2H, ArH), 7.44 (d, 1H, $J = 8.1$ Hz, indanone 6-H), 7.48 (s, 1H, indanone 4-H), 7.56–7.59 (m, 2H, ArH), 7.78 (d, 1H, $J = 8.1$ Hz, indanone 7-H).

*2-(Benzimidazol-2-ylthio)-6-chloro-2,3-dihydroinden-1-one hydrobromide (**5e**)*: Yield 61.4%, light yellow solid, m.p. 160 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.16 (dd, 1H, $J_1 = 4.2$ Hz, $J_2 = 18.2$ Hz, indanone 3-H), 3.81 (dd, 1H, $J_1 = 8.4$ Hz, $J_2 = 18.2$ Hz, indanone 3-H), 4.43 (dd, 1H, $J_1 = 4.2$ Hz, $J_2 = 8.4$ Hz, indanone 2-H), 7.22–7.28 (m, 2H, ArH), 7.45 (d, 1H, $J = 8.1$ Hz, indanone 4-H), 7.51–7.61 (m, 2H, ArH), 7.66 (dd, 1H, $J_1 = 2.1$ Hz, $J_2 = 8.1$ Hz, indanone 5-H), 7.83 (d, 1H, $J = 2.1$ Hz, indanone 7-H).

*2-(Benzimidazol-2-ylthio)-4,6-dichloro-2,3-dihydroinden-1-one hydrobromide (**4f**)*: Yield 51.1%, white solid, m.p. 200–203 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.29 (dd, 1H, $J_1 = 3.6$ Hz, $J_2 = 17.8$ Hz, indanone 3-H), 3.77 (dd, 1H, $J_1 = 8.0$ Hz, $J_2 = 17.8$ Hz, indanone 3-H), 4.88 (dd, 1H, $J_1 = 3.6$ Hz, $J_2 = 8.0$ Hz, indanone 2-H), 7.15–7.50 (m, 4H, ArH), 7.76 (d, 1H, $J = 1.5$ Hz, indanone 5-H), 8.04 (d, 1H, $J = 1.5$ Hz, indanone 7-H).

*2-(5-Nitrobenzimidazol-2-ylthio)-2,3-dihydroinden-1-one hydrobromide (**4g**)*: Yield 33.8%, white solid, m.p. 232–235 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.38 (dd, 1H, $J_1 = 4.8$ Hz, $J_2 = 17.6$ Hz, indanone 3-H), 3.87 (dd, 1H, $J_1 = 8.1$ Hz, $J_2 = 17.6$ Hz, indanone 3-H), 4.80 (dd, 1H, $J_1 = 4.8$ Hz, $J_2 = 8.1$ Hz, indanone 2-H), 7.42–7.47 (m, 2H, ArH), 7.54 (d, 1H, $J = 7.6$ Hz, indanone 4-H), 7.70 (d, 1H, $J = 7.6$ Hz, ArH), 7.68 (d, 1H, $J = 8.9$ Hz, ArH), 8.00 (dd, 1H, $J_1 = 2.2$ Hz, $J_2 = 8.9$ Hz, ArH), 8.17 (d, 1H, $J = 2.2$ Hz, ArH).

*2-(5-Nitrobenzimidazol-2-ylthio)-4,5,6-trimethoxy-2,3-dihydroinden-1-one hydrobromide (**4h**)*: Yield 41.9%, yellow solid, m.p. 181–183 °C. ^{1}H NMR ($CDCl_3$, 300 MHz,) δ: 3.02 (dd, 1H, $J_1 = 3.6$ Hz, $J_2 = 18.0$ Hz, indanone 3-H), 3.80 (dd, 1H, $J_1 = 8.1$ Hz, $J_2 = 18.0$ Hz, indanone 3-H), 3.95 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.44 (dd, 1H, $J_1 = 3.6$ Hz, $J_2 = 8.1$ Hz, indanone 2-H), 7.10 (s, 1H, indanone 7-H), 7.62 (d, 1H, $J = 8.7$ Hz, ArH), 8.19 (dd, 1H, $J_1 = 2.1$ Hz, $J_2 = 8.8$ Hz, ArH), 8.50 (d, 1H, $J = 2.1$ Hz, ArH).

*2-(5-Nitrobenzimidazol-2-ylthio)-5-chloro-2,3-dihydroinden-1-one hydrobromide (**4i**)*: Yield 57.4%, white solid, m.p. 186–188 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.21 (dd, 1H, $J_1 = 4.2$ Hz, $J_2 = 18.3$ Hz, indanone 3-H), 3.88 (dd, 1H, $J_1 = 8.4$ Hz, $J_2 = 18.3$ Hz, indanone 3-H), 4.43 (dd, 1H, $J_1 = 4.2$ Hz, $J_2 = 8.4$ Hz, indanone 2-H), 7.44–7.55 (m, 2H, ArH), 7.67 (d, 1H, $J = 7.5$ Hz, ArH), 7.85 (d, 1H, $J = 8.4$ Hz, ArH), 8.18 (d, 1H, $J = 8.5$ Hz, ArH), 8.54 (s, 1H, ArH).

*2-(5-Nitrobenzimidazol-2-ylthio)-6-chloro-2,3-dihydroinden-1-one hydrobromide (**4j**)*: Yield 37.1%, yellow solid. It was used directly in the next step without further purification.

*2-(5-Nitrobenzimidazol-2-ylthio)-6-fluoro-2,3-dihydroinden-1-one hydrobromide (**4k**)*: Yield 56.6%, yellow solid, m.p. 172–174 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.19 (dd, 1H, $J_1 = 4.2$ Hz, $J_2 = 18.0$ Hz, indanone 3-H), 3.87 (dd, 1H, $J_1 = 8.4$ Hz, $J_2 = 17.4$ Hz, indanone 3-H), 4.47 (dd, 1H, $J_1 = 4.2$ Hz, $J_2 = 8.4$ Hz, indanone 2-H), 7.46 (m, 1H, ArH), 7.52 (dd, 1H, $J_1 = 5.4$ Hz, $J_2 = 8.4$ Hz, ArH), 7.55 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 8.6$ Hz, ArH), 7.58 (d, 1H, $J = 8.9$ Hz, ArH), 8.18 (dd, 1H, $J_1 = 2.1$ Hz, $J_2 = 9.0$ Hz, ArH), 8.45 (d, 1H, $J = 2.1$ Hz, ArH).

*2-(5-Methoxybenzimidazol-2-ylthio)- 2,3-dihydroinden-1-one hydrobromide (**4l**)*: Yield 47.7%, white solid, m.p. 189–192 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.15 (dd, 1H, $J_1 = 4.4$ Hz, $J_2 = 18.0$ Hz, indanone 3-H), 3.85 (dd, 1H, $J_1 = 8.2$ Hz, $J_2 = 18.0$ Hz, indanone 3-H), 3.87 (s, 3H, OCH_3), 4.39 (dd, 1H, $J_1 = 4.5$ Hz, $J_2 = 8.2$ Hz, indanone 2-H), 6.91 (dd, 1H, $J_1 = 1.7$ Hz, $J_2 = 8.3$ Hz, ArH), 7.08 (d, 1H, $J = 1.8$ Hz, ArH), 7.46–7.51 (m, 3H, ArH), 7.69–7.74 (m, 1H, ArH), 7.86 (d, 1H, $J = 7.5$ Hz, ArH).

*2-(5-Methoxybenzimidazol-2-ylthio)-5-chloro-2,3-dihydroinden-1-one hydrobromide (**4m**)*: Yield 58.5%, yellow solid. It was used directly in the next step without further purification.

*2-(5-Methoxybenzimidazol-2-ylthio)-6-fluoro-2,3-dihydroinden-1-one hydrobromide (**4n**)*: Yield 42.9%, white solid. It was used directly in the next step without further purification.

*2-(Benzothiazol-2-ylthio)- 6-dimethoxy-2,3-dihydroinden-1-one (**4o**)*: Yield 45.0%, white solid, m.p. 112–114 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.34 (dd, 1H, $J_1 = 3.9$ Hz, $J_2 = 18.0$ Hz, indanone 3-H), 3.85 (dd, 1H, $J_1 = 8.1$ Hz, $J_2 = 18.0$ Hz, indanone 3-H), 3.87 (s, 3H, OCH_3), 4.73 (dd, 1H, $J_1 = 4.1$ Hz, $J_2 = 8.1$ Hz, indanone 2-H), 7.25 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 8.4$ Hz, ArH), 7.27 (d, 1H, $J = 8.4$ Hz, ArH), 7.33–7.37 (m, 2H, ArH), 7.39 (d, 1H, $J = 2.4$ Hz, ArH), 7.66–7.78 (m, 2H, ArH).

*2-(Benzothiazol-2-ylthio)-5,6-dimethoxy-2,3-dihydroinden-1-one (**4p**)*: Yield 37.3%, white solid, m.p. 127–129 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 3.32 (dd, 1H, $J_1 = 4.0$ Hz, $J_2 = 17.5$ Hz, indanone 3-H), 3.87 (dd, 1H, $J_1 = 7.5$ Hz, $J_2 = 17.5$ Hz, indanone 3-H), 3.93 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.74 (dd, 1H, $J_1 = 4.0$ Hz, $J_2 = 7.5$ Hz, indanone 2-H), 6.87 (s, 1H, indanone 4-H), 7.25 (s, 1H, indanone 7-H), 7.27–7.78 (m, 4H, ArH).

*2-(Benzothiazol-2-ylthio)-4,5,6-trimethoxy-2,3-dihydroinden-1-one (**4q**)*: Yield 55.7%, white solid, m.p. 129.5–132 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 3.43 (dd, 1H, $J_1 = 4.0$ Hz, $J_2 = 17.5$ Hz, indanone 3-H), 4.04 (dd, 1H, $J_1 = 8.0$ Hz, $J_2 = 17.5$ Hz, indanone 3-H), 4.07 (s, 3H, OCH_3), 4.10 (s, 3H, OCH_3), 4.14 (s, 3H, OCH_3), 4.87 (dd, 1H, $J_1 = 4.0$ Hz, $J_2 = 8.0$ Hz, indanone 2-H), 7.42 (s, 1H, indanone 7-H),

Table 3
Cardioprotective activity of compounds **5a**, **5g**, **5m** and **5o** against ischemic–reperfusion injury in SD rat hearts.

Compd.[a]	Dosage (mg/kg)	CK[b] (U/ml)	Infarct size[c] (%)
Ischemia–reperfusion	–	5.69 ± 1.18	69.36 ± 3.98
Cariporide	1.0	$3.53 \pm 0.97^{**}$	$45.74 \pm 3.33^{**}$
5a	1.0	$3.02 \pm 1.26^{**}$	$42.89 \pm 3.43^{**}$
5g	1.0	$4.12 \pm 1.34^{*}$	$49.18 \pm 3.62^{*}$
5m	1.0	$2.82 \pm 0.87^{**}$	$36.16 \pm 2.58^{**}$
	0.5	$3.34 \pm 1.05^{**}$	$40.87 \pm 2.48^{**}$
	0.25	$3.72 \pm 1.21^{**}$	$42.27 \pm 3.41^{**}$
5o	1.0	$3.38 \pm 0.96^{**}$	$43.24 \pm 2.84^{**}$

$^{*}p < 0.05$, $^{**}p < 0.01$ compared with ischemia–reperfusion group.

[a] Cariporide and the tested compounds were injected intravenously 5 min before LAD occlusion.

[b] The amount of creatine kinase (CK) was determined using a CK-NAC kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and a 722 grating photospectrometer (Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China). Serum CK activity was expressed as U/ml. Values are means ± SD, $n = 6$ or higher.

[c] Infarct size was expressed as the ratio of myocardial infarct weight to weight of ventricle at risk. Values are means ± SD, $n = 6$ or higher.

Table 4
IR, MS and ^{1}H NMR data of compounds **5a**–**5u**.

No.	IR (cm^{-1})	MS (m/z)[a]	^{1}H NMR (δ)
5a	3458, 3068, 2912, 1663, 1607, 1547, 1271, 854, 743	337.0 $[M+H]^+$	[b,d]3.08 (d, 1H, J = 17.7 Hz, H-3), 3.64 (dd, 1H, J_1 = 7.2 Hz, J_2 = 17.7 Hz, H-3), 5.22 (d, 1H, J = 7.2 Hz, H-2), 6.25 (bs, 6H, NH), 7.07–7.11 (m, 2H, ArH), 7.25–7.30 (m, 3H, ArH), 7.48–7.51 (m, 2H, ArH), 7.72 (dd, 1H, J_1 = 2.3 Hz, J_2 = 8.1 Hz, H-7), 11.80 (s, 1H, NH)
5b	3454, 3003, 2962, 1673, 1622, 1591, 1330, 1266, 828, 755	397.1 $[M+H]^+$	[b,e]2.95 (dd, 1H, J = 17.3 Hz, H-3), 3.56 (dd, 1H, J_1 = 7.2 Hz, J_2 = 17.3 Hz, H-3), 3.76 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 5.19 (d, 1H, J = 7.2 Hz, H-2), 6.30 (bs, 6H, NH), 6.92 (s, 1H, H-4), 7.10–7.12 (m, 2H, ArH), 7.30 (s, 1H, H-7), 7.47–7.49 (m, 2H, ArH), 12.05 (s, 1H, NH)
5c	3429, 3128, 2951, 1668, 1626, 1545, 1350, 1093, 860, 750	427.1 $[M+H]^+$	[b,d]3.28 (dd, 1H, J_1 = 6.0 Hz, J_2 = 17.7 Hz, H-3), 3.67 (s, 3H, OCH_3), 3.70–3.87 (m, 1H, H-3), 3.77 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 5.24 (d, 1H, J = 6.0 Hz, H-2), 7.27–7.30 (m, 2H, ArH), 7.33 (s, 1H, H-7), 7.95 (bs, 5H, NH), 11.70 (s, 1H, NH)
5d	3362, 3146, 2972, 1678, 1618, 1545, 1401, 1134, 829, 744	371.1 $[M+H]^+$	[b,d]3.29 (d, 1H, J = 18.3 Hz, H-3), 3.84 (dd, 1H, J_1 = 6.9 Hz, J_2 = 18.3 Hz, H-3), 5.26 (d, 1H, J = 6.9 Hz, H-2), 7.12–7.19 (m, 2H, ArH), 7.43–7.49 (m, 3H, ArH), 7.51 (s, 1H, H-4), 7.81 (bs, 4H, NH), 7.94 (d, 1H, J = 8.1 Hz, H-7), 12.50 (s, 1H, NH)
5e	3366, 3140, 2974, 1680, 1618, 1541, 1267, 1130, 812, 742	371.1 $[M+H]^+$	[b,d]3.29 (d, 1H, J = 18.0 Hz, H-3), 3.85 (dd, 1H, J_1 = 6.9 Hz, J_2 = 18.0 Hz, H-3), 5.26 (d, 1H, J = 6.0 Hz, H-2), 7.11–7.20 (m, 3H, ArH), 7.30 (bs, 2H, NH), 7.41–7.48 (m, 3H, ArH), 7.50 (bs, 3H, NH), 8.06 (d, 1H, J = 1.8 Hz, H-7), 11.50 (s, 1H, NH)
5f	3462, 3331, 1676, 1605, 1542, 1263, 1147, 848, 736	405.0 $[M+H]^+$	[b,d]3.27 (d, 1H, J = 18.0 Hz, H-3), 3.84 (dd, 1H, J_1 = 6.0 Hz, J_2 = 18.0 Hz, H-3), 5.27 (d, 1H, J = 6.0 Hz, H-2), 7.07–7.18 (m, 4H, ArH), 7.48 (bs, 4H, NH), 7.62 (d, 1H, J = 1.8 Hz, H-5), 8.07 (d, 1H, J = 1.8 Hz, H-7), 12.50 (bs, 1H, NH)
5g	3435, 3121, 1684, 1618, 1587, 1524, 1336, 1062, 819, 740	382.1 $[M+H]^+$	[b,d]3.36 (dd, 1H, J = 18.0 Hz, H-3), 3.94 (dd, 1H, J_1 = 6.6 Hz, J_2 = 18.0 Hz, H-3), 5.38 (d, 1H, J = 6.6 Hz, H-2), 7.36–7.50 (m, 3H, ArH), 7.68 (d, 1H, J = 8.6 Hz, H-7′), 7.80 (bs, 5H, NH), 7.96 (d, 1H, J = 7.6 Hz, H-7), 8.12 (dd, 1H, J_1 = 2.3 Hz, J_2 = 8.6 Hz, H-6′), 8.41 (d, 1H, J = 2.3 Hz, H-4′), 12.30 (bs, 1H, NH)
5h	3411, 3155, 2939, 1680, 1618, 1521, 1352, 1109, 820, 737	472.0 $[M+H]^+$	[b,d]3.17 (d, 1H, J = 17.7 Hz, H-3), 3.75 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.74–3.9 (m, 1H, H-3), 5.26 (d, 1H, J = 6.3 Hz, H-2), 7.35 (s, 1H, H-7), 7.63 (d, 1H, J = 8.7 Hz, H-7′), 7.85 (bs, 5H, NH), 8.05 (dd, 1H, J_1 = 2.1 Hz, J_2 = 8.7 Hz, H-6′), 8.39 (d, 1H, J = 2.1 Hz, H-4′)
5i	3416, 3172, 2941, 1680, 1619, 1520, 1336, 1109, 821, 734	416.1 $[M+H]^+$	[b,e]3.26 (d, 1H, J = 18.2 Hz, H-3), 3.86 (dd, 1H, J_1 = 6.8 Hz, J_2 = 18.1 Hz, H-3), 5.31 (d, 1H, J = 6.8 Hz, H-2), 7.42 (d, 1H, J = 8.2 Hz, H-6), 7.49 (s, 1H, H-4), 7.63 (d, 1H, J = 8.9 Hz, H-7′), 7.70 (bs, 5H, NH), 7.90 (d, 1H, J = 8.2 Hz, H-7), 8.06 (dd, 1H, J_1 = 2.2 Hz, J_2 = 8.8 Hz, H-6′), 8.38 (d, 1H, J = 2.2 Hz, H-4′), 12.60 (bs, 1H, NH)
5j	3393, 3151, 1680, 1616, 1521, 1336, 1068, 820, 737	416.0 $[M+H]^+$	[b,d]3.30 (d, 1H, J = 18.2 Hz, H-3), 3.89 (dd, 1H, J_1 = 6.8 Hz, J_2 = 18.0 Hz, H-3), 5.35 (d, 1H, J = 6.7 Hz, H-2), 7.42 (d, 1H, J = 8.2 Hz, H-4), 7.47 (dd, 1H, J_1 = 2.1 Hz, J_2 = 8.2 Hz, H-5), 7.66 (d, 1H, J = 8.9 Hz, H-7′), 7.75 (bs, 5H, NH), 8.06 (s, 1H, H-7), 8.09 (dd, 1H, J_1 = 2.2 Hz, J_2 = 8.9 Hz, H-6′), 8.39 (d, 1H, J = 2.2 Hz, H-4′), 12.80 (bs, 1H, NH)
5k	3442, 3114, 1687, 1622, 1589, 1521, 1335, 820, 742	400.1 $[M+H]^+$	[b,d]3.25 (d, 1H, J = 18.0 Hz, H-3), 3.84 (dd, 1H, J_1 = 6.6 Hz, J_2 = 18.0 Hz, H-3), 5.34 (d, 1H, J = 6.5 Hz, H-2), 7.25 (m, 1H, H-5), 7.47 (m, 1H, H-4), 7.50 (bs, 5H, NH), 7.64 (d, 1H, J = 8.9 Hz, H-7′), 7.74 (dd, 1H, J_1 = 2.5 Hz, J_2 = 8.9 Hz, H-7), 8.07 (dd, 1H, J_1 = 2.1 Hz, J_2 = 8.9 Hz, H-6′), 8.39 (d, 1H, J = 2.1 Hz, H-4′), 12.60 (bs, 1H, NH)
5l	3400, 3176, 2954, 1678, 1616, 1595, 1122, 829, 734	367.1 $[M+H]^+$	[c,d]3.15 (dd, 1H, J_1 = 4.3 Hz, J_2 = 18.0 Hz, H-3), 3.84 (dd, 1H, J_1 = 7.0 Hz, J_2 = 18.0 Hz, H-3), 3.86 (s, 3H, OCH_3), 4.40 (dd, 1H, J_1 = 4.5 Hz, J_2 = 7.2 Hz, H-2), 6.92 (dd, 1H, J_1 = 2.3 Hz, J_2 = 8.9 Hz, H-6′), 7.08 (d, 1H, J = 1.75 Hz, H-4′), 7.20–7.50 (bs, 6H, NH), 7.46–7.51 (m, 3H, ArH), 7.72 (m, 1H, ArH), 7.86 (d, 1H, J = 7.5 Hz, H-7), 12.40 (bs, 1H, NH)
5m	3420, 3147, 1680, 1620, 1151, 1070, 825	401.1 $[M+H]^+$	[b,d]3.27 (d, 1H, J = 17.7 Hz, H-3), 3.76 (s, 3H, OCH_3), 3.80 (d, 1H, J = 17.7 Hz, H-3), 5.19 (d, 1H, J = 6.3 Hz, H-2), 6.78 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.7 Hz, H-6′), 7.37–7.43 (m, 2H, ArH), 7.49 (s, 1H, H-4), 7.70 (bs, 5H, NH), 7.93 (m, 2H, ArH), 12.35 (bs, 1H, NH)
5n	3412, 3155, 2959, 1680, 1622, 1151, 818, 758	385.1 $[M+H]^+$	[b,d]3.24 (d, 1H, J = 17.7 Hz, H-3), 3.72 (d, 1H, J = 17.7 Hz, H-3), 3.78 (s, 3H, OCH_3), 5.19 (d, 1H, J = 6.2 Hz, H-2), 6.80 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.8 Hz, H-6′), 7.02 (s, 1H, H-4′), 7.23–7.29 (m, 1H, H-5), 7.40–7.44 (m, 2H, ArH), 7.76 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.9 Hz, H-7), 7.93 (bs, 5H, NH), 12.30 (bs, 1H, NH)
5o	3402, 3144, 2955, 1676, 1618, 1595, 1230, 1126, 817, 758	384.1 $[M+H]^+$	[b,d]3.26 (d, 1H, J = 17.5 Hz, H-3), 3.81 (s, 3H, OCH_3), 3.89 (dd, 1H, J_1 = 6.5 Hz, J_2 = 17.7 Hz, H-3), 5.43 (d, 1H, J = 6.0 Hz, H-2), 7.03 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.4 Hz, H-5), 7.29 (d, 1H, J = 8.4 Hz, H-4), 7.37–7.52 (m, 2H, ArH), 7.54 (d, 1H, J = 2.4 Hz, H-7), 7.85 (d, 1H, J = 8.0 Hz, H-4′), 7.90 (bs, 5H, NH), 8.04 (d, 1H, J = 7.9 Hz, H-7′)
5p	3400, 3076, 1702, 1603, 1585, 1310, 1110, 832, 747	414.1 $[M+H]^+$	[c,d]3.31 (d, 1H, J = 18.0 Hz, H-3), 3.77 (dd, 1H, J_1 = 6.5 Hz, J_2 = 18.0 Hz, H-3), 3.86 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 5.26 (d, 1H, J = 6.0 Hz, H-2), 6.70 (s, 1H, H-4), 7.17 (s, 1H, H-7), 7.24–7.39 (m, 2H, ArH), 7.60 (bs, 5H, NH), 7.72 (d, 1H, J = 7.9 Hz, H-4′), 7.83 (d, 1H, J = 8.1 Hz, H-7′), 11.30 (bs, 1H, NH)
5q	3398, 3154, 2994, 1676, 1607, 1556, 1107, 1035, 839, 758	444.1 $[M+H]^+$	[c,e]3.27 (d, 1H, J = 17.8 Hz, H-3), 3.75 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.86 (dd, 1H, J_1 = 6.7 Hz, J_2 = 17.8 Hz, H-3), 5.36 (d, 1H, J = 6.0 Hz, H-2), 7.37 (s, 1H, H-7), 7.40–7.43 (m, 1H, H-5′), 7.49–7.52 (m, 1H, H-6′), 7.88 (d, 1H, J = 8.0 Hz, H-4′), 8.04 (d, 1H, J = 7.9 Hz, H-7′)
5r	3405, 3144, 2959, 1678, 1610, 1587, 1132, 993, 794, 756	388.1 $[M+H]^+$	[b,e]3.37 (d, 1H, J = 18.0 Hz, H-3), 3.96 (dd, 1H, J_1 = 6.7 Hz, J_2 = 18.0 Hz, H-3), 5.42 (d, 1H, J = 6.5 Hz, H-2), 7.38–7.52 (m, 4H, ArH), 7.76 (d, 1H, J = 8.0 Hz, H-4′), 7.85 (bs, 5H, NH), 7.92 (d, 1H, J = 7.7 Hz, H-7), 8.03 (d, 1H, J = 8.0 Hz, H-7′), 11.50 (bs, 1H, NH)
5s	3398, 3144, 1678, 1607, 1567, 1236, 1130, 993, 827, 758	388.1 $[M+H]^+$	[b,d]3.38 (d, 1H, J = 18.3 Hz, H-3), 3.91 (dd, 1H, J_1 = 6.6 Hz, J_2 = 18.3 Hz, H-3), 5.41 (d, 1H, J = 6.0 Hz, H-2), 7.36–7.50 (m, 4H, ArH), 7.70 (bs, 5H, NH), 7.82 (d, 1H, J = 8.1 Hz, H-4′), 7.92 (d, 1H, J = 8.1 Hz, H-7′), 8.02 (d, 1H, J = 7.8 Hz, H-7)
5t	3396, 3144, 1678, 1616, 1589, 1130, 993, 811, 758	387.9 $[M+H]^+$	[b,d]3.36 (d, 1H, J = 18.3 Hz, H-3), 3.89 (dd, 1H, J_1 = 6.9 Hz, J_2 = 18.3 Hz, H-3), 5.41 (d, 1H, J = 6.0 Hz, H-2), 7.38–7.52 (m, 4H, ArH), 7.65 (bs, 5H, NH), 7.82 (d, 1H, J = 7.7 Hz, H-4′), 8.03–8.05 (m, 2H, ArH)
5u	3446, 3180, 1680, 1618, 1597, 1527, 1135, 993, 814, 758	372.1 $[M+H]^+$	[b,d]3.30 (d, 1H, J = 17.7 Hz, H-3), 3.89 (dd, 1H, J_1 = 7.4 Hz, J_2 = 17.8 Hz, H-3), 5.41 (d, 1H, J = 6.1 Hz, H-2), 7.26–7.33 (m, 1H, ArH), 7.38–7.51 (m, 2H, ArH), 7.60 (bs, 5H, NH), 7.75 (d, 1H, J = 8.5 Hz, H-7), 7.85 (d, 1H, J = 7.8 Hz, H-4′), 8.05 (d, 1H, J = 7.8 Hz, H-7′)

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

7.43–7.55 (m, 2H, ArH), 7.88 (d, 1H, $J = 8.0$ Hz, ArH), 7.91 (d, 1H, $J = 8.0$ Hz, ArH).

*2-(Benzothiazol-2-ylthio)-4-chloro-2,3-dihydroinden-1-one (**4r**)*: Yield 58.7%, yellow solid, m.p. 121–123 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 3.40 (dd, 1H, $J_1 = 4.5$ Hz, $J_2 = 18.0$ Hz, indanone 3-H), 3.93 (dd, 1H, $J_1 = 8.0$ Hz, $J_2 = 18.0$ Hz, indanone 3-H), 4.66 (dd, 1H, $J_1 = 4.5$ Hz, $J_2 = 18.0$ Hz, indanone 2-H), 7.27–7.36 (m, 2H, ArH), 7.41–7.44 (m, 1H, ArH), 7.58 (d, 1H, $J = 7.5$ Hz, ArH), 7.65–7.75 (m, 2H, ArH), 7.78 (d, 1H, $J = 7.5$ Hz, ArH).

*2-(Benzothiazol-2-ylthio)-5-chloro-2,3-dihydroinden-1-one (**4s**)*: Yield, 49.8%, yellow solid, m.p. 123–125 °C. ^{1}H NMR ($CDCl_3$, 300 MHz,) δ: 3.45 (dd, 1H, $J_1 = 4.8$ Hz, $J_2 = 17.7$ Hz, indanone 3-H), 3.90 (dd, 1H, $J_1 = 8.1$ Hz, $J_2 = 17.7$ Hz, indanone 3-H), 4.63 (dd, 1H, $J_1 = 4.8$ Hz, $J_2 = 8.1$ Hz, indanone 2-H), 7.26–7.48 (m, 4H, ArH), 7.59 (d, 1H, $J = 7.6$ Hz, ArH), 7.75 (d, 1H, $J = 7.8$ Hz, ArH), 7.79 (d, 1H, $J = 8.1$ Hz, ArH).

*2-(Benzothiazol-2-ylthio)-6-chloro-2,3-dihydroinden-1-one (**4t**)*: Yield 42.9%, yellow solid, m.p. 128–130 °C. ^{1}H NMR ($CDCl_3$, 300 MHz,) δ: 3.42 (dd, 1H, $J_1 = 4.5$ Hz, $J_2 = 17.4$ Hz, indanone 3-H), 3.88 (dd, 1H, $J_1 = 8.4$ Hz, $J_2 = 17.7$ Hz, indanone 3-H), 4.64 (dd, 1H, $J_1 = 4.5$ Hz, $J_2 = 8.4$ Hz, indanone 2-H), 7.26–7.38 (m, 2H, ArH), 7.42 (d, 1H, $J = 8.1$ Hz, indanone 4-H), 7.59 (d, 1H, $J = 7.9$ Hz, ArH), 7.62 (dd, 1H, $J_1 = 2.1$ Hz, $J_2 = 8.1$ Hz, ArH), 7.75 (d, 1H, $J = 7.9$ Hz, ArH), 7.82 (d, 1H, $J = 2.1$ Hz, ArH).

*2-(Benzothiazol-2-ylthio)-6-fluoro-2,3-dihydroinden-1-one (**4u**)*: Yield 37.4%, yellow solid, m.p. 149–152 °C. ^{1}H NMR ($CDCl_3$, 300 MHz,) δ: 3.41 (dd, 1H, $J_1 = 4.0$ Hz, $J_2 = 17.2$ Hz, indanone 3-H), 3.89 (dd, 1H, $J_1 = 8.2$ Hz, $J_2 = 17.2$ Hz, indanone 3-H), 4.66 (dd, 1H, $J_1 = 4.2$ Hz, $J_2 = 8.2$ Hz, indanone 2-H), 7.28–7.51 (m, 5H, ArH), 7.60 (d, 1H, $J = 7.9$ Hz, ArH), 7.74 (d, 1H, $J = 7.8$ Hz, ArH).

4.3. General procedure for the synthesis of compounds **5**

To a solution of (un)substituted 2-[benzimidazol (benzothiazol)-2-ylthio]-2,3-dihydroinden-1-one hydrobromide (**4**) (2 mmol) in methanol (30 ml) was added 5% HCl in methanol solution (0.5 mL) at 40 °C. To the resulting mixture was then added aminoguanidine hydrochloride (0.3 g, 2.7 mmol) and heated to reflux for 2 h. After decolorizing, the residue was purified by silica gel column chromatography ($CHCl_3/CH_3OH = 10:1$) to afford a solid product **5**.

For the physicochemical characters and spectral data of compounds **5a**–**5u**, see Tables 1 and 4.

4.4. 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 2.23 g, citric acid 0.86 g and glucose 2.47 g in 100 ml distilled H_2O). Platelet-rich plasma (PRP) was obtained by 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 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 μl) was added to 175 μl of propionate buffer (in mmol/l; 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 μl 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 (*Thermo Multiscan Spectrum*). The IC_{50} value was calculated according to the regression analysis. Each measurement was performed in triplicate for all molecules.

4.5. Cardioprotective effects in rat model of ischemic heart

Adult male and female Sprague–Dawley rats (280–300 g) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). Coronary artery occlusion was produced by ligating the left anterior descending coronary artery (LAD) for 1 h. After that, the coronary artery was reperfused by loosing the ligature. After 2 h of reperfusion, the coronary artery was reoccluded and 2 ml of a 1% *Evans* blue was injected via tail vein. Then the heart was removed and blood sample was collected.

The left ventricle of the removed heart was dissected free from other structures and sliced transversely into 1-mm thick sections. The sections were then incubated in 1% triphenyltetrazolium chloride for 15 min at 37 °C and then fixed for 20–24 h in a 10% formalin solution to determine the infarct size. The infarct size was calculated by the formula as follows:

$$\text{Myocardial infarct size\%} = (\text{the weight of undyed myocardium} / \text{the weight of left ventricle}) \times 100\%$$

The blood samples were centrifuged at 3000 *g* for 10 min. The supernatant serum was removed and stored in liquid nitrogen until the biochemical analysis was performed. Creatine kinase (CK) in serum was measured by TU-1800 (Purkinje general instrument Co., Ltd., China) using commercial kits (Jiancheng Bioengineering Institute, China).

Target compounds **5a**, **5g**, **5m**, **5o** and cariporide were intravenously given 5 min before reperfusion.

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