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Original article

Cardioselective anti-ischemic ATP-sensitive potassium channel (K_{ATP}) openers: benzopyranyl indoline and indole analogues

Sunkyung Lee [a], Kyu Yang Yi [a], Soo-Kyung Kim [a], Jeehee Suh [a], Nak Jeong Kim [a], Sung-eun Yoo [a,*], Byung Ho Lee [a], Ho Won Seo [a], Sun-Ok Kim [b], Hong Lim [b]

[a] *Medicinal Science Division, Korea Research Institute of Chemical Technology, 100 Jang-dong, Yoosung-gu, Taejon 305-600, South Korea*
[b] *Division of Life Science, AgroPharma Research Institute of Dongbu-Hannong Chemical Co., Taejon 305-600, South Korea*

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Abstract

This paper describes the design, syntheses, and biological evaluations of novel ATP-sensitive potassium channel (K_{ATP}) openers, benzopyranyl indoline and indole derivatives. Among those, two enantiomers of indoline-2-carboxylic ethyl esters (**14**, **18**) showed the best cardioprotective activities both in vitro and in vivo, while their vasorelaxation potencies were very low (concentration for 50% inhibition of vasorelaxation >30 μM). The cardioprotective effect of **14** was completely reversed by 5-hydroxydecanoate, a selective mitochondrial K_{ATP} blocker, indicating its provable protective mechanism through the mitochondrial K_{ATP} opening. In addition, we performed conformational analyses using ^{2}D-NMR, X-ray crystallography and molecular modeling to study the structure–activity relationships in this series of compounds.

Keywords: K_{ATP} opener; Benzopyranyl indole; Cardioselectivity; Mitochondrial K_{ATP}; Conformational analyses

1. Introduction

Ischemic preconditioning (PC) is a powerful endogenous protective mechanism against ischemic injury observed in a variety of organ systems [1], including heart, brain, spinal cord, retina, liver, lung, and skeletal muscle, which had been previously subjected to brief, sub-lethal periods of ischemia [2–4]. Ischemic PC is a multifactorial physiological process, requiring the interaction of numerous cellular signals, second messengers, and end-effector mechanisms [5]. Many researchers have identified the possible triggers, transducers, and end-effectors involved in ischemic PC, which allows mimicking of the physiological response by chemical agents [6], known as 'chemical PC' [7]. ATP-sensitive potassium channel (K_{ATP}) openers have shown to play a crucial role in ischemic PC, and this hypothesis is supported by studies showing the K_{ATP} blockers can attenuate this protective effect, suggesting K_{ATP} to have an important protective mechanism [8,9]. K_{ATP} channels are composed of a complex of two distinct subunits, an inward rectifying K^+ channel subunit (Kir 6.x) forming the channel pore and a sulfonylurea receptor (SUR) belonging to the ATP-binding cassette super family [10,11]. Diversity of K_{ATP} channels by the various combinations of their subunit composition may provide the possibility to target certain isoforms for the identification of tissue-selective compounds [12]. In particular, the importance of cardiac K_{ATP} channel opening in myocardial PC has been demonstrated by many researchers [13,14], even though it is still unclear how K_{ATP} activation can mediate PC. Whatever the mechanism of PC, it is

Abbreviations: PC, preconditioning; K_{ATP}, ATP-sensitive potassium channel; Kir, inward rectifying K^+ channel; SUR, sulfonylurea receptor; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; Boc, *t*-butyloxycarbonyl; LAH, lithium aluminum hydride; IC_{50}, concentration for 50% inhibition of vasorelaxation; LVDP, left ventricular developing pressure; HR, heart rate; TTC, time to contracture; i.v., intravenous; i.p., intraperitoneal; LDH, lactate dehydrogenase; IZ/AAR, infarction zone to area at risk; 5-HD, 5-hydroxydecanoate; M.p., melting point; THF, tetrahydrofuran; TFA, trifluoroacetic acid; LVP, left ventricular pressure; EDP, end diastolic pressure; LVEDP, left ventricular end diastolic pressure; LVSP, left ventricular systolic pressure; TTC, 2,3,5-triphenyltetrazolium chloride.

* Corresponding author.
E-mail address: seyoo@krict.re.kr (S.-e. Yoo).

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encouraging that K_{ATP} openers can mimic an endogenous protective factor, which further increase the enthusiasm for developing novel cardioprotective K_{ATP} openers [15].

The first cardioselective K_{ATP} opener to be described was BMS-180448 **1** (Fig. 1), benzopyranyl cyanoguanidine [16,17]. From studies at Bristol–Myers Squibb using the analogues of urea, benzimidazolone and indole derivatives [18], the imidazole analogue (BMS-191095, **2**) has been discovered which displayed more than a 20-fold improvement in selectivity for cardioprotective over vasorelaxant effects in vitro compared with BMS-180448 [19].

In our previous work, we found 6-aminobenzopyranyl cyanoguanidine analogue (**3**), showing good cardio- and neuro-protective effects without vasorelaxation activity [20] in contrast to SKP-450 (**4**) which is also a K-channel activator but with a strong blood pressure lowering activity [21,22]. Aiming at identifying cardioselective K_{ATP} openers and elucidating structure–activity relationships, we prepared several structurally constrained indole and indoline derivatives based on a benzopyran scaffold having an acetal moiety at the 2-position similarly, as the previously studied compound **3** and SKP-450 (**4**). Herein, we describe their design, syntheses, structure analyses using NMR (NOESY), X-ray crystallography, and molecular modeling as well as their biological activities.

2. Chemistry

2.1. Synthesis

Optically pure benzopyranyl indoline and indole derivatives substituted at the 2-position were prepared according to the procedure described in Scheme 1.

Indoline-2-carboxylic ester analogues **13**–**18** were prepared from previously described optically pure benzopyranyl epoxides by epoxide ring opening with the appropriate indolines in the presence of magnesium perchlorate in acetonitrile [20,23]. The reaction of indoline with epoxide proceeded smoothly in good yield (around 80%), and the resulting two separable diastereoisomers at indoline-2-position were separated by silica gel column chromatography. The absolute stereochemistry was confirmed by using commercially available (−)-(*S*)-indoline-2-carboxylic acid as a starting material. Various indoline-2-carboxylic esters **5**–**7** were prepared by acid-catalyzed esterification of indoline-2-carboxylic acid. The indoline-2-carboxylic acid analogue **24** was obtained from the ester compound **14** by a typical base hydrolysis.

The *N*-(2-methoxycarbonyl)indolylbenzopyran has been prepared from epoxide and indole in the presence of sodium hydride in low yield [18]. The major product in that reaction was reported to be a dehydrated compound at 3,4-position of benzopyran ring and a significant amount of a carboxylic acid product was also obtained presumably due to saponification of an ester group under the reaction condition. We tried the same reaction with indole and an epoxide under various conditions, but did not get any reasonable yield of the desired product. Fortunately, we found that the oxidation of indoline derivatives (**13**–**16**, **19**–**22**) with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) [24] provided corresponding indole derivatives **25**–**28** efficiently (85–91% yields).

For the preparation of highly constrained lactone analogues (**29**, **30**) several intramolecular cyclization reactions between the 3-hydroxyl group in the benzopyran ring and the ester group at the 2-position of indolines (**21**, **22**) were examined to come up with the method using aluminum diethylchloride in the presence of a base catalysis [25] which provided the desired products in good yields (85 and 81%).

Indoline-2-ether analogues (**31**, **32**) were prepared by a series of reactions from indoline-2-carboxylic acid; (1) *t*-butyloxycarbonyl (Boc) protection of the nitrogen atom, (2) reduction of the acid group to the alcohol using lithium aluminum hydride (LAH), (3) *O*-methylation of the alcohol to ether, (4) deprotection of the *N*-Boc group under acidic condition, and finally the coupling of methoxymethylindoline (**10**) with the epoxide (**12**) using magnesium perchlorate in acetonitrile.

2.2. Structure analysis

Previously the structure–activity relationship on K_{ATP} openers which have the aniline moiety

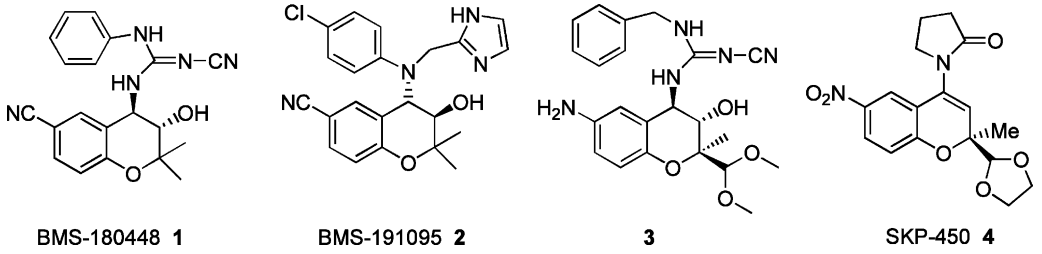
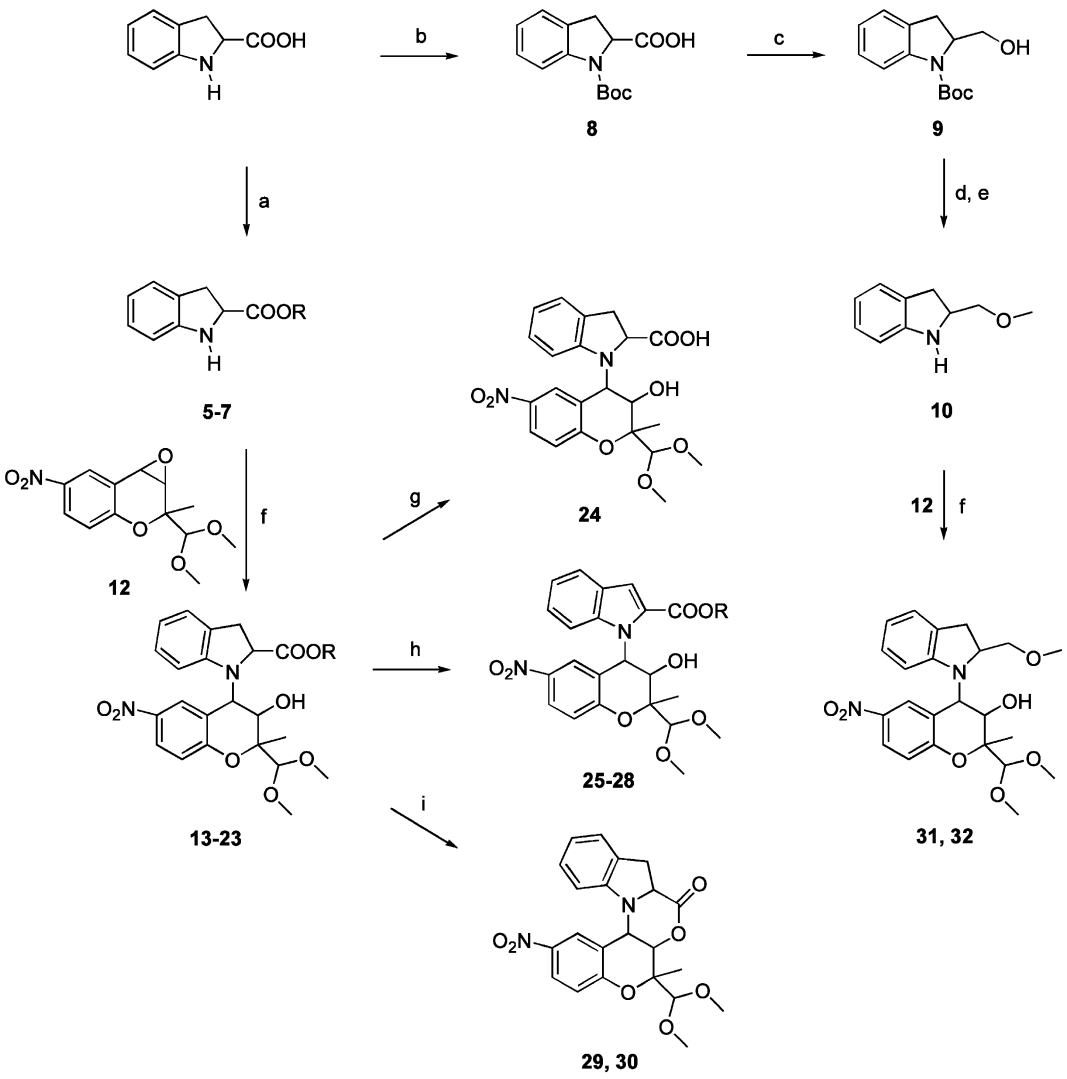


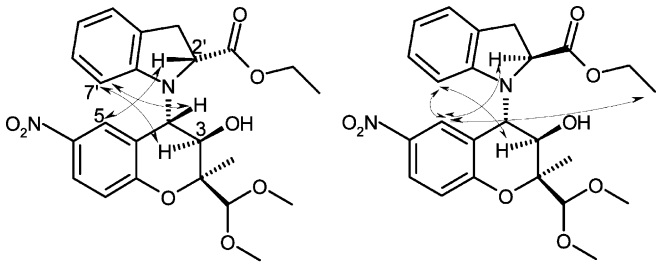
Fig. 1. K_{ATP} openers.



Scheme 1. Reagents and conditions: (a) $SOCl_2$, ROH (R = Me, Et, *i*Pr), reflux; (b) $(Boc)_2O$, NEt_3, THF, r.t.; (c) BH_3, THF, 0 °C → r.t.; (d) MeI, NaH, THF, 0 °C → r.t.; (e) TFA, CH_2Cl_2, r.t.; (f) $Mg(ClO_4)_2$, CH_3CN, r.t.; (g) NaOH, MeOH, r.t.; (h) DDQ, benzene, r.t.; (i) Et_2AlCl, *i*-Pr_2NH, CH_2Cl_2, r.t.

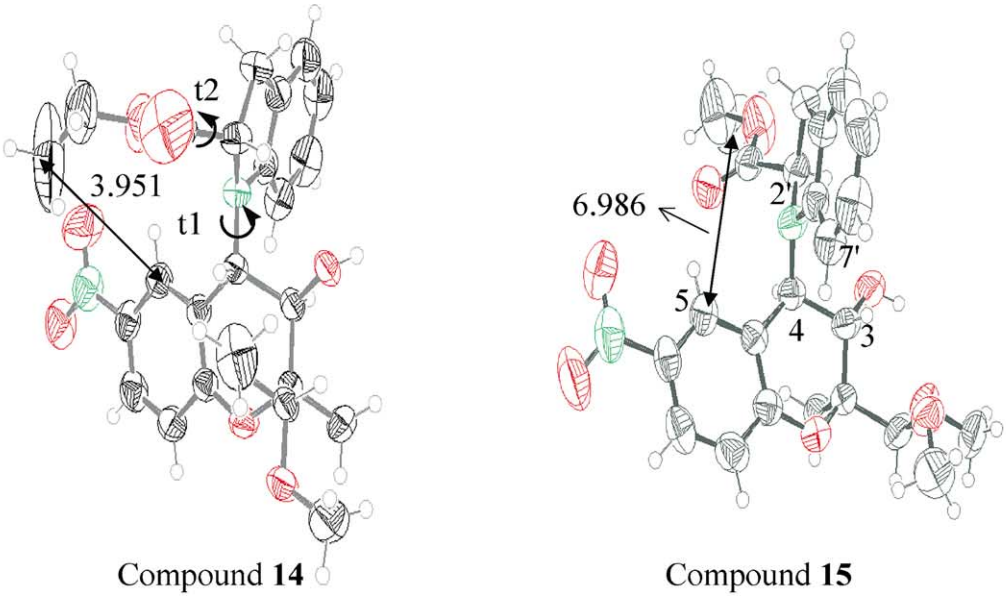
($PhNCH_2COOEt$) attached to C4 of the benzopyran ring has been done and several key points found from the study are as follows [19]; (1) The carboxylic acid analogue is devoid of anti-ischemic activity. (2) Increase in the size of ester groups causes attenuation of anti-ischemic potency. (3) There are restrictions as to the size of the pendent aryl/heterocyclic ring. (4) An sp^3 carbon adjacent to the aniline nitrogen is preferred. (5) Changing the ester to an imidazole retains full anti-ischemic activity with excellent selectivity. (6) The SARs for the vasorelaxant and anti-ischemic potencies are distinctively different.

To establish the pharmacophore of our series of compounds and to compare with the pharmacophore of other series of compounds, we carried out extensive conformational analyses experimentally and theoretically including 2-D NMR experiments (COSY and NOESY, Fig. 2), X-ray analyses (Fig. 3), and molecular modeling (Fig. 4) on the compound **14** which has good in vitro and in vivo cardioprotective activities with selectivity.



Compound **13** Compound **14**

Fig. 2. NOESY cross peaks. ^{2}D-NMR experiments were performed using Bruker 600 MHz spectrometer at 25 °C. Mixing times of 400 ms for NOESY were used.



Compound **14** Compound **15**

	t1	t2	7'H-5H	7'H-3H	7'H-4H	EtC-5C	2'H-5H
Compound **14**	-78.2	69.2	3.151 Å	2.408 Å	3.932 Å	3.951 Å	4.314 Å
Compound **15**	-49.0	-168.7	3.683 Å	2.754 Å	4.057 Å	6.986 Å	4.428 Å

[a]ORTEP diagram showed the X-ray structures. Torsion angles and distances were calculated using SYBYL (v. 6.7).

Fig. 3. X-ray crystallography of compound **14** and **15**. ORTEP diagram showed the X-ray structures. Torsion angles and distances were calculated using SYBYL (v. 6.7).

3. Pharmacology

The vasorelaxant potencies were determined by concentration for 50% inhibition of vasorelaxation (IC_{50}) values for relaxation of the methoxamine-contracted rat aorta, as described previously [20,26]. Cardioprotective effects in vitro were evaluated in globally ischemic, isolated, perfused rat hearts at 10 μM concentration [27,28]. Severity of ischemia was judged by following parameters; the recovery of contractile function (LVDP × HR, left ventricular developing pressure × heart rate) at the end of the reperfusion period, the time to contracture (TTC the time from the onset of global ischemia in which the first 5 mmHg increase in end diastolic pressure (EDP) is observed), and the amount of cumulative LDH (lactate dehydrogenase) release into the reperfusate. Anti-ischemic in vivo potencies were determined by measuring a ratio of myocardial infarction zone to area at risk (IZ/AAR), using ischemic myocardium damage rat model at 0.3 mg kg^{-1} via intravenous (i.v.) injection [20,29].

4. Results and discussion

Besides the indole analogues described to have good pharmacological profiles as a cardioprotective [18], we investigated the indoline analogues based on the report that an sp^3 carbon adjacent to the aniline is preferred rather than an sp^2 carbon [19].

Initially we synthesized four diastereomers of optically pure 6-nitobenzopyranylindoline-2-carboxylic acid ethyl ester derivatives (**13**–**16**) with the same (2*S*)-stereochemistry at the benzopyran as 6-aminobenzopyranyl-*N*-cyanoguanidine analogue (**3**) and evaluated their pharmacological activities [20]. We investigated the 6-

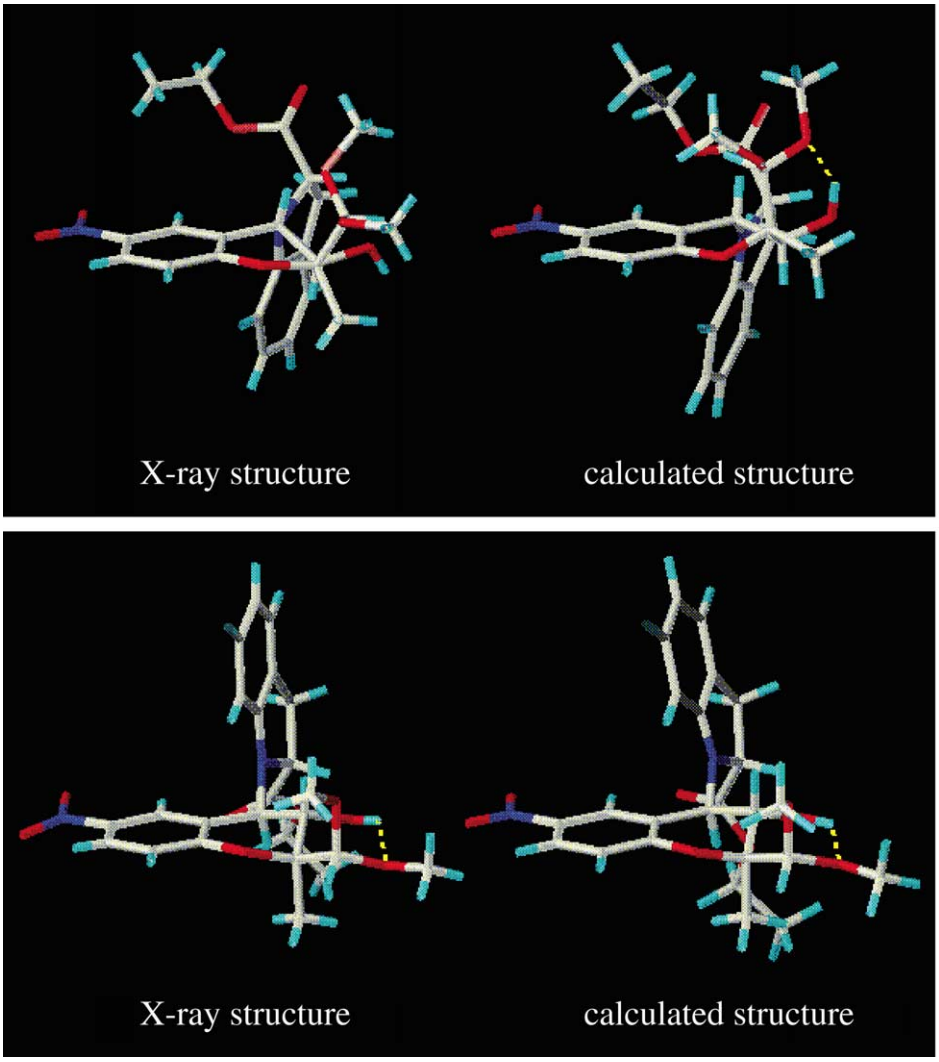


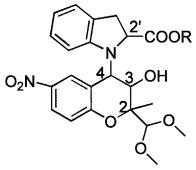
Fig. 4. Comparison of structures between X-ray and molecular modeling.

nitrobenzopyran analogues prior to the 6-amino compounds, based on the report that an electron withdrawing group at the 6-position of benzopyran ring seems to be essential for K_{ATP} opening activity [26]. While all four compounds showed much weaker vasorelaxation activity compared to BMS-180448 (**1**), only the compound **14** with (2*S*,3*R*,4*S*,2′*S*)-stereochemistry showed a comparable cardioprotective effect both in vitro and in vivo to BMS-180448 (Table 1). To determine the importance of the absolute stereochemistry of the compounds, we synthesized the enantiomer **18** and found that the cardioprotective and vasorelaxation activities were almost the same as **14**. Also, we prepared the diastereomer **17** in which the stereochemistry at the 2-position of the indoline ring is opposite and found that the cardioprotective activity was much weaker than **18**. These results indicate that the relative stereochemistry of diastereoisomers is crucial for determining cardioprotective activity, but the absolute stereochemistry is not in this case. While the compounds **14** and **18** showed the comparable cardioprotective efficacies to BMS-180448, the vasorelaxation potencies of those compounds were more than 20 times weaker than that of BMS-180448, demonstrating improved selectivity.

With the compound **14** as a standard, we then examined the effect of ester group by preparing the methyl and isopropyl analogues, and the acid itself, **20**, **23**, and **24**, respectively. In general, in vitro cardioprotective activities of the methyl **20** and ethyl **14** esters were comparable but the ethyl ester derivative was much weaker in the vasorelaxation activity to make the ethyl ester analogue more desirable overall. Furthermore the methyl ester analogue **20** did not demonstrate in vivo cardioprotective efficacy. Isopropyl ester analogue **23** showed only marginal protective effect, and the acid compound **24** completely lost the protective activity.

The indole analogues **25** and **27** with the same (2*S*,3*R*,4*S*)-stereochemistry as the compound **14** showed good in vitro cardioprotective activities, but

Table 1
Vasorelaxant potencies and cardioprotective effects of benzopyranyl indoline and indole analogues

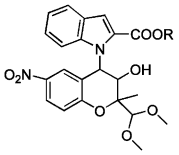
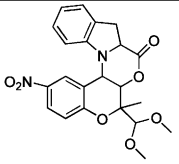
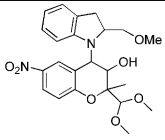
Compounds (Stereo, 2,3,4,2')	R	Vaso[a] IC_{50} (μM)	In vitro Cardioprotection at 10 μM[b] LVDP × HR (%)	EDP (mmHg)	TTC (min)	LDH (U/g)	In vivo Anti-Infarction[c] at 0.3 mg/Kg (IZ/AAR, %)
vehicle			23.0	43.4	20.3	29.9	61/40
BMS-180448		1.4 (1.3, 1.5)	67.6	16.5	27.8	17.1	39/39
 **13** (SRSR)	Et	16.2 (9.6, 23.9)	20.6	29.5	23.8	21.0	nd[d]
14 (SRSS)	Et	> 30	67.9	2 5.7	23.7	20.4	39/33
15 (SSRR)	Et	> 30	23.9	47.3	19.9	17.9	nd[d]
16 (SSRS)	Et	26.4 (16.4, 34.9)	23.4	28.2	19.2	16.9	nd[d]
17 (RSRS)	Et	> 30	27.2	54.7	20.2	18.4	nd[d]
18 (RSRR)	Et	> 30	64.1	11.3	27.1	9.2	39/34
19 (SRSR)	Me	11.5 (9.6, 14.6)	44.0	17.0	24.2	19.6	nd[d]
20 (SRSS)	Me	15.6 (13.2, 20.5)	62.2	21.0	24.5	10.4	54/40
21 (SSRR)	Me	16.2 (9.6, 22.5)	38.4	30.3	21.7	24.2	nd[d]
22 (SSRS)	Me	19.2 (15.4, 24.6)	34.9	40.0	22.1	30.8	nd[d]
23 (SRSS)	*i*-Pr	> 30	36.4	24.0	23.5	38.4	43/35
24 (SRSS)	H	> 30	31.2	34.0	21.5	26.1	52/37

no in vivo efficacies. The activities among diastereomers were also quite sensitive to the stereochemistry same as the indoline case.

Structurally highly constrained lactone analogues (**29**, **30**) did not show any significant cardioprotective activities. The ether analogues (**31**, **32**) showed excellent in vivo cardioprotective activities in the rat model but poor activities in the Langendorff model.

To elucidate the nature of the cardioprotective effects of this series of compounds, we first checked whether the cardioprotective effect was reversed by the action of 5-hydroxydecanoate (5-HD), a mitochondrial K_{ATP} blocker. As can be seen in Table 2, the cardioprotective effect of the compound **14** was completely reversed by 5-HD, indicating that the compound **14** exhibits its cardioprotective effect through the mechanism of opening of K_{ATP}, especially a mitochondrial K_{ATP} channel.

The NMR spectra of the compounds **13** and **14** indicate the existence of two rotamers in a 3:2 ratio. Other indoline-2-carboxylic esters exist as a mixture of

	**25** (SRS)	Et	> 30	55.7	24.7	26.4	31.4	51/37
	26 (SSR)	Et	22.3 (16.7, 28.6)	26.6	42.0	24.6	26.3	nd[d]
	27 (SRS)	Me	24.0 (19.2, 28.9)	79.5	9.5	22.0	16.7	nd[d]
	28 (SSR)	Me	5.0 (3.9, 6.6)	35.1	46.7	19.1	23.5	nd[d]
	**29** (SRSS)		17.7 (9.4, 23.1)	38.0	34.6	22.6	20.8	47/39
	30 (SSRR)		4.1 (3.9, 4.2)	36.9	40.0	21.5	23.8	48/37
	**31** (SRSR)		> 30	37.1	46.6	19.9	33.3	35/38
	32 (SRSS)		> 30	33.4	45.2	21.5	28.0	37/39

[a]Vasorelaxant potency was assessed by measurement of IC_{50} for inhibition of methoxamine-contracted rat aorta. IC_{50} value is presented as a mean with 95% confidence interval in parentheses, $n = 3$. [b]In vitro cardioprotective effects were evaluated measuring the contractile function (LVDP × HR), TTC, and LDH in the globally ischemic rat heart. Each value is an average of three or higher determinations and within ±10%. [c]In vivo anti-infarction effects were determined by measuring a ratio of myocardial IZ/AAR in ischemic myocardium damage rat model (0.3 mg kg^{-1}), $n = 3$ or higher. Each value given is an average and within ±10%. [d]Not determined.

rotamers, too. Besides, there are few interesting points to be mentioned. As shown in Fig. 2, NOEs were observed between 7H (δ, 5.53 ppm) in the indole ring and 3H (δ, 4.41), 5H (δ, 8.80) in the benzopyran part in the major conformer of **14** but not in the minor conformer. The chemical shift of 7H in the indole ring in the major conformer of **14** was 5.53 ppm, far shifted to upfield, presumably due to the shielding effect of the benzene ring in the benzopyran moiety. In the X-ray structure, the distances between 5H in the benzopyran and 7H in the indole ring of compound **14** and **15** were 3.15 and 3.68 Å, respectively. The chemical shift of 7H in the indole ring in NMR of **15** was 5.61 ppm, indicating the shielding effect. From NMR and X-ray analyses, two benzene rings of the benzopyran and indole in both compounds **14** (δ, 8.80) and **15** (δ, 8.90) seem to be close enough to show NOE and the shielding effects, even though those minor conformer are not in NMR (δ, 7.76 for **14**, 7.82 for **15**). The torsion angle between the benzopyran ring and indole ring in **14** was $-78.2°$ in the X-ray structure, while it was $-49.0°$ in the compound **15** (Fig. 3). The structures from molecular modeling study were matched well with the experimental ones from NMR and X-ray (Fig. 4).

In addition, there were NOEs between 5H (δ, 8.80) in the benzopyran and the CH_3 protons (δ, 1.35) in the

Table 2
The effects of K_{ATP} blockers on cardioprotection of compound **14**

Compounds	LVDP × HR (%)	EDP (mmHg)	CFR (%)	TTC (min)	LDH (U g^{-1})
Vehicle	15.5	55.3	83.4	19.8	33.6
14 (10 μM)	67.9	25.7	91.6	23.7	20.3
14 (10 μM)+5-HD (100 μM)	17.7	52.0	93.5	18.6	33.7

In vitro cardioprotective effects were evaluated measuring the contractile function (LVDP × HR), TTC, and LDH in the globally ischemic rat heart with or without K_{ATP} blocker, sodium 5-hydroxydecanoate (5-HD). Each value is an average of three determinations and within ±10%.

ester group of the major conformer of **14** (Fig. 2). The distance between those carbons was estimated to be 4.0 Å in the lowest energy conformer of compound **14** by molecular mechanic calculation which is very close to 3.95 Å measured in the X-ray structure (Fig. 3). The corresponding distance in the crystal structure of compound **15**, an inactive compound, was 6.99 Å. It is hard to know whether the major conformer of compound **14** is the active one or not, but this distance parameter could be used to differentiate the active compounds from inactive compounds.

Due to the existence of rotamers, it is not appropriate to correlate the conformational structures with the biological activities from this study. But it is apparent from NMR, X-ray, and molecular modeling analyses that two aryl rings in the active compound **14** seem to be perpendicular and closely located. We will continue the study to elucidate the structure–activity relationships in this and related series.

5. Conclusions

We prepared a series of optically active benzopyranyl indoline and indole derivatives in which various esters, acid, ethers and lactones are substituted at the 2-position and evaluated their cardioprotective and vasorelaxation activities. Two enatiomers of indoline-2-ethyl esters **14** and **18** showed the best cardioprotective activities both in vitro and in vivo among those, while their vasorelaxation potencies were very weak ($IC_{50} > 30$ μM). The cardioprotective effect of **14** was completely reversed by 5-HD, a selective mitochondrial K_{ATP} blocker, indicating its probable protective mechanism through the mitochodrial K_{ATP} opening.

6. Experimental

6.1. Chemistry

Melting points (m.p.) were determined on a capillary m.p. apparatus and are uncorrected. Anhydrous solvents were dried by conventional methods. Reagents of commercial quality were used from freshly opened containers unless otherwise stated. For purification of products by column chromatography, Merck Silica gel 60 (230–400 mesh) was used. ^{1}H-NMR spectra were recorded on a Varian Gemini 200 with TMS as an internal standard. Chemical shifts are reported in δ (ppm). 2-D NMR experiments were performed using Bruker 600 MHz spectrometer at 25 °C. Mixing times of 400 ms for NOESY were used. Mass spectra were obtained with a JEOL JMS-DX 303 instrument by using electron impact or chemical ionization techniques. Elemental analyses of key compounds were performed in the Analytical Department of Korea Research Institute of Chemical technology and C, H, and N values were found to be within ±0.4% of the calculated values.

6.2. General procedure for the syntheses of indoline-2-carboxylic acid esters (*5*–*8*)

To a solution of indoline-2-carboxylic acid (163 mg, 1 mmol) in an appropriate alcohol (40 mL) were added $SOCl_2$ (100 μL, 1.37 mmol) dropwise. The mixture was heated at reflux for 5 h, and all volatiles were removed in vacuo. The residue was diluted with water and extracted with CH_2Cl_2 (10 mL × 3), and the organic layers were combined. The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 4:1).

6.2.1. Indoline-2-carboxylic acid ethyl ester (*5*)

^{1}H-NMR ($CDCl_3$) δ; 1.29 (t, 3H, $J = 7.3$ Hz), 3.32 (m, 2H), 4.21 (q, 2H, $J = 7.2$ Hz), 4.39 (dd, 1H, $J = 9.5$, 6.1 Hz), 6.72 (m, 2H), 6.98–7.12 (m, 2H); MS 191 [M^+].

6.2.2. Indoline-2-carboxylic acid methyl ester (*6*)

^{1}H-NMR ($CDCl_3$) δ 3.38 (m, 1H), 3.74 (s, 3H), 4.36–4.47 (m, 2H), 6.72 (m, 2H), 7.01–7.13 (m, 2H); MS 177 [M^+].

6.2.3. Indoline-2-carboxylic acid isopropyl ester (*7*)

^{1}H-NMR ($CDCl_3$) δ 1.25 (s, 3H), 1.28 (s, 3H), 3.12–3.54 (m, 2H), 4.48 (dd, 1H, $J = 10.1$, 5.9 Hz), 5.08 (m, 1H), 6.83–6.96 (m, 2H), 7.08–7.16 (m, 2H);); MS 205 [M^+].

6.3. *N*-(*t*-butyloxycarbonyl)indoline-2-carboxylic acid (*8*)

To the solution of indoline-2-carboxylic acid (5 g, 42.8 mmol) in tetrahydrofuran (THF) (50 mL) were added triethylamine (12.8 mL, 91.8 mmol) and di-*t*-butyl dicarbonate (13.4 g, 6.4 mmol). The mixture was stirred at room temperature (r.t.) overnight, and concentrated under reduced pressure. The residue was dissolved in 1 N NaOH (20 mL) and washed with ether. The water layer was acidified with c-HCl, and then extracted with ether (15 mL × 3). The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give a white solid (7.6 g, 94.2%): ^{1}H-NMR ($CDCl_3$) δ 1.52 (s, 9H), 3.12 (dd, 1H, $J = 14.7$, 3.2 Hz), 3.52 (dd, 1H, $J = 14.8$, 11.6 Hz), 3.81 (dd, 1H, $J = 11.6$, 3.2 Hz), 6.93 (dd, 1H, $J = 7.8$, 7.4 Hz), 7.15 (m, 2H), 7.88 (brd, 1H); MS 263 [M^+].

6.4. N-(t-butyloxycarbonyl)-2-hydroxymethylindoline (9)

To a solution of acid **8** (500 mg, 1.9 mmol) in THF (15 mL) was added 1 N BH_3·THF (7.6 mL) dropwise at 0 °C via syringe. The solution was stirred at r.t. overnight, then all volatiles were removed in vacuo. The residue was dissolved in ethyl acetate (15 mL) and washed with 0.5 N NaOH (10 mL × 3). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 1:1) to give an off-white solid (450 mg, 96%): ^{1}H-NMR ($CDCl_3$) δ 1.58 (s, 9H), 2.83 (brd, 1H), 3.31 (dd, 1H, J = 16.3, 10.1 Hz), 3.58–3.80 (m, 2H), 4.56 (m, 1H), 6.94 (dd, 1H, J = 7.5, 7.5 Hz), 7.10–7.19 (m, 2H), 7.56 (br, 1H); MS 249 [M^+].

6.5. N-(t-butyloxycarbonyl)-2-methoxymethylindoline (10)

To a solution of alcohol **9** (400 mg, 1.6 mmol) in THF (15 mL) was added NaH (60% in oil, 84 mg, 2.1 mmol) at 0 °C. After stirring for 30 min, iodomethane (0.2 mL, 3.22 mmol) was added to the mixture. The reaction was stirred at r.t. overnight, and all volatiles were removed under reduced pressure. The residue was dissolved in dichloromethane (15 mL), washed with water (15 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 10:1) to give an off-white solid (260 mg, 61%): ^{1}H-NMR ($CDCl_3$) δ 1.57 (s, 9H), 2.98 (dd, 1H, J = 10.9, 2.4 Hz), 3.19–3.33 (m, 2H), 3.35 (s, 3H) 3.60 (dd, 1H, J = 10.3, 3.8 Hz), 4.57 (m, 1H), 6.97 (dd, 1H, J = 7.3, 7.3 Hz), 7.11–7.18 (m, 2H), 7.63 (br, 1H); MS 263 [M^+].

6.6. 2-Methoxymethylindoline (11)

The solution of compound **10** (260 mg, 1 mmol) in dichloromethane (7 mL) and TFA (0.8 mL) was stirred for 20 h at r.t., and all volatiles were removed in vacuo. The residue was dissolved in dichloromethane (10 mL) and carefully washed with saturated $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 4:1) to give an off-white solid (140 mg, 87%): ^{1}H-NMR ($CDCl_3$) δ 2. 69 (dd, 1H, J = 15.7, 6.7 Hz), 3.14 (dd, 1H, J = 15.7, 9.0 Hz), 3.39–3.44 (m, 5H), 4.13 (m, 1H), 6.61–6.74 (m, 1H), 6.99–7.15 (m, 2H); MS 163 [M^+].

6.7. General procedure for the syntheses of compounds (13-23)

The reaction mixture of an amine **5**–**7** (1 mmol), appropriate optically pure epoxide (282 mg, 1 mmol) [20], and $Mg(ClO_4)_2$ (224 mg, 1 mmol) in CH_3CN (0.5 mL) was stirred at r.t. for 6 h, and concentrated under reduced pressure. Resulting two diastereomers were separated by silica gel column chromatography (hexane:ethyl acetate = 4:1) to give a pale yellow foam each (75–94%). Some foams were crystallized from an appropriate solvent.

6.7.1. 1-[(2S,3R,4S)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-(2R)-2,3-dihydro-1H-indole-2-carboxylic acid ethyl ester (13)

M.p.: 126–127 °C (ethanol); ^{1}H-NMR ($CDCl_3$, 600 MHz) δ 0.94 (t, 1.2H, J = 7.1 Hz), 1.36 (t, 1.8H, J = 7.1 Hz), 1.64 (s, 3H), 3.20 (d, 0.4H, J = 10.0 Hz), 3.26 (dd, 0.6H, J = 11.1, 10.2 Hz), 3.33 (s, 1.8H), 3.40 (m, 1.6H), 3.49 (s, 1.2H), 3.51 (s, 1.8H), 3.58 (m, 0.4H), 3.65 (m, 0.4H), 3.72 (dd, 0.6H, J = 14.6, 10.3 Hz), 4.28 (m, 0.4H), 4.34 (m, 1.2H), 4.38 (m, 0.6H), 4.55–4.63 (m, 1.8H), 4.72 (s, 0.6H), 4.90 (dd, 0.6H, J = 12.0, 10.3 Hz), 4.98 (d, 0.4H, J = 10.3 Hz), 5.25 (s, 0.6H), 5.79 (d, 0.6H, J = 8.3 Hz), 6.56–6.63 (m, 1H), 6.73 (m, 1H), 6.87 (d, 0.4H, J = 8.9 Hz), 6.94 (d, 0.6H, J = 9.0 Hz), 7.05 (d, 0.6H, J = 7.3 Hz), 7.12 (m, 0.8H), 7.61 (s, 0.6H), 7.95 (dd, 0.6H, J = 9.4, 2.2 Hz), 7.97 (d, 0.4H, J = 10.8 Hz), 8.27 (s, 0.4H); MS 472 [M^+]; Anal. ($C_{24}H_{28}N_2O_8$) C, H, N.

6.7.2. 1-[(2S,3R,4S)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-(2S)-2,3-dihydro-1H-indole-2-carboxylic acid ethyl ester (14)

M.p.: 137–138 °C (ethanol); ^{1}H-NMR ($CDCl_3$, 600 MHz) δ 1.26 (m, 1.2H), 1.35 (m, 1.8H), 1.56 (s, 3H), 3.26 (br, 0.6H), 3.37 (s, 2.4H), 3.37 (m, 0.4H), 3.41 (m, 0.4H), 3.48 (s, 1.8H), 3.48 (m, 0.4H), 3.50 (s, 1.8H), 3.58 (m, 1H), 4.12 (q, 0.8H, J = 6.8 Hz), 4.22 (br, 0.6H), 4.34 (m, 1.2H), 4.41 (m, 1.4H), 4.68 (m, 0.6H), 4.74 (s, 0.6H), 5.14 (br, 0.6H), 5.26 (br, 0.4H), 5.53 (brd, 0.6H), 6.60 (m, 1H), 6.77 (m, 1H), 6.93 (d, 1H, J = 9.1 Hz), 7.04 (m, 0.6H), 7.12 (m, 0.8H), 7.76 (br, 0.4H), 8.01 (br, 0.4H), 8.07 (m, 0.6H), 8.80 (s, 0.6H); MS 472 [M^+]; Anal. ($C_{24}H_{28}N_2O_8$) C, H, N.

6.7.3. 1-[(2S,3S,4R)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-(2R)-2,3-dihydro-1H-indole-2-carboxylic acid ethyl ester (15)

M.p.: 196–197 °C (ethyl acetate–hexane); ^{1}H-NMR ($CDCl_3$) δ 1.23–1.47 (m, 6H), 3.28 (m, 2H), 3.55 (s, 3H), 3.62 (s, 3H), 4.35 (q, 2H), 4.80 (m, 1H), 5.16 (m, 1H), 5.61 (d, 0.6H, J = 8.3 Hz), 6.6–7.2 (m, 4.4H), 7.82 (d,

0.4H), 8.08 (m, 1H), 8.90 (d, 0.6H, $J = 2.0$ Hz); MS 472 [M^+]; Anal. ($C_{24}H_{28}N_2O_8$) C, H, N.

*6.7.4. 1-[(2S,3S,4R)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-(2S)-2,3-dihydro-1H-indole-2-carboxylic acid ethyl ester (**16**)*

M.p.: 115–118 °C (ethyl acetate–hexane); ^{1}H-NMR ($CDCl_3$) δ 1.35 (m, 6H), 3.38 (m, 2H), 3.64 (s, 3H), 3.66 (s, 3H), 4.29 (q, 2H, $J = 7.1$ Hz), 4.68 (m, 1H), 4.91 (m, 1H), 5.22 (s, 1H), 5.88 (d, 0.7H, $J = 7.3$ Hz), 6.6–7.2 (m, 4.3H), 7.68 (d, 0.7H, $J = 1.6$ Hz), 8.02 (m, 1H), 8.34 (d, 0.3H, $J = 1.7$ Hz); MS 472 [M^+]; Anal. ($C_{24}H_{28}N_2O_8$) C, H, N.

*6.7.5. 1-[(2R,3S,4R)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-(2S)-2,3-dihydro-1H-indole-2-carboxylic acid ethyl ester (**17**)*

M.p.: 125–127 °C (ethyl acetate–hexane); ^{1}H-NMR ($CDCl_3$) δ 0.94 (t, 1.2H, $J = 7.2$ Hz), 1.36 (t, 1.8H, $J = 7.0$ Hz), 1.64 (s, 3H), 3.1–3.3 (m, 1H), 3.33 (s, 1.8H), 3.41 (m, 1.6H), 3.49 (s, 1.2H), 3.51 (s, 1.8H), 3.55–3.8 (m, 1.4H), 4.25–5.0 (m, 5.6H), 5.25 (s, 0.6H), 5.78 (d, 0.6H, $J = 8.0$ Hz), 6.5–7.2 (m, 4.4H), 7.61 (s, 0.6H), 7.9–8.0 (m, 1H), 8.27 (br, 0.4H); MS 472 [M^+]; Anal. ($C_{24}H_{28}N_2O_8$) C, H, N.

*6.7.6. 1-[(2R,3S,4R)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-(2R)-2,3-dihydro-1H-indole-2-carboxylic acid ethyl ester (**18**)*

M.p.: 135–137 °C (ethyl acetate–hexane); ^{1}H-NMR ($CDCl_3$) δ 1.2–1.4 (m, 3H), 1.56 (s, 3H), 3.2–3.6 (m, 9.4H), 4.1–4.5 (m, 4H), 4.6–4.8 (m, 1H), 5.1–5.3 (br, 1H), 5.54 (br, 0.6H), 6.6–6.8 (m, 2H), 6.93 (d, 1H, $J = 9.2$ Hz), 7.0–7.2 (m, 1.4H), 7.77 (br, 0.4H), 8.0–8.1 (m, 1H), 8.80 (brs, 0.6H); MS 472 [M^+]; Anal. ($C_{24}H_{28}N_2O_8$) C, H, N.

*6.7.7. 1-[(2S,3R,4S)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-(2R)-2,3-dihydro-1H-indole-2-carboxylic acid methyl ester (**19**)*

M.p.: 96–97 °C (ethyl acetate–hexane); ^{1}H-NMR ($CDCl_3$) δ 1.64 (s, 3H), 3.10–3.60 (m, 8H), 3.95 (s, 3H), 4.58 (m, 1H), 4.85–5.16 (m, 2H), 5.79 (d, 0.6H, $J = 8.2$ Hz), 6.4–7.2 (m, 4.4H), 7.64 (d, 0.4H, $J = 2.0$ Hz), 8.04 (m, 1H), 8.31 (d, 0.6H, $J = 1.9$ Hz); MS 458 [M^+]; Anal. ($C_{23}H_{26}N_2O_8$) C, H, N.

*6.7.8. 1-[(2S,3R,4S)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-(2S)-2,3-dihydro-1H-indole-2-carboxylic acid methyl ester (**20**)*

M.p.: 99–100 °C (ethyl acetate–hexane); ^{1}H-NMR ($CDCl_3$) δ 1.62 (s, 3H), 3.38 (m, 2H), 3.64 (s, 3H), 3.67 (s, 3H), 3.89 (s, 3H), 4.41 (m, 1H), 4.62 (m, 1H), 5.06 (s, 1H), 5.52 (brd, 0.7H), 6.5–6.8 (m, 1.6H), 6.81 (d, 1H, $J = 8.0$ Hz), 6.92 (m, 1H), 7.64 (m, 0.7H), 8.08 (m, 1H), 8.26 (brd, 0.3H), 8.81 (brd, 0.7 H); MS 458 [M^+]; Anal. ($C_{23}H_{26}N_2O_8$) C, H, N.

*6.7.9. 1-[(2S,3S,4R)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-(2R)-2,3-dihydro-1H-indole-2-carboxylic acid methyl ester (**21**)*

M.p.: 174–176 °C (ethyl acetate–hexane); ^{1}H-NMR ($CDCl_3$) δ 1.32 (s, 3H), 3.28 (m, 2H), 3.54 (s, 3H), 3.61 (s, 3H), 3.86 (s, 3H), 4.41 (m, 1H), 4.79 (d, 1H, $J = 9.1$ Hz), 5.11 (m, 1H), 5.59 (d, 0.6H, $J = 7.8$ Hz), 6.60–7.20 (m, 4.4H), 7.81 (d, 0.4H, $J = 2.1$ Hz), 8.08 (m, 1H), 8.84 (d, 0.6H, $J = 2.2$ Hz); MS 458 [M^+]; Anal. ($C_{23}H_{26}N_2O_8$) C, H, N.

*6.7.10. 1-[(2S,3S,4R)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-(2S)-2,3-dihydro-1H-indole-2-carboxylic acid methyl ester (**22**)*

M.p.: 108–109 °C (ethyl acetate–hexane); ^{1}H-NMR ($CDCl_3$) δ 1.57 (s, 3H), 3.26 (m, 1H), 3.46 (m, 1H), 3.64 (s, 3H), 3.68 (s, 3H), 3.89 (s, 3H), 4.30 (m, 1H), 4.60 (s, 1H), 4.69 (d, 1H, $J = 10.1$ Hz), 4.91 (m, 1H), 6.63 (m, 1H), 6.72 (m, 1H), 6.94 (m, 1H), 7.08 (m, 2H), 7.68 (d, 0.3H, $J = 1.9$ Hz), 8.01 (dd, 1H, $J = 7.9$, 2.0 Hz), 8.39 (d, 0.7H, $J = 2.1$ Hz); MS 458 [M^+]; Anal. ($C_{23}H_{26}N_2O_8$) C, H, N.

*6.7.11. 1-[(2S,3R,4S)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-(2S)-2,3-dihydro-1H-indole-2-carboxylic acid isopropyl ester (**23**)*

M.p.: 95–96 °C (ethyl acetate–hexane); ^{1}H-NMR ($CDCl_3$) δ 1.27 (m, 6H), 1.57 (s, 3H), 3.37 (m, 1H), 3.48 (s, 3H), 3.49 (s, 3H), 3.58 (m, 1H), 4.02 (m, 1H), 4.13 (d, 1H, $J = 7.1$ Hz), 4.44 (s, 1H), 4.68 (m, 1H), 5.12 (d, 1H, $J = 6.9$ Hz), 5.58 (m, 0.3H), 6.6–6.8 (m, 2H), 6.94 (d, 1H, $J = 7.8$ Hz), 7.06 (m, 1H), 7.6–7.7 (m, 1H), 8.09 (dd, 1H, $J = 9.1$, 2.6 Hz), 8.42 (d, 0.7H, $J = 2.6$ Hz); MS 486 [M^+]); Anal. ($C_{25}H_{30}N_2O_8$) C, H, N.

*6.8. 1-[(2S,3R,4S)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-(2S)-2,3-dihydro-1H-indole-2-carboxylic acid (**24**)*

To the solution of KOH (29 mg, 0.5 mmol) in water (30 μM) and ethanol (1.1 mL) was added, the compound

14 (170 mg, 0.36 mmol) and the reaction mixture was stirred at r.t. for 6 h. The reaction was diluted with water (10 mL) and acidified with c-HCl, then extracted with ethyl acetate (10 mL × 3). The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give an off-white solid (150 mg, 94%): m.p.: >200 °C (decomposed); ^{1}H-NMR (CD_3OD) δ 1.47 (s, 3H), 3.31 (s, 3H), 3.38 (s, 3H), 3.51 (m, 2H), 4.34 (m, 1H), 4.65 (s, 1H), 5.04 (m, 1H), 5.73 (m, 1H), 6.56 (m, 1H), 6.73 (m, 1H), 6.91 (d, 1H), 6.97 (m, 2H), 8.04 (dd, 1H, $J = 7.6$, 1.4 Hz), 8.66 (m, 1H); MS 444 [M^+]; Anal. $C_{22}H_{24}N_2O_8$) C, H, N.

6.9. General procedure for the syntheses of compounds (**25**–**28**)

To the solution of indoline compounds (0.25 mmol) in benzene (10 mL) was added DDQ (100 mg, 0.44 mmol) and the reaction was stirred at r.t. for 6 h. The reaction mixture was diluted with ethyl acetate (10 mL) and washed with 10% NaOH (10 mL) and water (10 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 2:1) to give an off-white solid in 85–91% yields.

6.9.1. 1-[(2S, 3R, 4S)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-1H-indole-2-carboxylic acid ethyl ester (**25**)

M.p.: 184–185 °C (ethanol); ^{1}H-NMR ($CDCl_3$) δ 1.44 (t, 3H, $J = 7.1$ Hz), 1.62 (s, 3H), 3.47 (d, 2H, $J =$ 6.0 Hz), 3.52 (s, 3H), 3.55 (s, 3H), 4.27–4.42 (m, 3H), 4.61 (s, 1H), 6.46 (d, 1H, $J = 8.1$ Hz), 6.98–7.12 (m, 3H), 7.25 (d, 1H, $J = 8.4$ Hz), 7.45 (s, 1H), 7.68 (m, 1H), 8.12 (dd, 1H, $J = 7.3$, 0.8 Hz); MS 470 [M^+]; Anal. ($C_{24}H_{26}N_2O_8$) C, H, N.

6.9.2. 1-[(2S, 3S, 4R)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-1H-indole-2-carboxylic acid ethyl ester (**26**)

M.p.: 185–186 °C (ethanol); ^{1}H-NMR ($CDCl_3$) δ 1.45 (t, 3H, $J = 7.3$ Hz), 1.47 (s, 3H), 3.36 (d, 2H, $J =$ 2.2 Hz), 3.59 (s, 3H), 3.60 (s, 3H), 4.40 (q, 2H, $J = 7.1$ Hz), 4.46 (s, 1H), 4.75 (dd, 1H, $J = 10.2$, 2.2 Hz), 6.55 (d, 1H, $J = 7.5$ Hz), 7.07 (m, 3H), 7.45 (s, 1H), 7.73 (m, 2H), 8.10 (dd, 1H, $J = 9.4$, 2.4 Hz); MS 470 [M^+]; Anal. ($C_{24}H_{26}N_2O_8$) C, H, N.

6.9.3. 1-[(2S, 3R, 4S)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-1H-indole-2-carboxylic acid methyl ester (**27**)

M.p.: 178–179 °C (ethanol); ^{1}H-NMR ($CDCl_3$) δ 1.63 (s, 3H), 3.48 (d, 1H, $J = 10.5$ Hz), 3.54 (s, 3H), 3.56 (s, 3H), 3.96 (s, 3H), 4.36 (m, 1H), 4.62 (s, 1H), 6.47 (d, 1H, $J = 7.3$ Hz), 7.06 (m, 2H), 7.33 (m, 1H), 7.46 (s, 1H), 7.70 (m, 2H), 8.11 (dd, 1H, $J = 7.9$, 2.4 Hz); MS 456 [M^+]; Anal. ($C_{23}H_{24}N_2O_8$) C, H, N.

6.9.4. 1-[(2S, 3S, 4R)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-1H-indole-2-carboxylic acid methyl ester (**28**)

M.p.: 194–195 °C (ethanol); ^{1}H-NMR ($CDCl_3$) δ 1.49 (s, 3H), 3.36 (d, 1H, $J = 2.3$ Hz), 3.60 (s, 3H), 3.62 (s, 3H), 3.98 (s, 3H), 4.48 (s, 1H), 4.76 (dd, 1H, $J =$ 10.2, 2.2 Hz), 6.59 (d, 1H, $J = 8.0$ Hz), 7.08 (m, 3H), 7.45 (s, 1H), 7.72 (m, 2H), 8.11 (dd, 1H, $J = 8.9$, 2.1 Hz); MS 456 [M^+]; Anal. ($C_{23}H_{24}N_2O_8$) C, H, N.

6.10. (6S, 6aR, 8aS, 13cS)-Dimethoxymethyl-6-methyl-2-nitro-6a,8a,9,13c-tetrahydro-6H-5,7-dioxa-13b-aza-indeno[2,1-c]phenanthren-8-one (**29**)

To the solution of compound **20** (92 mg, 0.2 mmol) in dichloromethane (4 mL) were added diisopropylamine (30 μL, 0.22 mmol) and 1.8 M solution of diethyl aluminum chloride in toluene (110 μL, 0.2 mmol) at −20 °C. The reaction mixture was stirred at −20 °C for 2 h, then at r.t. for 4 h, and poured over ice-cold saturated solution of $NaHCO_3$ (15 mL). The mixture was extracted with ethyl acetate (10 mL × 3), dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 4:1) to yield a pale yellow solid (72 mg, 85%): m.p.: 117–118 °C (ethyl acetate–hexane); ^{1}H-NMR ($CDCl_3$) δ 1.54 (s, 3H), 3.37 (s, 3H), 3.41 (m, 1H), 3.48 (s, 3H), 3.54 (m, 1H), 4.58 (m, 1H), 4.67 (m, 1H), 5.14 (d, 1H), 5.81 (m, 1H), 6.62 (d, 1H, $J = 8.1$ Hz), 6.82 (m, 2H), 7.05 (d, 1H, $J = 9.1$ Hz), 7.17 (m, 1H), 8.13 (dd, 1H, $J = 9.8$, 2.3 Hz), 8.46 (d, 1H, $J = 2.2$ Hz); MS 426 [M^+]; Anal. ($C_{22}H_{27}N_2O_7$) C, H, N.

6.11. (6S, 6aS, 8aR, 13cR)-Dimethoxymethyl-6-methyl-2-nitro-6a,8a,9,13c-tetrahydro-6H-5,7-dioxa-13b-aza-indeno[2,1-c]phenanthren-8-one (**30**)

The same reaction for the preparation of compound **29** was employed using compound **21** as a starting material, to give a pale yellow foam (69 mg, 81%): m.p.: 128–129 °C (ethyl acetate–hexane); ^{1}H-NMR ($CDCl_3$) δ 1.66 (s, 3H), 3.24 (m, 1H), 3.38 (s, 3H), 3.48 (m, 1H), 3.54 (s, 3H), 3.82 (d, 1H, $J = 11.4$ Hz), 4.55 (d, 1H, $J =$ 11.2 Hz), 4.57 (s, 1H), 4.79 (d, 1H, $J = 11.6$ Hz), 6.61 (d, 1H, $J = 7.7$ Hz), 6.94 (m, 2H), 7.23 (m, 2H), 8.09 (m, 2H); MS 426 [M^+]; Anal. ($C_{22}H_{27}N_2O_7$) C, H, N.

6.12. 1-[(2S,3R,4S)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-(2R)-2,3-dihydro-2-methoxymethyl-1H-indole (**31**)

The compound **31** and **32** were prepared by the general procedure for **13**–**23** using compound **11** as a

starting material. Resulting two diastereisomers were separated by silica gel column chromatography (hexane:ethyl acetate = 3:1): m.p.: 94–95 °C (ethanol); ^{1}H-NMR ($CDCl_3$) δ 1.56 (s, 3H), 2.9–3.1 (m, 2H), 3.44 (s, 3H), 3.49 (s, 3H), 3.52–3.9 (m, 5H), 4.1–4.3 (m, 2H), 4.43 (s, 1H), 4.60 (m, 1H), 4.90 (dd, 1H, $J = 11.2$, 1.9 Hz), 5.52 (dd, 1H, $J = 7.0$, 1.8 Hz), 6.55 (m, 1H), 6.72 (m, 1H), 7.0 (m, 2H), 8.06 (dd, 1H, $J = 8.8$, 1.9 Hz), 8.47 (d, 1H, $J = 1.9$ Hz); MS 444 [M^+]; Anal. ($C_{23}H_{28}N_2O_7$) C, H, N.

6.13. 1-[(2S,3R,4S)-3,4-Dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2H-1-benzopyran-4-yl]-(2S)-2,3-dihydro-2-methoxymethyl-1H-indole (32)

M.p.: 117–118 °C (ethanol); ^{1}H-NMR ($CDCl_3$) δ 1.63 (s, 3H), 2.9–3.3 (m, 2H), 3.41 (s, 3H), 3.51 (s, 3H), 3.54–3.7 (m, 4H), 4.1–4.45 (m, 3H), 4.64 (m, 1H), 4.90 (m, 1H), 5.64 (dd, 1H, $J = 11.5$, 1.9 Hz), 6.56 (m, 1H), 6.94 (m, 1H), 7.0–7.2 (m, 2H), 7.67 (d, 0.6H, $J =$ 1.9 Hz), 8.00 (m, 1H), 8.34 (d, 1H, $J = 1.9$ Hz); MS 444 [M^+]; Anal. ($C_{23}H_{28}N_2O_7$) C, H, N.

6.14. Molecular modeling

All molecular modeling techniques and conformational studies described herein were performed on Silicongraphics workstation (Origin R1000, 256 Mbytes memory, 2 CPU, 180 MHz IP27 processor) and PC, using SYBYL (v. 6.7) (Tripos Associate Inc., 1699 S. Hanley Road, Suite 303, St. Louis, MO 63144-2913, USA) and CACHE (Compute-Aided Chemistry, Oxford Molecular Group Inc., 11350 McLormick Road, Executive Plaza III, Suite 1100, Hunt Valley, MD 21030) programs.

Initial geometry for conformational analysis was obtained from Tripos force field using distance-dependent dielectric function (The distance-dependent value was used for a dielectric constant to simulate the effect of solvent.), and 0.05 kcal mol^{-1} energy gradient convergence. In order to obtain low energy conformers, random search was performed. Options for random search were 3000 iteration cycles, 100 kcal mol^{-1} energy cut-off, and chirality checking. After we selected low energy conformers, the geometry was re-optimized using semi-empirical method with PM3 charge. And using MM3 force field in CACHE program, optimized map was calculated, rotating torsion angle, t_1 between chromane and indole ring, and t_2 between indole ring and ester side chain by 5° increments.

6.15. Pharmacology

6.15.1. Relaxation of methoxamine contracted rat aorta

To measure the peripheral vasodilation potencies, we determined IC_{50} values for relaxation of the methoxamine-contracted rat aorta. Experimental details of method are described [20].

6.15.2. Cardioprotective activity in isolated ischemic rat heart model

The anti-ischemic effects of the compounds on isolated rat hearts were determined, according to the published methods [25,26] after some modification. Male Sprague–Dawley rats (300–450 g) were anesthetized with sodium pentobarbital (100 mg kg^{-1}, intraperitoneal (i.p.)). The jugular vein was injected with heparin (1000 U kg^{-1}) and then the trachea was incubated. While rats were mechanically ventilated with a rodent ventilator (Model 7025, Ugobasile, Italy), their hearts were perfused in situ with oxygenated modified Krebs–Henseleit bicarbonate buffer (described herein) via retrograde aortic cannulation. The hearts were then excised and quickly moved to a Langendorff apparatus (H.S.E., Germany), where they were perfused with oxygenated modified Krebs–Henseleit bicarbonate buffer containing (in mM) NaCl 116, $NaHCO_3$ 24.9, KCl 4.7, $MgSO_4$ 1.1, KH_2PO_4 1.17, $CaCl_2$ 2.52, glucose 8.32 and pyruvate 2.0 at a constant perfusion pressure (85 mmHg). A latex balloon filled with solution (ethanol: water = 1:1 (v/v)) and attached to a metal cannula was placed in the left ventricle through pulmonary vein and connected to a Isotec pressure transducer (H.S.E., Germany) for measurement of left ventricular pressure (LVP). The hearts were allowed to equilibrate for 15 min, at which time left ventricular end diastolic pressure (LVEDP) was adjusted to 5 mm Hg and this balloon volume was maintained throughout the experiment. Then, baseline contractile function, HR, and coronary flow (extra corporeal electromagnetic flow probe, Narco Bio-System, USA) were measured. Cardiac contractile function was determined by multiplying HR by LVDP, calculated by subtracting LVEDP from left ventricular systolic pressure (LVSP). Throughout the experiment, all these parameters were measured, and calculated before and 10 min after pretreatment with each compound and 30 min after the onset of reperfusion with buffer. Data on reperfusion were further expressed as the percentage to pretreatment. After stabilization for 15 min, the hearts were pretreated for 10 min with compounds (10 μM, 0.04% DMSO) or vehicle (0.04% DMSO) before onset of global ischemia. Test compounds included in the perfusate were administered directly into the oxygenator of the Langendorff apparatus. Then the hearts were subjected to global ischemia by completely shutting off the perfusate for 30 min. Severity of ischemia was determined as the TTC (min) during global ischemia in which the first 5 mmHg increase in EDP was observed. Then, the hearts were reperfused and, 30 min later, contractile function (LVDP × HR) and cumulative reperfusion LDH release were measured. LDH was measured as a sensitive index

for loss of cell viability with a kit supplied by Boerhinger Mannheim.

6.15.3. In vivo cardioprotective activity in ischemic myocardium rat model

To measure cardioprotective potencies in vivo, we determined the % ratio of myocardial IZ/AAR (%) in ischemic myocardium rat model [27]. Experimental details of method are described [20]. Briefly, male rats (350–450 g) were anesthetized with pentobarbital (75 mg kg^{-1}, i.p.). After a left thoracotomy operation, the rats were allowed to stabilize for 20 min. Vehicles or test compounds were administered into the rats via the catheter inserted into the femoral vein at 30 min before the occlusion. Left ventricle occlusion was maintained for 45 min, following reperfusion for 90 min. The coronary artery was reoccluded and 2 mL of a 1% Evans blue was i.v. injected. Rats were then sacrificed by i.v. injection of pentobarbital, and the hearts were removed. The slices of the left ventricles were analyzed using a Hi-scope installed with an image analyzing program (Image Pro Plus) to calculate the AAR, and then 1,2,3-triphenyltetrazolium chloride staining to obtain the infarct zone area.

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