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Short communication

Synthesis and vasorelaxant activity of 4-(cyclic amido)-2*H*-naphtho[1,2-*b*]pyrans

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Abstract

A series of 4-(cyclic amido)-2*H*-naphtho[1,2-*b*]pyrans related to cromakalim (**1**) has been prepared and their vasorelaxant activities on isolated rat thoracic aorta precontracted with phenylephrine have been evaluated. The relaxant mechanism of **3a** was found not through ATP-sensitive K^+ channels as cromakalim, but through opening voltage-sensitive K^+ channels.

Keywords: cromakalim; vasorelaxant activity; ATP-sensitive K^+ channels; voltage-sensitive K^+ channels

1. Introduction

Since cromakalim (**1**), the first benzopyran type potassium channel activator, has been found to be a potent antihypertensive agent, potassium channel activators related to cromakalim have attracted much attention in the past decade [1–3]. To date, many benzopyran derivatives based on cromakalim and their pharmacological potency have been extensively studied and some potassium channel activators are under development for therapeutic application in various diseases, especially in hypertension, asthma, and urinary incontinence [4–13]. In the recent report, pyranoquinoline (**2**), an analog of cromakalin, does not possess potassium channel activating properties, but possess pure calcium channel blocking activity instead [14]. Comparing the structure of **1** and **2**, increasing the size of the benzopyran nucleus resulted in different mechanism of the vasorelaxant activitiy (Fig. 1). Therefore, we prepared naphthopyran (**3a**), in which the benzopyran nucleus was replaced by the naphthopyran nucleus, in order to examine the vasorelaxant activity and the mechanism. As in literature reports on cromakalim analogs, the *gem*-dimethyl group at C2 and an electron-withdrawing group at C6 were important for good activity. Thus, in the naphthopyran series we prepared, these two moieties still left unchanged. In this paper, we wish to report the synthesis and vasorelaxant activities of a series of analogs around **3a** as well as the possible mechanisms for the activity.

2. Chemistry

Compounds were prepared as shown in Fig. 2. Condensation of 1-naphthol with 2-methyl-1,3-butadiene (isoprene) in the presence of orthophosphoric acid gave 3,4-dihydro-2,2-dimethyl-naphthopyran (**4**) and 2-(3-methyl-2-butenyl)-1-naphthol (**5**) [15]. Compound **5** was intramolecularly cyclised using HCOOH to give compound **4**. The requisite compound **4** was totally obtained in 30% yield. Nitration or bromination of **4** afforded nitro derivative **6a** and bromo derivative **6b**, respectively. The cyano derivative **6c** was prepared from **6b** by reaction with CuCN in 1-methyl-2-pyrro-

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lidinone. Treatment of **6** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) gave naphthopyrans (**7**). Reaction of the naphthopyrans (**7**) with *N*-bromosuccinimide (NBS) in aqueous DMSO gave bromohydrins (**8**), which was converted to the key intermediate 3,4-epoxides (**9**) by reaction with NaOH in aqueous dioxane.

The opening of epoxides (**9**) with 2-pyrrolidone, 2-piperidone, or 2-pyridone in the presence of NaH in DMSO yielded the **3**, **10** and **11b**, respectively, using the conditions described by Evans et al. [1]. Compound **11a** was obtained by treatment of epoxide (**9a**) with 2-pyridone and pyridine in alcohol, using the conditions

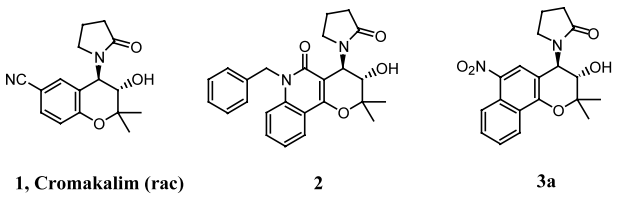
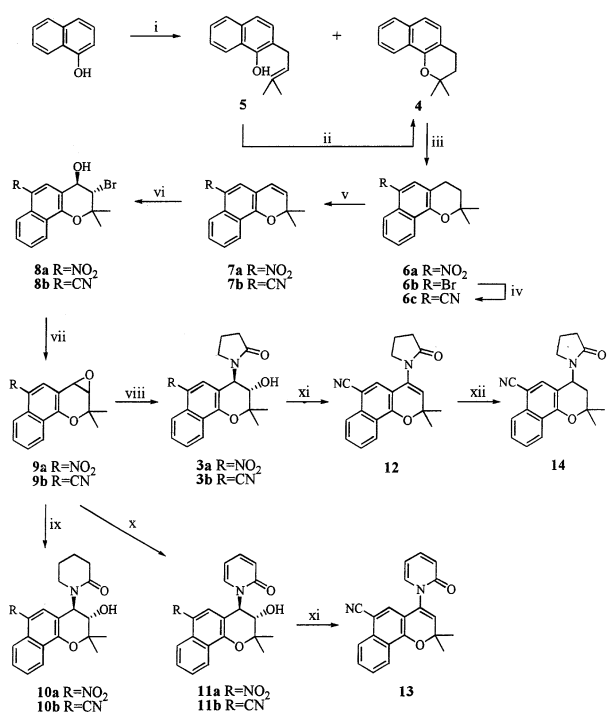


Fig. 1. Structures for **1**, **2**, and **3a**.



[a]Reagents: (i) isoprene, orthophosphoric acid, xylene, (ii) HCOOH, Δ; (iii) HNO_3, HOAc or Br_2, CCl_4; (iv) CuCN, *N*-methylpyrrolidinone; (v) DDQ, benzene, reflux; (vi) NBS, DMSO; (vii) NaOH, dioxane; (viii) NaH, 2-pyrrolidone, DMSO; (ix) NaH, 2-piperidone, DMSO; (x) for **11a**, 2-pyridone, pyridine, EtOH, reflux; for **11b**, NaH, 2-pyridone, DMSO; (xi) MsCl, Et_3N; $KOBu^t$, THF; (xii) H_2, Pd/C

Fig. 2. Synthesis of 4-(cyclic amido)-2*H*-naphthol[1,2-*b*]pyrans. Reagents: (i) isoprene, orthophosphoric acid, xylene; (ii) HCOOH, Δ; (iii) HNO_3, HOAc or Br_2, CCl_4; (iv) CuCN, *N*-methylpyrrolidinone; (v) DDQ, benzene, reflux; (vi) NBS, DMSO; (vii) NaOH, dioxane; (viii) NaH, 2-pyrrolidone, DMSO; (ix) NaH, 2-piperidone, DMSO; (x) for **11a** 2-pyridone, pyridine, EtOH, reflux, for **11b**, NaH, 2-pyridone, DMSO; (xi) MsCl, Et_3N, $KOBu^t$, THF; (xii) H_2, Pd/C.

described by Gericke et al. [5]. Compounds **3**, **10**, and **11** are all racemates. The ^1H NMR spectra of products **3**, **10**, and **11** showed the coupling constants of 9.5–10.1 Hz between C3 and C4 protons, and this is consistent with *trans* geometry. The alkenes **12** and **13** were obtained from **3b** and **11b** by elimination of the corresponding mesylate with $KOBu^t$ in THF. Hydrogenation over Pd–C of **12** provided **14**.

3. Biological results and discussion

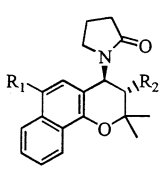
The vasorelaxant activities of these new compounds were evaluated in isolated rat thoracic aorta precontracted with phenylephrine (3×10^{-7} M) [16], and are shown in Table 1 in comparison with cromakalim.

These compounds were demonstrated significant relaxant activity on rat thoracic aorta, but less potent than cromakalim. In general, 6-nitro analogs (**3a**, **10a**, **11a**) showed better activity than 6-cyano analogs (**3b**, **10b**, **11b**). Enlargement in size of the cyclic amide to a 2-piperidone moiety (**10a**, **10b**) retained or enhanced the vasorelaxant activity. However, changing the 2-piperidone ring to 2-pyridone ring (**11a**, **11b**) decreased the activity. Elimination of water from **3b** and **11b** resulted in an increase of activity as observed for **12** and **13**. Thus, the dihydronaphthopyran (**14**) was considerably less potent than **3b**. The EC_{50} value of the most potent compound **10a** in this series is 0.22 μM.

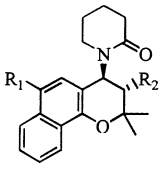
Compound **3a** was selected to examine the mechanism of relaxing effect in comparison with cromakalim. The relaxing effect of **3a** was not affected by glibenclamide (an ATP-sensitive K^+ channel blocker), but suppressed significantly by tetrabutylammonium (TBA, a non-selective K^+ channel blocker). It was suggested that mechanism of **3a** was not through ATP-sensitive K^+ channel as cromakalim but still related to potassium channel opener. Thus, further investigation was carried out to evaluate the effect of **3a** on various potassium channel blockers. The results showed **3a** was not effected by apamin (a small-conductance Ca^{2+}-dependent K^+-channel blocker) [17] and charybdotoxin (ChTX, a large-condutance Ca^{2+}-dependent K^+-channel blocker) [18], but by 4-aminopyridine (4-AP, a selective Kv channel blocker) [19]. Treatment of 4-AP shifted the concentration–response curves of **3a** to the right in a concentration-related fashion and in a manner suggestive of competitive antagonism (Fig. 3). The EC_{50} values for **3a** in control and in the presence of 0.1 and 0.3 mM 4-AP were 0.26 ± 0.05, 11.1 ± 1.4 and 35.7 ± 2.6 μM, respectively. The lack of effects of apamin and ChTX on compound **3a**-induced relaxation suggested that **3a**-induced vasorelaxation in rat aorta is caused principally through opening Kv channels.

Pyranoquinoline **2** has been reported to possess pure calcium channel blocking activity [14]. However, less

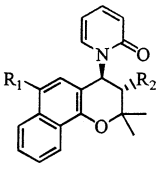
Table 1
Vasorelaxant activities of 4-(cyclic amido)-2*H*-naphtho[1,2-*b*]pyrans.



3a,3b,12,14



10a,10b



11a,11b,13

Compound	R_1	R_2	Formula	Analysis [a]	EC_{50} (μM) [b]	*n* [c]
3a	NO_2	OH	$C_{19}H_{20}N_2O_5$	C,H,N	0.26 ± 0.05	7
3b	CN	OH	$C_{20}H_{20}N_2O_3$	C,H,N	2.34 ± 1.50	3
10a	NO_2	OH	$C_{20}H_{22}N_2O_5 \cdot 1/4H_2O$	C,H,N	0.22 ± 0.03	8
10b	CN	OH	$C_{21}H_{22}N_2O_3$	C,H,N	0.74 ± 0.13	7
11a	NO_2	OH	$C_{20}H_{18}N_2O_5 \cdot 1/2H_2O$	C,H,N	0.39 ± 0.08	6
11b	CN	OH	$C_{21}H_{18}N_2O_3$	C,H,N	1.32 ± 0.46	4
12	CN	$\Delta^{3,4}$	$C_{20}H_{18}N_2O_2 \cdot 1/2H_2O$	C,H,N	1.02 ± 0.36	4
13	CN	$\Delta^{3,4}$	$C_{21}H_{16}N_2O_2$	C,H,N	0.34 ± 0.14	7
14	CN	H	$C_{20}H_{20}N_2O_2$	C,H,N	3.95 ± 1.17	4
Cromakalim	–	–	–	–	0.064 ± 0.012	6

[a] Analyses for the elements indicated were within $\pm 0.4\%$ of the theoretical values.
[b] Data expressed as 50% effective concentration to relax rat isolated thoracic aorta precontractions with phenylephrine, mean with $\pm$ S.E.M.
[c] Number of determinations.

effective response of **3a** in KCl-precontracted vessels than in phenylephrine-precontracted vessels suggested that channel blocking property was not contributed to **3a**-induced vasorelaxation.

4. Conclusions

We prepared a series of 4-(cyclic amido)-2*H*-naphtho[1,2-*b*]pyrans related to cromakalim and demonstrated that these compounds possessed good vasorelaxant activities on isolated rat thoracic aorta precontracted with phenylephrine. The relaxant mechanism of **3a**, analog of cromakalim, was found not through ATP-sensitive K^+ channels as cromakalim, but through opening voltage-sensitive K^+ channels.

5. Experimental protocols

5.1. Chemistry

Melting points were determined with a Yanaco micromelting point apparatus and are uncorrected. Infrared spectra were obtained on a Nicolet Avatar-320 FTIR spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian Gemini-200 spectrometer. Chemical shifts are reported in parts per million (δ) units relative to internal tetramethylsilane. Elemental analyses were performed on Perkin–Elmer CHN-2400 analyser and analyses indicated by the symbols of the elements were within $\pm 0.4\%$ of the theoretical values. Column chromatography was performed with E. Merck 70–230 or 230–400 mesh silica gel.

5.1.1. 3,4-Dihydro-2,2-dimethyl-2H-naphtho[1,2-b]-pyran (4)

A solution of isoprene (10 mL) in xylene (15 mL) was added dropwise to the mixture of 1-naphthol (10 g, 83.3 mmol) and orthophosphoric acid (85%, 15 mL) in xylene (15 mL) at 30–35 °C during 2 h. After the mixture was stirred for further 6 h, ether (100 mL) was added. The mixture was then washed with water, dried over Na_2SO_4, and evaporated. The residue was chromatographied on silica gel column eluting with hexane

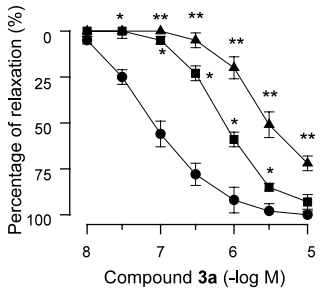


Fig. 3. Concentration–response curves for compound **3a** in PE-precontracted aortic rings in the absence (●) and presence of 4-aminopyridine (4-AP, ■: 0.1 mM; ▲: 0.3 mM) for 45 min, respectively. Results are expressed as percentages of relaxation%). Each point is the mean $\pm$ S.E.M. ($n = 6$–8). (*, $P < 0.05$ and **, $P < 0.01$ represent significant difference when compared with control groups).

to yield colourless oil product **4** (1.3 g) and uncyclised product **5** (4.3 g), respectively. The crude **5** was added HCOOH (85%, 50 mL) and the mixture was heated on a water bath at 60 °C for 4 h. After cooling, the mixture was diluted with H_2O and extracted with ether. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was subjected to vacuum distillation to give product **4** (3.1 g). The desired naphthopyran (**4**) was totally obtained as a colourless oil (4.4 g) in 30% overall yield: b.p. 122 °C (0.07 Torr); ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 6H, $2 \times CH_3$), 1.90 (t, $J = 6.8$ Hz, 2H, H-3), 2.88 (t, $J = 6.8$ Hz, 2H, H-4), 7.17 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.32 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.38–7.48 (m, 2H, Ar–H), 7.70–7.78 (m, 1H, Ar–H), 8.18–8.26 (m, 1H, Ar–H).

*5.1.2. 3,4-Dihydro-2,2-dimethyl-6-nitro-2H-naphtho-[1,2-b]pyran (**6a**)*

Concentrated HNO_3 (2.6 mL) was added dropwise to a solution of **4** (6.0 g, 28.3 mmol) in Ac_2O (30 mL) at below 10 °C. A lot of yellow precipitates were observed and the reaction mixture was continued stirring for 1 h. The mixture was then poured into ice water and filtered to give crude residue which was purified by column chromatography eluting with 5% EtOAc in hexane to give **6a** (4.6 g, 63%) as a yellow solid: m.p. 139–140 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 6H, $2 \times CH_3$), 1.94 (t, $J = 6.6$ Hz, 2H, H-3), 2.92 (t, $J = 6.6$ Hz, 2H, H-4), 7.48–7.56 (m, 1H, H-9), 7.61–7.69 (m, 1H, H-8), 8.24 (s, 1H, H-5), 8.28–8.33 (m, 1H, H-10), 8.73–8.78 (m, 1H, H-7).

*5.1.3. 6-Bromo-3,4-dihydro-2,2-dimethyl-2H-naphtho-[1,2-b]pyran (**6b**)*

Br_2 (1.6 mL, 31.1 mmol) in CCl_4 (5 mL) was added dropwise to a solution of **4** (6.0 g, 28.3 mmol) in CCl_4 (50 mL) at 5–10 °C. After the reaction mixture was continued stirring for 1 h and the solvent was evaporated. The residue was purified by column chromatography eluting with 5% EtOAc in hexane to give **6b** (7.2 g, 89%) as a white solid: m.p. 59–60 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 6H, $2 \times CH_3$), 1.87 (t, $J = 6.8$ Hz, 2H, H-3), 2.85 (t, $J = 6.8$ Hz, 2H, H-4), 7.48–7.63 (m, 2H, H-8 and H-9), 7.52 (s, 1H, H-5), 8.15–8.20 (m, 1H, H-10), 8.29–8.33 (m, 1H, H-7).

*5.1.4. 6-Cyano-3,4-dihydro-2,2-dimethyl-2H-naphtho-[1,2-b]pyran (**6c**)*

A mixture of **6b** (4.0 g, 13.7 mmol) and CuCN (1.9 g, 20.6 mmol) in 1-methyl-2-pyrrolidinone (40 mL) was refluxed for 2 h. After cooling, the solution was diluted with EtOAc and washed with water. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel chromatography eluting with 5% EtOAc in hexane to give **6c** (2.1 g, 64%) as a white solid: m.p. 98–100 °C; IR (KBr) 2215 (CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 6H, $2 \times CH_3$), 1.89 (t, $J = 6.7$ Hz, 2H, H-3), 2.85 (t, $J = 6.7$ Hz, 2H, H-4), 7.24–7.63 (m, 2H, H-8 and H-9), 7.61 (s, 1H, H-5), 8.05–8.10 (m, 1H, H-10), 8.22–8.27 (m, 1H, H-7).

*5.1.5. 2,2-Dimethyl-6-nitro-2H-naphtho[1,2-b]pyran (**7a**)*

A mixture of **6a** (4.5 g, 15.7 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (3.8 g, 16.7 mmol) in benzene (100 mL) was refluxed for 3 days. After cooling, the solution was filtered and evaporated. The residue was purified by silica gel chromatography eluting with 5% EtOAc in hexane to give **7a** (3.6 g, 80%) as a yellow solid: m.p. 89–90 °C; ^{1}H NMR ($CDCl_3$) δ 1.52 (s, 6H, $2 \times CH_3$), 5.69 (d, $J = 10.0$ Hz, 1H, H-3), 6.36 (d, $J = 10.0$ Hz, 1H, H-4), 7.43–7.51 (m, 1H, H-9), 7.57–7.65 (m, 1H, H-8), 8.10 (s, 1H, H-5), 8.21–8.26 (m, 1H, H-10), 8.68–8.73 (m, 1H, H-7).

*5.1.6. 6-Cyano-2,2-dimethyl-2H-naphtho[1,2-b]pyran (**7b**)*

Compound **7b** was prepared using the same procedure as for **7a**, and was obtained in 93% yield as a white solid: m.p. 178–179 °C; IR (KBr) 2217 (CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.53 (s, 6H, $2 \times CH_3$), 5.67 (d, $J = 9.9$ Hz, 1H, H-4), 6.36 (d, $J = 9.9$ Hz, 1H, H-3), 7.47–7.63 (m, 2H, H-8 and H-9), 7.55 (s, 1H, H-5), 8.05–8.09 (m, 1H, H-10), 8.20–8.25 (m, 1H, H-7).

*5.1.7. 3-Bromo-4-hydroxy-3,4-dihydro-2,2-dimethyl-6-nitro-2H-naphtho[1,2-b]pyran (**8a**)*

A mixture of **7a** (3.5 g, 13.7 mmol) and NBS (2.7 g, 15.2 mmol) in DMSO (20 mL) and H_2O (0.5 mL) was stirred at room temperature (r.t.) for 4 h. The reaction mixture was then diluted with H_2O and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The crude bromohydrin was purified by silica gel chromatography eluting with 15% EtOAc in hexane to give **8a** (4.1 g, 85%) as a yellow solid: m.p. 146–147 °C; ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 3H, CH_3), 1.74 (s, 3H, CH_3), 3.35 (d, $J = 4.6$ Hz, 1H, OH), 4.19 (d, $J = 9.1$ Hz, 1H, H-3), 5.01 (dd, $J = 9.1$, 4.6 Hz, 1H, H-4), 7.49–7.57 (m, 1H, H-9), 7.62–7.71 (m, 1H, H-8), 8.20–8.25 (m, 1H, H-10), 8.53 (s, 1H, H-5), 8.62–8.66 (m, 1H, H-7).

*5.1.8. 3-Bromo-6-cyano-4-hydroxy-3,4-dihydro-2,2-dimethyl-2H-naphtho[1,2-b]pyran (**8b**)*

Compound **8b** was prepared using the same procedure as for **8a**, and was obtained in 81% yield as a white solid: m.p. 180 °C (sub); IR (KBr) 3474, 2215 (CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 3H, CH_3), 1.75 (s, 3H, CH_3), 4.20 (d, $J = 9.16$ Hz, 1H, H-3), 5.01 (d, $J = 9.16$ Hz, 1H, H-4), 7.51–7.60 (m, 1H, H-9), 7.62–7.70 (m, 1H, H-8), 8.05 (s, 1H, H-5), 8.07–8.11 (m, 1H, H-10), 8.19–8.24 (m, 1H, H-7).

*5.1.9. 3,4-Dihydro-2,2-dimethyl-3,4-epoxy-6-nitro-2H-naphtho[1,2-b]pyran (**9a**)*

A mixture of **8a** (3.5 g, 10.0 mmol) and NaOH (4.0 g, 0.1 mol) in dioxane (100 mL) and H_2O (20 mL) was stirred at r.t. for 4 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The crude epoxide was purified by silica gel chromatography eluting with 15% EtOAc–hexane to give **9a** (2.6 g, 95%) as a yellow solid: m.p. 113–114 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3H, CH_3), 1.72 (s, 3H, CH_3), 3.64 (d, $J = 4.4$ Hz, 1H, H-4), 4.05 (d, $J = 4.4$ Hz, 1H, H-3), 7.48–7.56 (m, 1H, H-9), 7.63–7.72 (m, 1H, H-8), 8.24–8.29 (m, 1H, H-10), 8.44 (s, 1H, H-5), 8.69–8.74 (m, 1H, H-7).

*5.1.10. 6-Cyano-3,4-dihydro-2,2-dimethyl-3,4-epoxy-2H-naphtho[1,2-b]pyran (**9b**)*

Compound **9b** was prepared using the same procedure as for **9a**, and was obtained in 90% yield as a white solid: m.p. 93–94 °C; IR (KBr) 2213 (CN) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 3H, CH_3), 1.73 (s, 3H, CH_3), 3.63 (d, $J = 4.4$ Hz, 1H, H-4), 4.02 (d, $J = 4.4$ Hz, 1H, H-3), 7.51–7.59 (m, 1H, H-8), 7.62–7.71 (m, 1H, H-9), 7.88 (s, 1H, H-5), 8.11–8.15 (m, 1H, H-10), 8.21–8.26 (m, 1H, H-7).

*5.1.11. trans-3,4-Dihydro-2,2-dimethyl-6-nitro-4-(2-oxopyrrolidin-1-yl)-2H-naphtho[1,2-b]pyran-3-ol (**3a**)*

The pyrrolidinone (0.94 g, 11.0 mmol) was treated with 60% NaH (0.44 g, 11.0 mmol) in DMSO (5 mL) and epoxide (**9a**) (3.0 g, 11.0 mmol) in DMSO (30 mL) was then added at r.t. under N_2. After the reaction mixture was stirred for 5 h, water was added cautiously, and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The crude residue was purified by silica gel chromatography eluting with 50% EtOAc in hexane to give **3a** (1.33 g, 34%) as a yellow solid: m.p. 252–254 °C; IR (KBr) 3195, 1650 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3H, CH_3), 1.69 (s, 3H, CH_3), 2.05 (br, 1H, OH), 2.06–2.17 (m, 2H, NCH_2CH_2), 2.55–2.66 (m, 2H, CH_2CO), 3.02–3.11 (m, 1H, NC*H*H), 3.28–3.36 (m, 1H, NCH*H*), 3.87 (d, $J = 10.0$ Hz, 1H, H-3), 5.44 (d, $J = 10.0$ Hz, 1H, H-4), 7.56–7.61 (m, 1H, H-9), 7.69–7.75 (m, 1H, H-8), 8.00 (s, 1H, H-5), 8.30 (d, $J = 8.44$ Hz, 1H, H-10), 8.71 (d, $J = 8.64$ Hz, 1H, H-7).

*5.1.12. trans-6-Cyano-3,4-dihydro-2,2-dimethyl-4-(2-oxopyrrolidin-1-yl)-2H-naphtho[1,2-b]pyran-3-ol (**3b**)*

Compound **3b** was prepared using the same procedure as for **3a**, and obtained in 75% yield as a white solid: m.p. 250 °C (sub); IR (KBr) 3259, 2217 (CN), 1659 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 2.06–2.19 (m, 2H, NCH_2CH_2), 2.62 (t, $J = 8.18$ Hz, 2H, CH_2CO), 2.68 (br, 1H, OH), 2.96–3.08 (m, 1H, NC*H*H), 3.30–3.42 (m, 1H, NCH*H*), 3.87 (d, $J = 10.1$ Hz, 1H, H-3), 5.39 (d, $J = 10.1$ Hz, 1H, H-4), 7.46 (s, 1H, H-5), 7.53–7.61 (m, 1H, H-9), 7.63–7.71 (m, 1H, H-8), 8.08–8.13 (m, 1H, H-7), 8.23–8.27 (m, 1H, H-10).

*5.1.13. trans-3,4-Dihydro-2,2-dimethyl-6-nitro-4-(2-oxopiperidin-1-yl)-2H-naphtho[1,2-b]pyran-3-ol (**10a**)*

In the same procedure to that described for **3a**, starting from **9a** and 2-piperidone, compound **10a** was obtained in 40% yield as a yellow solid: m.p. 256–258 °C; IR (KBr) 3333, 1616 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 1.75–1.94 (m, 4H, $NCH_2CH_2CH_2$), 2.27 (br, 1H, OH), 2.56–2.67 (m, 2H, CH_2CO), 2.85–2.96 (m, 1H, NC*H*H), 3.04–3.16 (m, 1H, NCH*H*), 3.89 (d, $J = 10.0$ Hz, 1H, H-3), 6.07 (d, $J = 10.0$ Hz, 1H, H-4), 7.54–7.62 (m, 1H, Ar–H-9), 7.68–7.76 (m, 1H, Ar–H-8), 8.04 (s, 1H, Ar–H-5), 8.27–8.32 (m, 1H, Ar–H-10), 8.69–8.73 (m, 1H, Ar–H-7).

*5.1.14. trans-6-Cyano-3,4-dihydro-2,2-dimethyl-4-(2-oxopiperidin-1-yl)-2H-naphtho[1,2-b]pyran-3-ol (**10b**)*

In the same procedure to that described for **3a**, starting from **9b** and 2-piperidone, compound **10b** was obtained in 27% yield as a white solid: m.p. 181 °C; IR (KBr) 3334, 2218 (CN), 1616 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 1.75–1.95 (m, 4H, $NCH_2CH_2CH_2$), 2.58 (s, 1H, OH), 2.64 (t, $J = 6.80$ Hz, 2H, CH_2CO), 2.82–2.93 (m, 1H, NC*H*H), 3.05–3.17 (m, 1H, NCH*H*), 3.89 (d, $J = 10.1$ Hz, 1H, H-3), 6.03 (d, $J = 10.1$ Hz, 1H, H-4), 7.50 (s, 1H, H-5), 7.53–7.62 (m, 1H, H-9), 7.63–7.72 (m, 1H, H-8), 8.09–8.13 (m, 1H, H-7), 8.22–8.27 (m, 1H, H-10).

*5.1.15. trans-3,4-Dihydro-2,2-dimethyl-6-nitro-4-(1,2-dihydro-2-oxo-1-pyridyl)-2H-naphtho[1,2-b]pyran-3-ol (**11a**)*

A mixture of epoxide (**9a**) (0.5 g, 1.85 mmol), 2-pyridone (0.26 g, 2.77 mmol), and pyridine (0.15 mL, 1.85 mmol) in EtOH (20 mL) was refluxed for 3 h. After cooling, the solvent was evaporated and the residue was purified by silica gel chromatography to give **11a** (120 mg, 18%) as a yellow solid: m.p. 260–262 °C; IR (KBr) 3288, 1656 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 3H, CH_3), 1.69 (s, 3H, CH_3), 3.51 (br, 1H, OH), 3.97 (d, $J = 9.50$ Hz, 1H, H-3), 6.24 (dt, $J = 6.80$, 1.40 Hz, 1H, Ar–H), 6.48 (d, $J = 9.50$ Hz, 1H, H-4), 6.69 (d, $J = 9.20$, 1.40 Hz, 1H, Ar–H), 6.92 (d, $J = 6.80$, 1.80 Hz, 1H, Ar–H), 7.40 (ddd, $J = 9.20$, 6.80, 1.80 Hz, 1H, Ar–H), 7.58–7.67 (m, 1H, H-9), 7.67–7.78 (m, 1H, H-8), 7.78 (s, 1H, H-5), 8.33–8.37 (m, 1H, H-10), 8.67 (d, $J = 8.54$ Hz, 1H, H-7).

*5.1.16. trans-6-Cyano-3,4-dihydro-2,2-dimethyl-4-(1,2-dihydro-2-oxo-1-pyridyl)-2H-naphtho[1,2-b]pyran-3-ol (**11b**)*

In the same procedure to that described for preparing **3a**, starting from **9b** and 2-pyridone, compound **11b** was obtained in 45% yield as a white solid: m.p. 255 °C (sub); IR (KBr) 3281, 2218 (CN), 1657 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.41 (s, 3H, CH_3), 1.68 (s, 3H, CH_3), 2.96 (br, 1H, OH), 3.96 (d, $J = 9.61$ Hz, 1H, H-3), 6.24 (dt, $J = 6.80$, 1.40 Hz, 1H, Ar–H), 6.44 (d, $J = 9.61$ Hz, 1H, H-4), 6.69–6.74 (m, 1H, Ar–H), 6.89 (dd, $J = 6.80$, 1.4 Hz, 1H, Ar–H), 7.28 (s, 1H, H-5), 7.37–7.46 (m, 1H, Ar–H), 7.59–7.67 (m, 1H, H-9), 7.68–7.76 (m, 1H, H-8), 8.10–8.15 (m, 1H, H-7), 8.29–8.33 (m, 1H, H-10).

*5.1.17. 6-Cyano-2,2-dimethyl-4-(2-oxopyrrolidin-1-yl)-2H-naphtho[1,2-b]pyran (**12**)*

A mixture of **3a** (0.15 g, 0.45 mmol), Et_3N (0.3 mL, 2.23 mmol), and methansulfonyl chloride (0.17 mL, 2.23 mmol) in CH_2Cl_2 (30 mL) was stirred at r.t. for overnight. The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, and evaporated to yield white solid residue. The residue was then dissolved in THF (20 mL), and KOBu^t (80 mg, mmol) was added. After the reaction mixture was stirred at r.t. for overnight, water was added cautiously, and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated. The crude residue was purified by silica gel chromatography eluting with 30% EtOAc in hexane to give **12** (0.12 g, 85%) as a white solid: m.p. 180 °C; IR (KBr) 2216 (CN), 1697 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.56 (s, 6H, $2 \times CH_3$), 2.15–2.30 (m, 2H, NCH_2CH_2), 2.58 (t, $J = 8.30$ Hz, 2H, CH_2CO), 3.64 (t, $J = 6.94$ Hz, 2H, NCH_2), 5.63 (s, 1H, H-3), 7.48–7.56 (m, 1H, H-9), 7.51 (s, 1H, H-5), 7.58–7.66 (m, 1H, H-8), 8.05–8.09 (m, 1H, H-7), 8.20–8.26 (m, 1H, H-10).

*5.1.18. 6-Cyano-2,2-dimethyl-4-(1,2-dihydro-2-oxo-1-pyridyl)-2H-naphtho[1,2-b]pyran (**13**)*

Compound **13** was prepared using the same procedure as for **12**, and was obtained in 88% yield as a white solid: m.p. 232 °C; IR (KBr) 2217 (CN), 1666 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.62 (s, 3H, CH_3), 1.69 (s, 3H, CH_3), 5.77 (s, 1H, H-3), 6.26 (dt, $J = 6.68$, 1.30 Hz, 1H, NCHC*H*), 6.61–6.66 (m, 1H, CHCO), 7.17–7.21 (m, 1H, NCH), 7.23 (s, 1H, H-5), 7.40–7.49 (m, 1H, C*H*CHCO), 7.49–7.66 (m, 2H, H-8 and H-9), 8.02–8.07 (m, 1H, H-7), 8.22–8.28 (m, 1H, H-10).

*5.1.19. 6-Cyano-3,4-dihydro-2,2-dimethyl-4-(2-oxopyrrolidin-1-yl)-2H-naphtho[1,2-b]pyran (**14**)*

A mixture of **12** (50 mg, 1.57 mmol) and Pd–C (5%, 5 mg) in EtOAc (20 mL) was stirred in an atmosphere of H_2 at r.t. for 2 days. The reaction mixture was then filtered, evaporated, and recrystallised from EtOAc–hexane to give **14** (42 mg, 83%) as a white solid: m.p. 175 °C; IR (KBr) 2217 (CN), 1684 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 1.96–2.11 (m 2H, NCH_2CH_2), 2.01 (d, $J = 9.32$ Hz, 2H, H-3), 2.43–2.64 (m 2H, CH_2CO), 2.90–3.01 (dt, $J = 9.58$, 6.54 Hz, 1H, NCH_2), 3.15–3.27 (dt, $J = 9.58$, 7.50 Hz, 1H, NCH_2), 5.61(t, $J = 9.32$ Hz, 1H, H-4), 7.49 (s, 1H, H-5), 7.49–7.57 (m, 1H, H-9), 7.59–7.67 (m, 1H, H-8), 8.05–8.10 (m, 1H, H-7), 8.21–8.26 (m, 1H, H-10).

5.2. In vitro assay for vasorelaxant potency

Male Sprague–Dawley rats weighing 250–300 g were killed by stunning, following by decapitation. The thoracic aorta was isolated and excess fat and connective tissue were removed. The vessels were cut into rings of about 5 mm in length and mounted in organ baths containing 5 mL of Krebs' solution of the following composition (mM): NaCl 118.2, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.7 and $CaCl_2$ 2.5. The tissue bath solution was maintained at 37 °C and bubbled with a 95% O_2–5% CO_2 mixture. Two stainless steel hooks were inserted into the aortic lumen, one was fixed while the other was connected to a transducer. The aorta were equilibrated in the medium for 90 min with three changes of Krebs' solution and maintained under an optimal tension of 1.8–2.0 g before specific experimental protocols were initiated. Contractions were recorded isometrically via a force–displacement transducer connected to a Gould polygraph (Model RS3400). The vessels were precontracted by phenylephrine (PE) or KCl. When the contractile responses attained steady state (10–15 min), drugs were added in increasing cumulative concentrations to generate concentration–relaxation curves [16]. The vasorelaxations induced by each concentration of vasodilators were expressed as a percentage of relaxation against PE induced muscle tone.

Different kinds of potassium channel blockers were used to evaluate the involvement of K^+ channel properties to compound **3a**. Twenty minutes after the PE challenge, the rings were exposed to glibenclamide (10 μM), tetrabutylammonium (TBA, 1 mM), 4-aminopyridine (4-AP, 0.1 and 0.3 mM), apamin (1 μM) or charybdotoxin (ChTX, 1 μM) for 45 min [20–22]. Then cromakalim or **3a** were added to construct another concentration–response curves.

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