Bioorganic & Medicinal Chemistry 17 (2009) 855–866

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Discovery and structure–activity relationships of a novel series of benzopyran-based K_{ATP} openers for urge urinary incontinence

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ARTICLE INFO

Article history:
Received 8 January 2008
Revised 11 November 2008
Accepted 13 November 2008
Available online 27 November 2008

Keywords:
Urge urinary incontinence
KATP channel openers
Benzopyran

ABSTRACT

A novel series of benzopyran derivatives were synthesized and evaluated as K_{ATP} channel openers. Structure–activity relationships were investigated around 4-position of the benzopyran nucleus. Optimization of 4-substituent with some heterocyclic rings led to compound **13b** bearing a benzo[*d*]isoxazol-3-one moiety as a potent and selective K_{ATP} channel opener in vitro. In two anesthetized rat models of myogenic bladder overactivity, compound **13b** was found to inhibit spontaneous bladder contractions.

1. Introduction

Ion channels play a fundamental role in the hormeostasis of cell function through the regulation of the transmembrane movement of ions. Cellular activity can be affected by modifications of the activities of the ion channels. This leads to changes in membrane potential difference. Potassium channels are a diverse and ubiquitous group of ion channels.[1] They principally regulate the resting membrane potential of the cell and attenuate the level of excitation of cells. A functional K_{ATP} channel is a hetero-octamer assembled from four inward rectifying potassium channel subunits (Kir6.2) and four sulfonylurea receptor (SUR) subunits.[2] There are two SUR genes, SUR1 and SUR2. SUR1/Kir6.2 channels are found in the pancreas and brain. Two major splice variants arise from the SUR2 gene, SUR2A and SUR2B, that differ only at the C-terminal 42 amino acids. SUR2A/Kir6.2 channels are found in cardiac and skeletal tissues whereas SUR2B/Kir6.2 channels are found in smooth muscles of many tissues including bladder.[3] A number of diseases or conditions may be treated with potassium channel openers.[4] This includes overactive bladder, urinary incontinence, male erectile dysfunction, female sexual disorders, premature labor, benign prostate hyperplasia (BPH), dysmenorrhea, neurodegeneration, stroke, pain, coronary artery disease, angina, ischemia, eating disorders, irritable bowl syndrome, alopecia.

Urinary incontinence (UI) is a disease that can affect the overall quality of life of a patient. Overactive bladder (OAB) is the most prevalent form of UI, with reported prevalence rate from 40% to 70% of all diagnosed UI cases.[5] OAB is characterized by the symptoms of increased urinary frequency, urgency, and involuntary loss of urine. A primary cause of OAB is an oversensitive bladder that contracts unexpectedly and involuntarily. The ideal pharmaceutical agent should suppress the involuntary contraction while leaving the normal voiding contractions intact. ATP-sensitive potassium channel openers (KCO) could serve as such agents. The ATP-sensitive potassium channels (K_{ATP}) are expressed in bladder smooth muscle and function as key regulators of the resting membrane potential in these cells. Compounds that selectively open these channels hyperpolarize the cell and decrease cellular excitability, resulting in suppression of involuntary bladder contractions, while leaving the normal micturition circuitry intact.[6]

A number of structurally distinct series of K_{ATP} channel openers have been described. Among them, *N*-cyanoguanidine, benzopyran, tertiary carbinol, dihydropyridine and squarate scaffolds have been reported to activate bladder K_{ATP} channel in vitro and to inhibit bladder function in vivo.[7] Clinical studies have been conducted on newer generation of K_{ATP} channel openers such as ZD-6169 and ZD-0947 for treatment of OAB.

The present work aimed at developing novel benzopyrans structurally related to cromakalim (**1**, Fig. 1) by varying nature of 4-substitution at the core structure. Particular attention was paid to identification of structural requirements leading to improvement of potency and selectivity on bladder. Benzopyrans represent the most thoroughly studied KCOs. Previous structure-activity studies indicated that 2, 4 and 6-positions of the nucleus are highly susceptible to modulate biological activity. Initial screening of

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doi:10.1016/j.bmc.2008.11.055

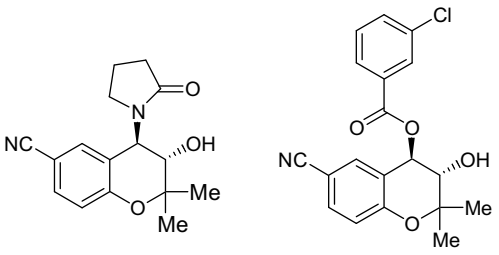


Figure 1.

in-house compounds with benzopyran core resulted in compound **2** (Fig. 1) bearing a 3-chloro-benzoate group as a potent K_{ATP} channel opener (EC_{50} = 0.31 μM). We envisaged that modification on this template should generate our starting point to search structurally novel, more potent and selective K_{ATP} channel openers. Our strategy was to replace the benzoate group of compound **2** with heterocycles or heterocycles attached via a heteroatom linker. In this paper, we describe synthesis, extensive SAR investigations and discovery of a potent and selective benzopyran bearing benzo[*d*]isoxazol-3-one moiety as K_{ATP} channel opener targeting UI.

2. Chemistry

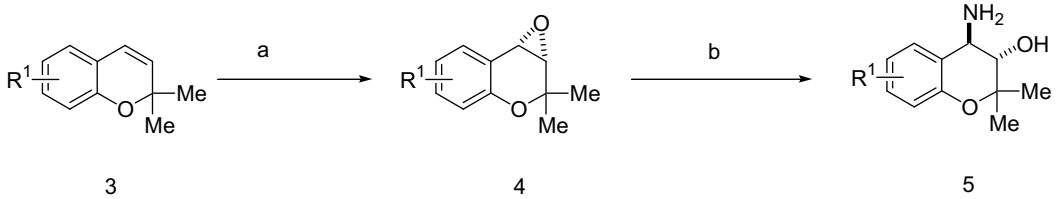
The preparations of all compounds in this paper are shown in Schemes 1–3. Epoxides **4** were prepared from asymmetric epoxidation of chromenes **3** by Jocobsen's catalyst (Scheme 1).[8] Opening of epoxides **4** with ammonia afforded amino-alcohols **5**.[9] Analogues **6a–6i** reported in this paper were prepared by following the literature known methods (Scheme 2, condition a).[10] Compounds **10a** and **10b** were prepared by reacting amino-alcohols **5** with corresponding 2-methoxy-1*H*-quinazolin-4-ones **9** in refluxing toluene (Scheme 2, condition b). Treatment of amino-alcohols **5** with acid anhydrides **14** in neat condition at 120 °C generated corresponding phthalimide derivatives **13o** and **13p** in moderate yields (Scheme 2, condition c). Scheme 3 showed the general procedure for preparation of compounds **8a–j, 10c–g, 12a–i** and **13a–n**. Opening of epoxides **4** with appropriately substituted heterocyclic nucleophiles in the presence of a base such as K_2CO_3 in DMF gave corresponding hydroxyl chromans. The required nucleophiles **7**, **11a** and **11b** were commercially available or prepared by the literature known methods.[11] Yield of epoxide-opening reaction is critically dependent on nature of nucleophiles. Generally the reaction proceeds in good yield when a S-containing nucleophile is used. Using N or O containing nucleophiles results in longer reaction time, higher temperature and decreased chemical yield. While K_2CO_3-induced opening of epoxides **4** with heterocyclic substrates was successful for preparation of a variety of analogues, it gave very low yield for preparation of analogues **8g** and **8h**. Using pyridine in refluxing EtOH provided an alternative way to yield the desired products (**8g–j**) in good yields with easy purification. When heterocyclic nucleophiles **11b** were 1*H*-indazol-3-ylamines or benzo[*d*]isoxazol-3-ylamines, opening of epoxides **4** only yielded corresponding **12** with NH as the linker between benzopyran core and the heterocycles. However, benzo[*d*]isoxazol-3-ones **11b** as the nucleophiles reacted with epoxides **4** in the presence of K_2CO_3 to afford a pair of regio-isomers in almost 1:1 ratio as O-alkylated derivatives **12** and N-alkyated products **13**. ^{13}C NMR spectra were used for differentiation of these two isomers. For example, **12a** and **13b** were isolated from the epoxide-opening reaction. Compound **12a** presented a characteristic C signal next to the linker O at 72.6 ppm, which was 26 ppm downfield than that of the N-linked isomer **13b**.

3. Results and discussion

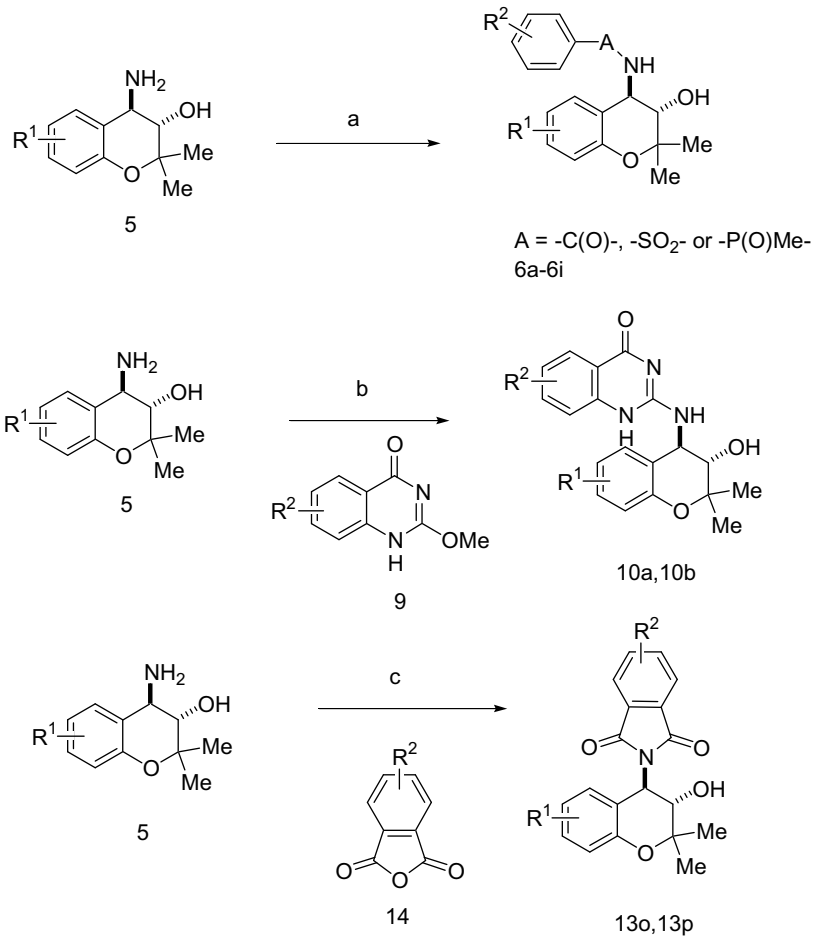
Compounds were assayed for their activity as K_{ATP} channel openers using primary TE671 human medulloblastoma cells.[12] The effect of test compounds on K_{ATP} channels were evaluated on a fluorometric imaging plate reader (FLIPR, Molecular Devices) at room temperature. Following stabilization, increasing concentrations of test compounds were superfused and isometic force was measured. Glyburide, a K_{ATP} channel blocker, was added to a final concentration to check the specificity of the test compound as a K_{ATP} channel openers. Hyperpolarization resulting from K_{ATP} channel opening was observed as a decrease in fluorescent intensity.

All of the benzopyrans below are presented as K_{ATP} channel openers. The K_{ATP} channel activation activity for all of these compounds is reversed by glyburide. The initial effort was directed at expanding SAR on structure **2** (**6a** in Table 1). Replacement of 3-chloro-benzoate group of **6a** with 3-chloro-benzamide group at 4-position of the benzopyran core resulted in slight increase in activity, as shown for compound (±)-**6b**. Shifting of 3-Cl to 4-Cl substitution on the phenyl ring resulted in a slight loss of activity (**6c**). Replacement of Cl with CN group diminished the potency (**6d**). Partial loss in activity was observed for the compounds with bis-substitutions, as illustrated in **6e** and **6f**. Replacement of the phenyl group with a pyridinyl group seemed to be tolerated for K_{ATP} activity (**6g**). While partial loss in activity was observed for **6h** with sulfonamide group as the linker, phosphinic amide group (**6i**) as the linker was detrimental to activity. Resolution of enantiomers (+)-**6b** and (−)-**6b** showed that absolute stereochemistry was critical for K_{ATP} activity as activity resided predominantly with (3*S*,4*R*) enantiomer (−)-**6b**. Based on this information, all compounds in Tables 2–4 were prepared as single enantiomer with (3*S*,4*R*) configuration.

Structurally modifying the carboxyl group of **2** to a five-membered heterocyclic ring such as pyrazole or imidazole resulted in less potent compounds (Table 2). For 6-CN substituted benzopyran analogues, introduction of a Cl group on the side chain phenyl ring improved activity comparing with non-substituted phenyl group, as illustrated in **8a–c** versus **8d–e**. Pyrazole derivative **8a** seemed to be the most potent analogue in this series with EC_{50} 1.50 μM, while imidazole analogues **8b** and **8c** were less active with EC_{50} 2.75 and 7.45 μM correspondingly. Introduction of a methyl group



Scheme 1. Reagents and conditions: (a) Jacobsen's catalyst, bleach in DCM; (b) NH_3 in MeOH, rt.



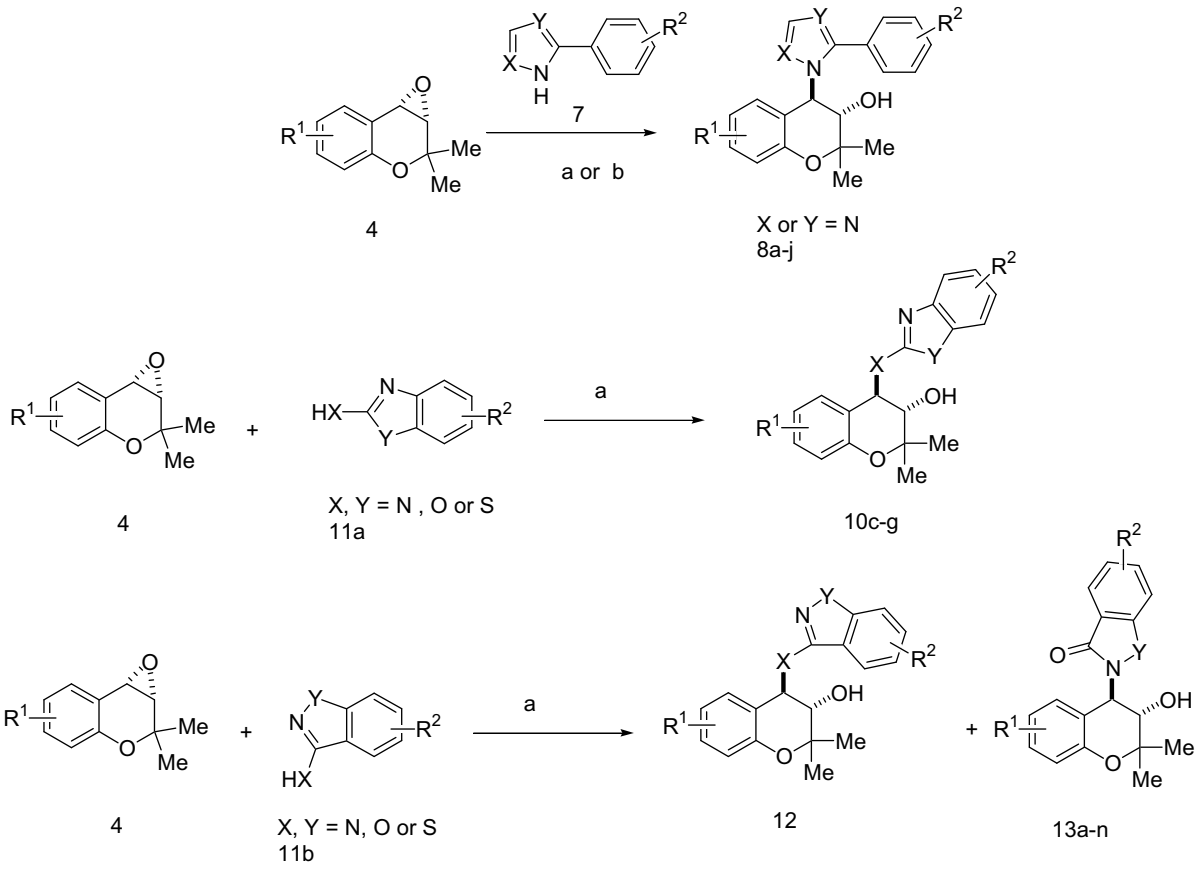
Scheme 2. Reagents and conditions: (a) Ar-A–Cl, TEA in DCM, 0 °C–rt; (b) toluene, refluxing; (c) neat, 120 °C.

on the imidazole ring was detrimental to activity (**8d**). For the benzopyran core, replacement of 6-CN with 6-Cl group abolished activity (**8f**). 6-Aza-chroman analogues **8h–i** were tolerated for activity comparing with their corresponding 6-CN substituted chroman analogues. Among 6-aza-chroman analogues, pyrazole **8i** seemed to be more active than imidazoles **8h** and **8j**.

Extensive SAR was also conducted by replacing the benzoate group of compound **2** with heterocycles attached via a heteroatom linker, as shown in Table 3. Quinazolinone derivatives **10a** (EC_{50} 3.71 μM) and **10b** (EC_{50} 2.29 μM) were ~10-fold less potent than the open chain analogue **6b**. Benzoxazole derivative **10c** was substantially less active with EC_{50} 16.5 μM. Introduction of benzoimidazole sulfanyl side chain resulted in a series of compounds **10d–g** with decreased activity (EC_{50} from 2.60 to 14.7 μM) comparing with compound **6b**. Benzoisoxazoleoxy (**12a**, EC_{50} 7.85 μM), benzo[*d*]-isothiazoleoxy (**12b**, EC_{50} 16.1 μM), benzo-isoxazolylamino (**12c**, EC_{50} 6.18 μM) and indazolylamino (**12d**, EC_{50} 6.85 μM) were also not well tolerated for sub-micro molar activity comparing with compound **6b**. Substitution at 6-position of the benzopyran core was also investigated. While 6-benzosulfone analogue **12e** showed good activity with EC_{50} 1.37 μM, further substitution of a *meta*-F group on the phenyl group resulted in tremendous loss in activity (**12f**, EC_{50} 7.77 μM). Replacement of 6-CN group with 6-sulfonamide (**12g**), 6,7-dichloro (**12h**) or 6-benzophenone (**12i**) group led to nearly inactive compounds.

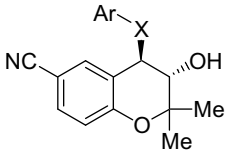
Further elucidation of structural and electronic properties of the pharmacophore was accomplished by exploring a series of benzo[*d*]isoxazol-3-ones and their close analogues shown in Table 4. While benzo[*d*]isoxazol-3-one **13a** and benzo[*d*]isothiazol-3-one **13d** bearing no substitution on the phenyl ring showed weak activities (EC_{50} 6.94 μM for **13a** and EC_{50} 14.1 μM for **13d**), substitution on the phenyl ring with a Cl group at either 5′ or 6′-position of **13a** and a NO_2 group at 5′-position of **13d** resulted in great improvement of activity, as shown in **13b** (EC_{50} 0.67 μM), **13c** (EC_{50} 2.44 μM) and **13e** (EC_{50} 4.10 μM). 5′-Cl substituted derivative **13b** was about 4-fold more potent than its 6'-Cl substituted isomer **13c**, 5-fold more potent than cromakalim, 10-fold more potent than pinacidil and almost equipotent as P1075. Brief survey of 6-substitution of the benzopyran core revealed that a variety of substitutions were well tolerated for KCO activity. As illustrated in **13f-i**, these compounds containing 6-benzosulfone or 6-benzosulfonamide substitution still maintained potent activity with EC_{50} ~1 μM. Other substitutions shown in Table 4 including 6,7-di-Cl (**13j**, EC_{50} 10.3 μM) and 6-benzophenone (**13k**, EC_{50} 6.45 μM) groups resulted in substantial loss of activity. Additional substitution such as F, Cl or methoxy group on the benzosulfone group of **13f** was detrimental to activity, as shown for **13l–n**. Finally, more than 10-fold drop of activity was observed by replacement of the benzo[*d*]isoxazol-3-one core with phthalimide moiety (**13o**, EC_{50} 3.16 μM and **13p**, EC_{50} 6.04 μM).

A selective binding of KCOs to different types of K_{ATP} channels in different organs is one of the most demanding challenges in K_{ATP}-channel research. On the basis of successful cloning of KIR and of SUR, it is widely accepted that SUR subtypes SUR1, SUR2A and SUR2B are responsible for differential effects of KCOs on each type of K_{ATP} channels. The small molecules prepared here targeted SUR2B/Kir6.2 channel in smooth muscles such as bladder. Thus, selected potent KCOs from screening assay were further evaluated



Scheme 3. Reagents and conditions: (a) K_2CO_3, DMF rt–100 °C; (b) pyridine, EtOH, reflux for **8g–8j**.

Table 1
SAR study of compounds **6** for K_{ATP} channel openers on TE671 cell



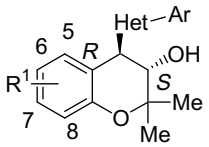
6

Compounds	–X–Ar	Ar	Stereochemistry	EC_{50} (μM)[b]
Pinacidil[a]				3.54
P1075[a]				0.23
Cromakalim[a]				1.25
6a	–OC(O)–Ar	3-Cl–Phenyl	(±)	0.31
(±)-**6b**	–NHC(O)–Ar	3-Cl–Phenyl	(±)	0.12
(+)-**6b**	–NHC(O)–Ar	3-Cl–Phenyl	(3*R*,4*S*)	2.07
(−)-**6b**	–NHC(O)–Ar	3-Cl–Phenyl	(3*S*,4*R*)	0.07
6c	–NHC(O)–Ar	4-Cl–Phenyl	(±)	0.78
6d	–NHC(O)–Ar	4-CN–Phenyl	(±)	21.1
6e	–NHC(O)–Ar	3,4-Methylenedioxy-phenyl	(3*S*,4*R*)	0.81
6f	–NHC(O)–Ar	3,4-Di-Cl-phenyl	(3*S*,4*R*)	7.06
6g	–NHC(O)–Ar	4-Cl-3-Pyridinyl	(3*S*,4*R*)	0.56
6h	–$NHSO_2$–Ar	3-Cl-Phenyl	(3*S*,4*R*)	1.54
6i	–NHP(O)(Me)–Ar	Phenyl	(3*S*,4*R*)	18.6

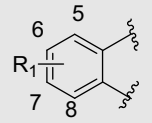
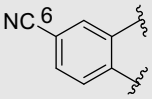
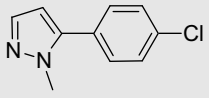
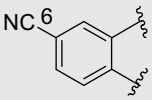
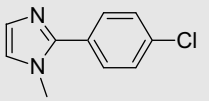
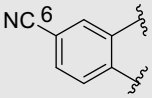
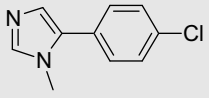
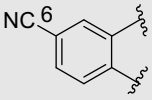
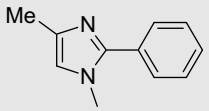
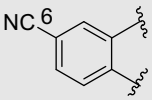
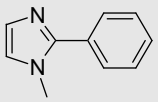
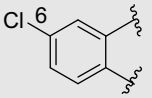
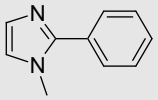
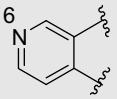
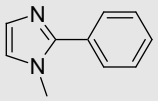
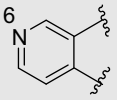
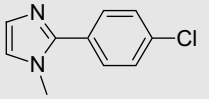
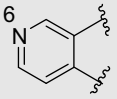
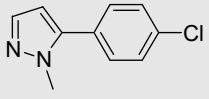
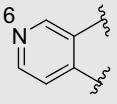
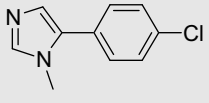
[a] Available from Sigma.
[b] Average value for pinacidil, P1075 and cromakalim on 14 tests and average value for other compounds on 3 tests.

for SUR2B selectivity over SUR1 and SUR2A. In these studies, β-TC-6 cell assay was used as the indicator of SUR2/Kir6.2 activity while COS-7 assay was used as the indicator of SUR2A/$Kir_{6.2}$ activity. Table 5 summarized the selectivity of the most potent compounds from screening. Benzo[*d*]isoxazol-3-one based benzopyrans (**13b**, **13f**, **13g**, **13i**) showed good SUR2B/Kir selectivity over SUR1/Kir. All compounds in Table 5 showed better selectivity than pinicidil and cromakalim. The most selective compound **13b** presented comparable SUR2B/Kir6.2 against SUR1/Kir6.2 selectivity as P1075. On the other hand, the benzopyran analogues were found

Table 2
SAR study of compounds **8** for K_{ATP} channel openers on TE671 cell



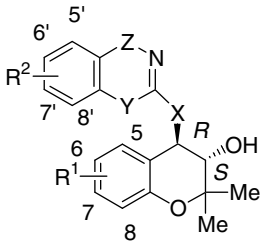
8

Compounds		-Het-Ar	EC_{50} (μM)[a]
Pinacidil			5.82
P1075			0.38
Cromakalim			3.48
8a			1.50
8b			2.75
8c			7.45
8d			>30
8e			13.0
8f			>30
8g			12.9
8h			4.33
8i			1.64
8j			13.8

[a] Average value on ⩾3 tests.

to be ~6-fold SUR2B/Kir selective against SUR2A/Kir6.2, which indicated that discrimination between cardiac (SUR2A/Kir6.2) and smooth muscle (SUR2B/Kir6.2) was feasible.

Table 3
SAR study of compounds **10** and **12** for K_{ATP} channel openers on TE671 cell



10 and **12**

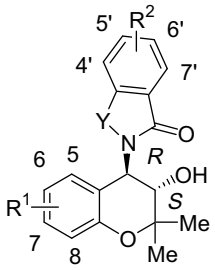
Compounds	R^1	R^2	X	Y	Z	EC_{50} (μM)[a]
Pinacidil						5.82
P1075						0.38
Cromakalim						3.48
10a	6-CN	—	NH	NH	C(O)	3.71
10b	6-CN	6′-Cl	NH	NH	C(O)	2.29
10c	6-CN	6′-Cl	NH	O	—	16.5
10d	6-CN	6′-OEt	S	NH	—	2.60
10e	6-CN	6′-F	S	NH	—	8.44
10f	6-CN	6′-NO_2	S	NH	—	3.09
10g	6-CN	6′-Cl	S	NH	—	14.7
12a	6-CN	6′-Cl	O	—	O	7.85
12b	6-CN	—	O	—	S	16.1
12c	6-CN	6′-Cl	NH	—	O	6.18
12d	6-CN	7′-Cl	NH	—	NH	6.85
12e	6-SO_2Ph	7′-Cl	NH	—	O	1.37
12f	6-SO_2-*m*-F-Ph	7′-Cl	NH	—	O	7.77
12g	6-SO_2NEt_2	7′-Cl	NH	—	O	> 30
12h	6,7-Di-Cl	7′-Cl	NH	—	O	24.8
12i	6-C(O)Ph	7′-Cl	NH		O	20.4

[a] Average value on ⩾3 tests.

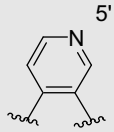
Compound **13b** was also evaluated in vitro using rat bladder strip assay (organ bath)[12] as shown in Fig. 2. Compound **13b** was found to be more active than other two KCOs, pinacidil and ZD-0947 with EC_{50} 2.04 μM. For comparison, P1075 in the same test had EC_{50} 0.14 μM.

According to all in vitro data, compound **13b** was identified as a potent and selective K_{ATP} channel openers in vitro. It was then evaluated in two in vivo models of overactive bladder. The detailed procedures are described in the experimental section. Compound **13b** was first evaluated in a rat acetic acid model (Fig. 3). Cystometry was performed using anesthetized female Sprague-Dawley rats with exhibiting unstable bladder contractions due to saline infusion. Administration of **13b** (lower panel) at 0.3 mg/kg/1mL via iv using 10% of PEG 300 and 20% of HPBCD as vehicle caused a clear reduction in the number of unstable contractions comparable to P1075 (middle panel), which demonstrates that **13b** is capable of suppressing spontaneous bladder contractions at the testing concentration. Compound **13b** is further evaluated in a rat partial outlet obstruction model. Cystometry was performed using anesthetized female Sprague-Dawley rats with bladder hypertrophy and exhibiting unstable bladder contractions due to intravesical outflow obstruction. Rats hypertrophied bladder cystometry plots before and after treatment with and P1075 and **13b** are presented in Fig. 4. The pretreatment plot (upper panel) shows spontaneous spikes which develop during the bladder filling phase. The lower panel shows the cystometry after oral administration of compound **13b** at 3 mg/kg. The spontaneous bladder contractions observed during the filling phase have been significantly reduced. For comparison, P1075 in the same model shows the similar pharmacological effect on the rat hyperophied bladder as shown in the middle panel. Thus, **13b** demonstrated its ability to prevent the spontaneous contractions in the hypertrophied rat bladder during filling.

Table 4
SAR study of compounds **13** for K_{ATP} channel openers on TE671 cell



13

Compounds	R_1	R_2	Y	EC_{50} (μM)[a]
Pinacidil				5.82
P1075				0.38
Cromakalim				3.48
13a	6-CN	—	O	6.94
13b	6-CN	5′-Cl	O	0.67
13c	6-CN	6′-Cl	O	2.44
13d	6-CN	—	S	14.1
13e	6-CN	5′-NO_2	S	4.10
13f	6-SO_2Ph	5′-Cl	O	0.75
13g	6-SO_2Ph	6′-Cl	O	1.56
13h	6-SO_2-Piperidinyl	5′-Cl	O	1.08
13i	6-SO_2NEt_2	5′-Cl	O	0.82
13j	6,7-Di-Cl	5′-Cl	O	10.3
13k	6-C(O)Ph	5′-Cl	O	6.45
13l	6-SO_2-*p*-MeO-Ph	5′-Cl	O	7.33
13m	6-SO_2-*p*-Cl-Ph	5′-Cl	O	7.99
13n	6-SO_2-*m*-F-Ph	5′-Cl	O	1.71
13o	6-CN	5′-Cl	C(O)	3.16
13p	6-CN		C(O)	6.04

[a] Average value on ⩾3 tests.

Table 5
Selectivity of SUR2B/Kir6.2 against SUR1/$Kir_{6.2}$ and SUR2A/$Kir_{6.2}$

Compounds	SUR2B TE-671 EC_{50} (μM)	SUR1 β-TC- 6 EC_{50} (μM)	SUR2A COS-7 EC_{50} (μM)	SUR1/ SUR2B	SUR2A/ SUR2B
Pinacidil	3.54	60.8	21.6	17	6.1
Cromakalim	1.25	33.7	4.89	27	3.9
P1075	0.23	60.5	0.62	263	2.7
13b	0.67	99.8	1.67	149	6.4
13f	0.75	44.3	2.44	59	3.3
13g	1.56	31.2	8.15	20	5.2
13i	0.82	53.3	3.19	65	3.8

4. Conclusion

We have identified a novel series of benzopyrans as K_{ATP} channel openers. Extensive SAR studies revealed that benzo[*d*]isoxazol-3-one as 4-subsutition of the benzopyran core was effective pharmacophore for potent activity. The best compound **13b** was demonstrated to be potent in cell and rat bladder strip functional assays with smooth muscle selectivity. It was also efficacious in two rat models of bladder overactivity.

5. Experimental

5.1. Chemistry. General procedures

^{1}H NMR spectra were recorded at 400 MHz or 300 MHz with *d*-chloroform or MeOD-d_4 as solvent on a Brucker AVANCE300 or AVANCE400 spectrometer. Chemical shifts are reported in ppm downfield from TMS as an internal standard. Thin-layer chromatography was carried out using 2.5 × 7.5 cm Silica Gel 60 (250 μM layer) plates with UV detection. Anhydrous sodium sulfate was employed to dry organic extracts prior to concentration by rotary evaporation. Flash chromatography was usually done using a CombiFlash companion system with pre-packed silica gel cartridges purchased from AnaLogix. Solvents from J.T. Baker or Aldrich and all other commercially available reagents were used without further purification. Melting points were taken using a Thomas-Hoover MelTemp apparatus without any correction. Microanalysis was done by Quantitative Technologies Inc., Whitehouse, NJ. Mass spectra were obtained on a Hewlett–Packard 5989A quadrupole mass spectrometer. HPLC analysis was carried on Agilent 1100 Series LC/MSD equipment. High resolution mass spectra were obtained on M-Scan's VG Analytical ZAB 2SE high field mass spectrometer.

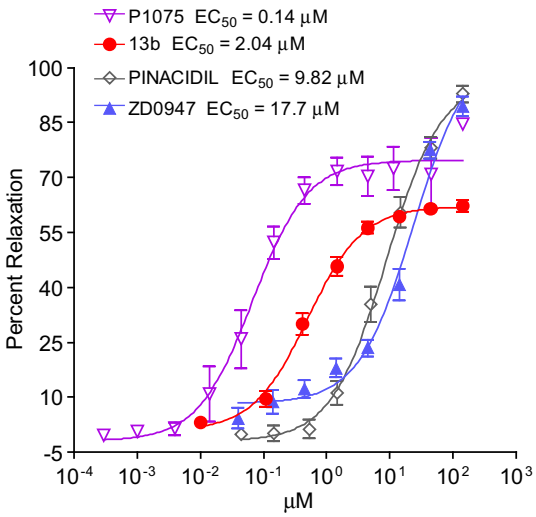


Figure 2. Organ bath study on rat bladder strips.

Compounds **6a–6h** were prepared according to the literatures. The spectroscopic data were the same as the reported data.[10]

5.1.1. (3*S*,4*R*)-*N*-(6-Cyano-3-hydroxy-2,2-dimethyl-chroman-4-yl)-methylphenylphosphinic amide (6i)

(3*S*,4*R*)-4-Amino-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile **5** (325 mg, 1.50 mmol) in DCM (10 mL) at 0 °C was treated with TEA (316 μL, 2.25 mmol) followed by methylphenylphosphinic chloride (261 mg, 1.50 mmol). The reaction was then slowly warmed to room temperature over 2 h. Saturated $NaHCO_3$ was added and the aqueous phase was extracted 2× with DCM. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated as a yellow solid, which was then purified by silica gel column chromatography to as yield the title compound as a pale yellow solid (400 mg, 75%).

^{1}H NMR: ($CDCl_3$) δ 8.15–7.52 (m, 5H), 7.10 (d, *J* = 7.5 Hz, 1H), 6.62 (d, *J* = 7.5 Hz, 1H), 6.08 (s, 1H), 4.23 (d, *J* = 9.5 Hz, 1H), 3.15 (d, *J* = 9.5 Hz, 1H), 1.55–1.42 (m, 9H). MS (*m*/*z*): MH^+ 357.

5.1.2. (3*S*,4*R*)-4-[5-(4-Chloro-phenyl)-pyrazol-1-yl]-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile (8a)

(*S*,*S*)-2,2-Dimethyl-1a,7b-dihydro-2*H*-1,3-dioxa-cyclopropa[*a*]-naphthalene-6-carbonitrile **4** (500 mg, 2.49 mmol) and 5-(4-chloro-phenyl)-1*H*-pyrazole **7** (445 mg, 2.50 mmol) in DMF (5 mL) were treated with K_2CO_3 (520 mg, 3.75 mmol) at 70 °C for 4 h. The reaction was cooled down and the solid was filtered off. The filtrate

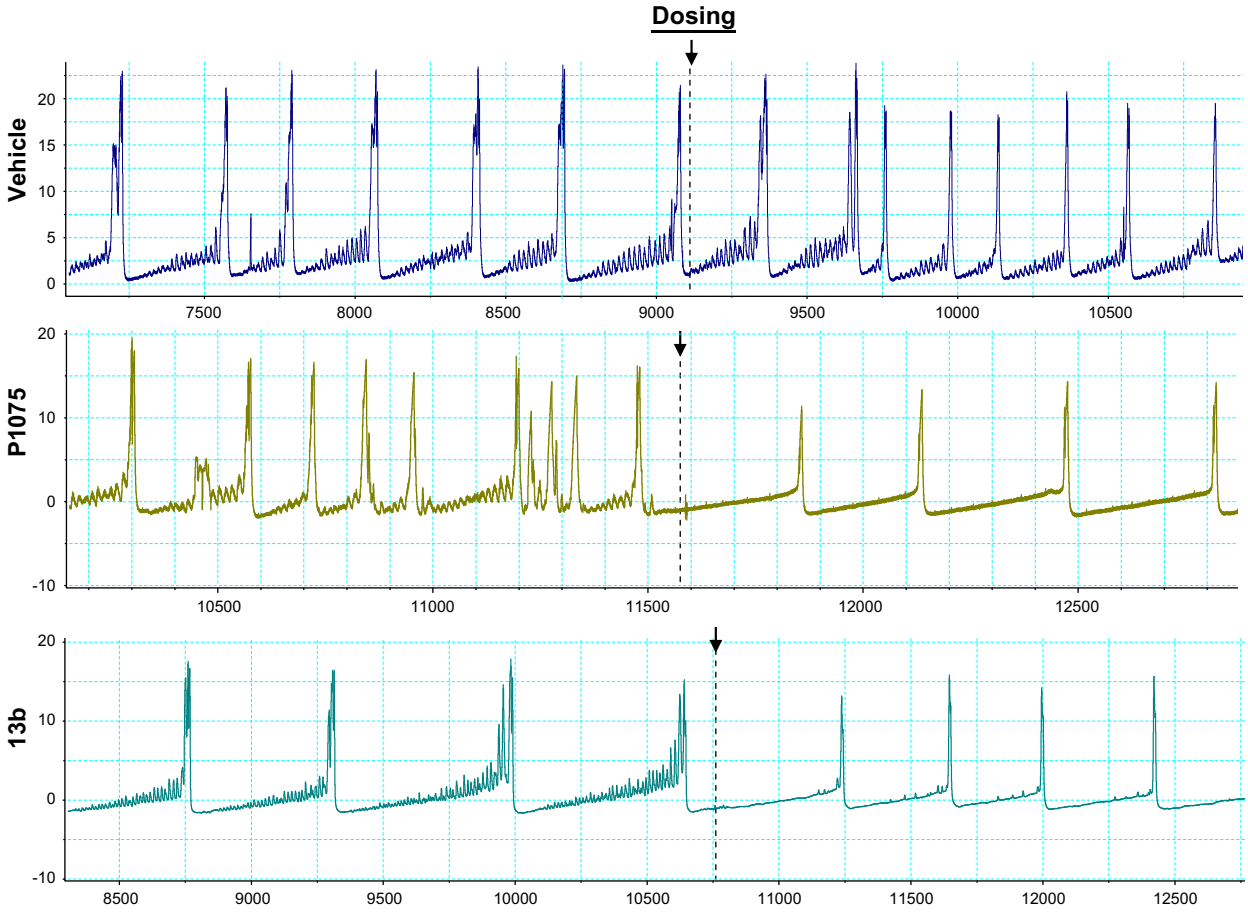


Figure 3. Cystometry on acetic acid treated rats.

was then partitioned between EtOAc and water. The organic layer was further washed with sat. NH_4Cl solution, water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated as a yellow solid, which was then purified by silica gel column chromatography to as yield the title compound as a yellow solid (420 mg, 45%).

^{1}H NMR ($CDCl_3$) δ 8.00 (s, 1H), 7.70 (m, 2H), 7.55 (m, 1H), 7.50 (s, 1H), 7.30 (m, 2H), 7.10 (s, 1H), 7.00 (m, 1H), 5.30 (m, 1H), 4.00 (m, 1H), 1.50 (s, 3H), 1.30 (s, 3H). MS (*m*/*z*): MH^+ 380.

5.1.3. (3*S*,4*R*)-4-[2-(4-Chloro-phenyl)-imidazol-1-yl]-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile (8b)

The same procedure for **8a** was followed to give the title compound as a white solid. Yield 41%.

^{1}H NMR: ($CDCl_3$) δ 7.72 (m, 3H), 7.45–7.35 (m, 4H), 6.85 (m, 2H), 5.90 (br, 1H), 5.30 (m, 1H), 4.02 (m, 1H), 1.65 (s, 3H), 1.28 (s, 3H). MS (*m*/*z*): MH^+ 380.

5.1.4. (3*S*,4*R*)-4-[5-(4-Chloro-phenyl)-imidazol-1-yl]-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile (8c)

The same procedure for **8a** was followed to give the title compound as a yellow solid. Yield 55%.

^{1}H NMR: ($CDCl_3$) δ 7.70 (m, 2H), 7.52 (s, 1H), 7.49 (m, 1H), 7.35 (m, 2H), 7.10 (s, 1H), 6.95 (m, 1H), 6.65 (s, 1H), 5.28 (d, J = 3.6 Hz, 1H), 4.30 (m, 1H), 2.75 (m, 1H), 1.60 (s, 3H), 1.35 (s, 3H). MS (*m*/*z*): MH^+ 380.

5.1.5. (3*S*,4*R*)-3-Hydroxy-2,2-dimethyl-4-(4-methyl-2-phenyl-imidazol-1-yl)-chroman-6-carbonitrile (8d)

The same procedure for **8a** was followed to give the title compound as a white solid. Yield, 50%.

^{1}H NMR: ($CDCl_3$) δ 7.72 (m, 1H), 7.35 (m, 5H), 6.82 (d, J = 7.5 Hz, 1H), 6.72 (s, 1H), 6.31 (s, 1H), 5.35 (d, J = 7.8 Hz, 1H), 4.05 (d, J = 7.8 Hz, 1H), 2.16 (s, 3H), 1.65 (s, 3H), 1.28 (s, 3H). MS (*m*/*z*): MH^+ 360.

5.1.6. (3*S*,4*R*)-3-Hydroxy-2,2-dimethyl-4-(2-phenyl-imidazol-1-yl)-chroman-6-carbonitrile (8e)

The same procedure for **8a** was followed to give the title compound as a white solid. Yield 48%.

^{1}H NMR: ($CDCl_3$) δ 7.82 (d, J = 3.5 Hz, 2H), 7.45 (m, 3H), 7.31 (d, J = 6.5 Hz, 1H), 6.82 (d, J = 7.5 Hz, 1H), 6.60 (s, 1H), 6.32 (s, 1H), 5.32 (d, J = 7.5 Hz, 1H), 3.88 (d, J = 7.5 Hz, 1H), 1.55 (s, 3H), 1.25 (s, 3H). MS (*m*/*z*): MH^+ 346.

5.1.7. (3*S*,4*R*)-6-Chloro-2,2-dimethyl-4-(2-phenyl-imidazol-1-yl)-chroman-3-ol (8f)

The same procedure for **8a** was followed to give the title compound as a white solid. Yield 37%.

^{1}H NMR: ($CDCl_3$) δ 7.82 (d, J = 7.5 Hz, 1H), 7.55 (d, J = 7.4 Hz, 1H), 7.38 (m, 4H), 7.02 (s, 1H), 6.78 (d, J = 8.5 Hz, 1H), 6.71 (s, 1H), 6.40 (s, 1H), 5.42 (d, J = 8.0 Hz, 1H), 4.12 (d, J = 7.1 Hz, 1H), 1.58 (s, 3H), 1.22 (s, 3H). MS (*m*/*z*): MH^+ 355.

5.1.8. (3*S*,4*R*)-2,2-Dimethyl-4-(2-phenyl-imidazol-1-yl)-3,4-dihydro-2*H*-pyrano[3,2-*c*]pyridin-3-ol (8g)

(*S*,*S*)-2,2-Dimethyl-1a,7b-dihydro-2*H*-1,3-dioxa-6-aza-cyclopropa[*a*]naphthalene **4** (450 mg, 2.54 mmol) and 2-phenyl-1*H*-imidazole **7** (366 mg, 2.55 mmol) in EtOH (10 mL) were treated with pyridine (1 mL). The reaction was refluxed for 10 hrs. The solvent was removed *in vacuo* and the residue was purified by silica

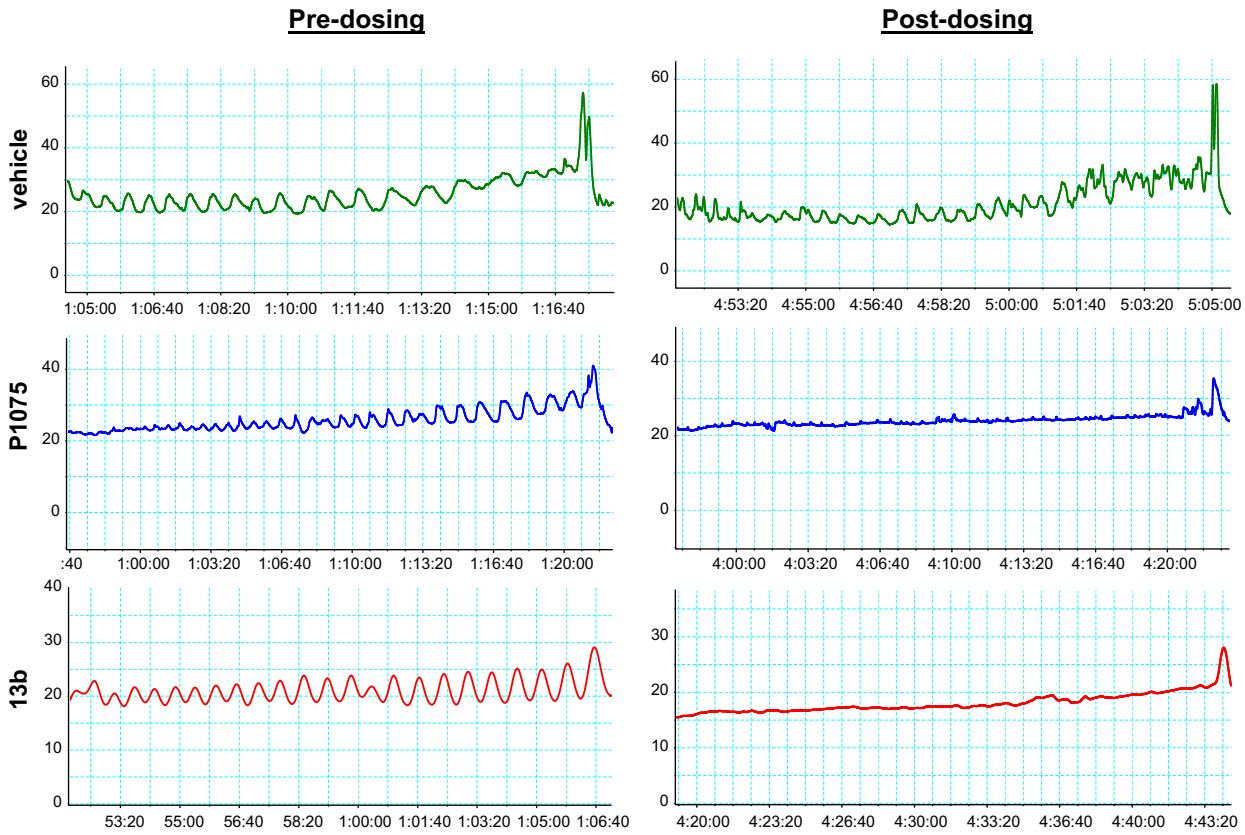


Figure 4. Cystometry on partially obstructed rats.

gel chromatography to give the title compound as a brown solid (340 mg, 42%).

^{1}H NMR: ($CDCl_3$) δ 8.20 (m, 1H), 7.80 (m, 2H), 7.50 (s, 1H), 7.40 (m, 3H), 6.75 (m, 2H), 6.40 (s, 1H), 6.55 (s, 1H), 5.45 (m, 1H), 3.75 (m, 1H), 1.55 (s, 3H), 1.22 (s, 3H). MS (m/z): MH^+ 322.

5.1.9. (3*S*,4*R*)-4-[2-(4-Chloro-phenyl)-imidazol-1-yl]-2,2-dimethyl-3,4-dihydro-2*H*-pyrano[3,2-*c*] pyridin-3-ol (8h)

The same procedure for **8g** was followed to give the title compound as a yellow solid. Yield 32%.

^{1}H NMR: ($CDCl_3$) δ 8.20 (m, 1H), 7.70 (m, 2H), 7.55 (s, 1H), 7.30 (m, 2H), 6.80 (s, 1H), 6.70 (m, 1H), 6.65 (s, 1H), 6.55 (s, 1H), 5.45 (m, 1H), 4.00 (m, 1H), 1.60(s, 3H), 1.25 (s, 3H). MS (m/z): MH^+ 356.

5.1.10. (3*S*,4*R*)-4-[5-(4-Chloro-phenyl)-pyrazol-1-yl]-2,2-dimethyl-3,4-dihydro-2*H*-pyrano[3,2-*c*]pyridin-3-ol (8i)

The same procedure for **8g** was followed to give the title compound as a white solid. Yield 30%.

^{1}H NMR: ($CDCl_3$) δ δ 8.20 (m, 1H), 7.70 (s, 2H), 7.60 (s, 1H), 7.32 (m, 2H), 7.22 (m, 2H), 6.70 (m, 2H), 5.00 (d, J = 3.6 Hz, 1H), 3.72 (d, J = 3.6 Hz, 1H), 1.60 (s, 3H), 1.30 (s, 3H). MS (m/z): MH^+ 356.

5.1.11. (3*S*,4*R*) 4-[5-(4-Chloro-phenyl)-imidazol-1-yl]-2,2-dimethyl-3,4-dihydro-2*H*-pyrano[3,2-*c*]pyridin-3-ol (8j)

The same procedure for **8g** was followed to give the title compound as a white solid. Yield 25%.

^{1}H NMR: (CD_3OD) δ 8.20 (d, J = 1.5 Hz, 1H), 7.93 (s, 1H), 7.72 (d, J = 3.0 Hz, 2H), 7.70 (s, 1H), 7.30 (d, J = 3.0 Hz, 2H), 6.90 (d, J = 1.5 Hz, 1H), 6.72 (s, 1H), 4.45 (d, J = 3.3 Hz, 1H), 4.30 (d, J = 3.3 Hz, 1H), 1.57 (s, 3H), 1.32 (s, 3H). MS (m/z): MH^+ 356.

5.1.12. (3*S*,4*R*)-4-(6-Chloro-4-oxo-1,4-dihydro-quinazolin-2-ylamino)-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile (10b)

4-Amino-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile **5** (1.09 g, 5 mmol) and 6-chloro-2-methoxy-1*H*-quinazolin-4-one (1.05 g, 5 mmol) in toluene (15 mL) in a seal tube was heated at 150 °C for 4 h. The solvent was removed and the residue was purified by silica gel chromatography with hexanes and ethyl acetate to yield the title compound as white solid (812 mg, 41%).

^{1}H NMR: ($CDCl_3$) δ 7.95 (s, 1H), 7.65 (m, 1H), 7.60 (d, J = 7.5 Hz, 1H), 7.51 (s, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.32 (d, J = 8.0 Hz, 1H), 5.31 (d, J = 4.0 Hz, 1H), 3.72 (d, J = 7.5 Hz, 1H), 1.58 (s, 3H), 1.38 (s, 3H). MS (m/z): MH^+ 398.

5.1.13. (3*S*,4*R*)-3-Hydroxy-2,2-dimethyl-4-(4-oxo-1,4-dihydro-quinazolin-2-ylamino)-chroman-6-carbonitrile (10a)

The same procedure for **10b** was followed to give the title compound as a white solid. Yield 45%.

^{1}H NMR: ($CDCl_3$) δ 6.95–7.85 (m, 7H), 5.20 (s, br, 1H), 5.05 (d, J = 8.5 Hz, 1H), 4.10 (d, J = 8.5 Hz, 1H), 1.55 (s, 3H), 1.41 (s, 3H). MS (m/z): MH^+ 363.

5.1.14. (3*S*,4*R*)-4-(5-Chloro-benzooxazol-2-ylamino)-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile (10c)

The same procedure for **8a** was followed to give the title compound as a white solid. Yield 60%.

^{1}H NMR: ($CDCl_3$) δ 7.65 (s, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.12 (d, J = 8.0 Hz, 2H), 7.05 (d, J = 6.5 Hz, 1H), 6.92 (d, J = 7.5 Hz, 1H), 6.42 (s, 1H), 5.05 (d, J = 7.1 Hz, 1H), 3.82 (d, J = 7.1 Hz, 1H), 1.55 (s, 3H), 1.35 (s, 3H). MS (m/z): MH^+ 372.

5.1.15. (3*S*,4*R*)-4-(5-Ethoxy-benzothiazol-2-ylamino)-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile (10d)

The same procedure for **8a** was followed to give the title compound as a white solid. Yield, 61%.

^{1}H NMR: ($CDCl_3$) δ 7.70 (s, 1H), 7.50–7.40 (m, 2H), 7.10 (s, 1H), 6.90 (m, 2H), 5.05 (m, 1H), 4.10 (m, 2H), 3.80 (m, 1H), 1.55 (s, 3H), 1.40 (m, 3H), 1.30 (s, 3H). MS (*m*/*z*): MH^+ 396.

5.1.16. (3*S*,4*R*)-4-(5-Fluoro-benzothiazol-2-ylamino)-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile (10e)

The same procedure for **8a** was followed to give the title compound as a white solid. Yield, 48%.

^{1}H NMR: ($CDCl_3$) δ 7.70 (s, 1H), 7.45–7.40 (m, 2H), 7.25 (m, 1H), 7.00 (m, 1H), 6.90 (m, 1H), 5.95 (br, 1H), 5.05 (d, J = 5.1 Hz, 1H), 3.80 (d, J = 5.1 Hz, 1H), 1.50 (s, 3H), 1.32 (s, 3H). MS (*m*/*z*): MH^+ 370.

5.1.17. (3*S*,4*R*)-3-Hydroxy-2,2-dimethyl-4-(5-nitro-benzothiazol-2-ylamino)-chroman-6-carbonitrile (10f)

The same procedure for **8a** was followed to give the title compound as a white solid. Yield 60%.

^{1}H NMR: ($CDCl_3$) δ 8.42 (s, 1H), 8.20 (m, 1H), 7.70 (s, 1H), 7.50 (m, 2H), 6.90 (m, 1H), 5.15 (m, 1H), 3.80 (m, 1H), 1.55 (s, 3H), 1.35 (s, 3H). MS (*m*/*z*): MH^+ 397.

5.1.18. (3*S*,4*R*)-4-(5-Chloro-1*H*-benzoimidazol-2-ylsulfanyl)-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile (10g)

The same procedure for **8a** was followed to give the title compound as a white solid. Yield 55%.

^{1}H NMR: ($CDCl_3$) δ 10.50 (br, 1H), 8.00 (s, 1H), 7.50–7.40 (m, 4H), 7.20 (m, 1H), 6.90 (m, 1H), 4.78 (m, 1H), 4.10 (m, 1H), 1.60 (s, 6H). MS (*m*/*z*): MH^+ 396.

5.1.19. (3*S*,4*R*)-4-(6-Chloro-benzo[*d*]isoxazol-3-yloxy)-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile (12a) and (3*S*,4*R*)-4-(6-chloro-3-oxo-3*H*-benzo[*d*]isoxazol-2-yl)-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile (13b)

The same procedure for **8a** was followed to give the title compounds as white solids in ~1:1 ratio. Total yield, 66%.

Compound **12a**: ^{1}H NMR: ($CDCl_3$) δ 7.70 (s, 1H), 7.60–7.50 (m, 2H), 7.35–7.30 (m, 2H), 6.95 (d, J = 8.2 Hz, 1H), 5.90 (d, J = 6.0 Hz, 1H), 4.15 (m, 1H), 3.60 (d, J = 2.0 Hz, 1H), 1.55 (s, 3H), 1.40 (s, 3H). ^{13}C NMR: ($CDCl_3$) δ 164.5, 163.2, 147.1, 135.9, 132.2, 129.8, 125.8, 125.1, 123.4, 120.3, 117.0, 114.6, 110.3, 103.9, 92.1, 75.9, 72.6, 21.5, 21.3. MS (*m*/*z*): MH^+ 371.

Compound **13b**: $[\alpha]_D$ +86.6° (c = 6.2, MeOH); ^{1}H NMR: ($CDCl_3$) δ 7.70 (d, J = 8.5 Hz, 1H), 7.50 (d, J = 8.5 Hz, 2H), 7.30–7.25 (m, 3H), 6.95 (d, J = 8.2 Hz, 1H), 5.62 (d, J = 8.5 Hz, 1H), 4.20 (d, J = 8.5 Hz, 1H), 3.20 (br, 1H), 1.55 (s, 3H), 1.35 (s, 3H). ^{13}C NMR: ($CDCl_3$) δ 162.9, 161.2, 157.5, 138.6, 132.4, 130.1, 129.8, 125.8, 121.6, 118.8, 116.5, 116.2, 114.7, 103.9, 87.4, 77.0, 46.6, 22.2, 21.5. MS (*m*/*z*): MH^+ 371. Anal. Calcd for $C_{19}H_{15}N_2O_4Cl$: C, 61.55; H, 4.08; N, 7.56. Found: C, 61.38; H, 3.98; N, 7.75.

5.1.20. (3*S*,4*R*)-4-(Benzo[*d*]isothiazol-3-yloxy)-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile (12b) and 2-(6-chloro-3-hydroxy-2,2-dimethyl-chroman-4-yl)-benzo[*d*]isothiazol-3-one (13d)

The same procedure for **8a** was followed to give the title compounds as white solids in ~1:1 ratio. Total yield, 60%.

Compound **12b**: ^{1}H NMR: ($CDCl_3$) δ 7.98 (d, J = 8.1 Hz, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.72 (s, 1H), 7.61 (t, J = 7.5 Hz, 1H), 7.53 (d, J = 8.1 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 6.98 (d, J = 8.5 Hz, 1H), 6.12 (d, J = 7.5 Hz, 1H), 4.82 (d, J = 2.1 Hz, 1H), 4.12 (d, J = 7.5 Hz, 1H), 1.59 (s, 3H), 1.46 s, 3H). MS (*m*/*z*): MH^+ 353.

Compound **13d**: ^{1}H NMR: ($CDCl_3$) δ 7.90 (m, 1H), 7.80 (m, 1H), 7.70 (s, 1H), 7.60–7.40 (m, 3H), 6.95 (m, 1H), 6.10 (m, 1H), 4.80 (m, 1H), 4.10 (m, 1H), 1.55 (s, 3H), 1.40 (s, 3H). MS (*m*/*z*): MH^+ 353.

5.1.21. (3*S*,4*R*)-4-(6-Chloro-benzo[*d*]isoxazol-3-ylamino)-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile (12c)

The same procedure for **8a** was followed to give the title compounds as a white solid. Yield, 55%.

^{1}H NMR: (DMSO) δ 8.00 (s, 1H), 7.70 (s, 1H), 7.60–7.50 (m, 3H), 6.95 (m, 1H), 5.80 (m, 1H), 4.65 (m, 1H), 3.88 (m, 1H), 1.45 (s, 3H), 1.20 (s, 3H). MS (*m*/*z*): MH^+ 370.

5.1.22. (3*S*,4*R*)-4-(5-Chloro-1*H*-indazol-3-ylamino)-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile (12d)

The same procedure for **8a** was followed to give the title compounds as a white solid. Yield, 61%.

^{1}H NMR: ($CDCl_3$) δ 7.50 (m, 1H), 7.15 (m, 2H), 7.05 (m, 2H), 6.85 (m, 1H), 5.85 (m, 1H), 5.35 (m, 1H), 1.62 (s, 3H), 1.38 (s, 3H). MS (*m*/*z*): MH^+ 351.

5.1.23. (3*S*,4*R*)-6-Benzenesulfonyl-4-(5-chloro-benzo[*d*]-isoxazol-3-ylamino)-chroman-3-ol (12e)

The same procedure for **8a** was followed to give the title compounds as a white solid. Yield, 40%.

^{1}H NMR: ($CDCl_3$) δ 7.90 (s, 1H), 7.75 (d, J = 6.5 Hz, 2H), 7.60 (s, 1H), 7.55–7.30 (m, 5H), 7.20 (d, J = 7.0 Hz, 1H), 6.70 (d, J = 7.0 Hz, 1H), 5.70 (d, J = 4.5 Hz, 1H), 4.90 (m, 1H), 4.15 (m, 1H), 3.90 (d, J = 4.5 Hz, 1H), 1.45 (s, 3H), 1.25 (s, 3H). MS (*m*/*z*): MH^+ 485.

5.1.24. (3*S*,4*R*)-4-(5-Chloro-benzo[*d*]isoxazol-3-ylamino)-6-(3-fluoro-benzenesulfonyl)-2,2-dimethyl-chroman-3-ol (12f)

The same procedure for **8a** was followed to give the title compounds as a white solid. Yield, 35%.

^{1}H NMR: ($CDCl_3$) δ 7.90 (s, 1H), 7.60–7.20 (m, 9H), 6.80 (d, J = 5.5 Hz, 1H), 5.60 (d, J = 5.5 Hz, 1H), 4.90 (m, 1H), 4.20 (br, 1H), 3.90 (d, J = 5.5 Hz, 1H), 1.50 (s, 3H), 1.35 (s, 3H). MS (*m*/*z*): MH^+ 503.

5.1.25. (3*S*,4*R*)-4-(5-Chloro-benzo[*d*]isoxazol-3-ylamino)-3-hydroxy-2,2-dimethyl-chroman-6-sulfonic acid diethylamide (12g)

The same procedure for **8a** was followed to give the title compound as a white solid. Yield, 51%.

^{1}H NMR: ($CDCl_3$) δ 7.80 (s, 1H), 7.55 (d, J = 4.5 Hz, 1H), 7.45 (m, 2H), 7.20(m, 1H), 6.90 (d, J = 5.0 Hz, 1H), 5.00 (s, 1H), 4.90 (s, 1H), 3.90 (m, 1H), 3.60 (m, 1H), 3.10 (m, 4H), 1.50 (s, 3H), 1.30 (s, 3H), 1.00 (t, J = 4.5 Hz, 6H). MS (*m*/*z*): MH^+ 480.

5.1.26. (3*S*,4*R*)-6,7-Dichloro-4-(5-chloro-benzo[*d*]isoxazol-3-ylamino)-2,2-dimethyl-chroman-3-ol (12h)

The same procedure for **8a** was followed to give the title compound as a white solid. Yield, 37%.

^{1}H NMR: ($CDCl_3$) δ 7.55 (s, 1H), 7.45 (d, J = 4.5 Hz, 1H), 7.30 (d, J = 4.5 Hz, 2H), 6.90 (d, J = 4.5 Hz, 1H), 6.85 (m, 1H), 5.00 (m, 1H), 4.85 (m, 1H), 4.25 (m, 1H), 1.50 (s, 3H), 1.35 (s, 3H). MS (*m*/*z*): MNa^+ 435.

5.1.27. (3*S*,4*R*)-4-(5-Chloro-benzo[*d*]isoxazol-3-ylamino)-3*S*-hydroxy-2,2-dimethyl-chroman-6-yl-phenyl-methanone (12i)

The same procedure for **8a** was followed to give the title compound as a white solid. Yield, 31%.

^{1}H NMR: ($CDCl_3$) δ 7.90 (s, 1H), 7.70 (s, 1H), 7.50 (m, 4H), 7.30 (m, 3H), 7.25 (m, 1H), 6.75 (d, J = 4.5 Hz, 1H), 5.65 (d, J = 4.5 Hz, 1H), 5.00 (m, 1H), 4.20 (br, 1H), 3.70 (d, J = 4.5 Hz, 1H), 1.45 (s, 3H), 1.30 (s, 3H). MS (*m*/*z*): MH^+ 449.

5.1.28. (3*S*,4*R*)-3-Hydroxy-2,2-dimethyl-4-(3-oxo-3*H*-benzo[*d*]-isoxazol-2-yl)-chroman-6-carbonitrile (13a)

^{1}H NMR: ($CDCl_3$) δ 7.71 (s, 1H), 7.65–7.48 (m, 4H), 7.30 (m, 1H), 6.95 (d, *J* = 5.1 Hz, 1H), 5.90 (d, *J* = 5.1Hz, 1H), 4.25 (m, 1H), 3.80 (d, *J* = 2.0 Hz, 1H), 1.55 (s, 3H), 1.40 (s, 3H). MS (*m*/*z*): MH^+ 337.

5.1.29 (3*S*,4*R*)-4-(6-Chloro-3-oxo-3*H*-benzo[*d*]isoxazol-2-yl)-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile (13c)

The same procedure for **8a** was followed to give the title compound as a white solid. Yield, 32%.

^{1}H NMR: ($CDCl_3$) δ 7.65 (m, 1H), 7.55 (m, 1H), 7.35 (s, 1H), 7.20 (m, 2H), 6.95 (m, 1H), 5.62 (m, 1H), 4.40 (m, 1H), 4.20 (m, 1H), 1.60 (s, 3H), 1.35 (s, 3H). MS (*m*/*z m*/*z*): MNa^+ 393.

5.1.30. (3*S*,4*R*)-3-Hydroxy-2,2-dimethyl-4-(6-nitro-3-oxo-3*H*-benzo[*d*]isothiazol-2-yl)-chroman-6-carbonitrile (13e)

The same procedure for **8a** was followed to give the title compound as a white solid. Yield, 45%.

^{1}H NMR: ($CDCl_3$) δ 9.95 (br, s, 1H), 9.05 (s, 1H), 8.58 (d, *J* = 8.5 Hz, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 8.5 Hz, 1H), 7.61 (s, 1H), 7.08 (d, *J* = 8.8 Hz, 1H), 4.56 (m, 1H), 3.02 (m, 1H), 1.65 (s, 3H), 1.45 (s, 3H). MS (m/z): MH^+ 398.

5.1.31. (3*S*,4*R*)-2-(6-Benzenesulfonyl-3-hydroxy-2,2-dimethyl-chroman-4-yl)-6-chloro-benzo[*d*]isoxazol-3-one (13f)

The same procedure for **8a** was followed to give the title compound as a white solid. Yield, 34%.

^{1}H NMR: ($CDCl_3$) δ 7.75 (m, 3H), 7.60 (d, *J* = 6.5Hz, 1H), 7.55–7.35 (m, 4H), 7.20 (d, *J* = 7.0Hz, 1H), 7.10 (s, 1H), 6.90 (d, *J* = 7.0 Hz, 1H), 5.60 (d, *J* = 6.5 Hz, 1H), 4.15 (m, 1H), 4.00 (d, *J* = 4.5 Hz, 1H), 1.55 (s, 3H), 1.30 (s, 3H). MS (*m*/*z*): MH^+ 486.

5.1.32. (3*S*,4*R*)-2-(6-Benzenesulfonyl-3-hydroxy-2,2-dimethyl-chroman-4-yl)-5-chloro-4-benzo[*d*] isoxazol-3-one (13g)

The same procedure for **8a** was followed to give the title compound as a white solid. Yield, 41%.

^{1}H NMR: ($CDCl_3$) δ 7.95 (s, 1H), 7.85 (d, *J* = 5.5 Hz, 2H), 7.80 (d, *J* = 6.0 Hz, 1H), 7.55–7.40 (m, 6H), 6.96 (d, *J* = 6.0 Hz, 1H), 5.85 (d, *J* = 4.5 Hz, 1H), 4.10 (m, 1H), 3.90 (m, 1H), 1.50 (s, 3H), 1.40 (s, 3H). MS (*m*/*z*): MH^+ 486.

5.1.33. (3*S*,4*R*)-6-Chloro-2-[3-hydroxy-2,2-dimethyl-6-(piperidine-1-sulfonyl)-chroman-4-yl]-benzo[*d*] isoxazol-3-one (13h)

The same procedure for **8a** was followed to give the title compounds as white solids (~1:1). Total yield, 67%.

Compound **13h**: ^{1}H NMR: ($CDCl_3$) δ 7.65 (d, *J* = 6.0 Hz, 1H), 7.55 (d, *J* = 6.5 Hz, 2H), 7.30 (s, 1H), 7.20 (d, *J* = 6.5 Hz, 1H), 7.15 (s, 1H), 7.00 (d, *J* = 6.5 Hz, 1H), 5.70 (d, *J* = 7.0 Hz, 1H), 4.20 (m, 1H), 3.90 (d, *J* = 4.5 Hz, 1H), 2.75 (t, *J* = 1.5 Hz, 4H), 1.60 (s, 3H), 1.50 (m, 4H), 1.35 (m, 2H), 1.35 (s, 3H). MS (*m*/*z*): MNa^+ 515.

O-linked regio-isomer: ^{1}H NMR: ($CDCl_3$) δ 7.75 (s, 1H), 7.60 (d, *J* = 5.5 Hz, 2H), 7.50 (m, 2H), 7.25 (d, *J* = 5.5 Hz, 1H), 7.00 (d, *J* = 6.0 Hz, 1H), 5.90 (d, *J* = 4.5 Hz, 1H), 4.20 (m, 1H), 3.90 (s, 1H), 2.90 (t, *J* = 2.5 Hz, 4H), 1.60 (m, 4H), 1.50 (s, 3H), 1.40 (m, 2H), 1.35 (s, 3H). MS (*m*/*z*): MNa^+ 515.

5.1.34. (3*S*,4*R*)-(5-Chloro-3-oxo-3*H*-benzo[*d*]isoxazol-2-yl)-3-hydroxy-2,2-dimethyl-chroman-6-sulfonic acid diethylamide (13i)

The same procedure for **8a** was followed to give the title compounds as white solids (~1:1). Total yield, 56%.

Compound **13i**: ^{1}H NMR: ($CDCl_3$) δ 7.65 (m, 2H), 7.55 (d, *J* = 6.5 Hz, 1H), 7.40 (s, 1H), 7.15 (d, *J* = 6.5 Hz, 1H), 6.95 (d, *J* = 6.5 Hz, 1H), 5.70 (d, *J* = 7.0 Hz, 1H), 4.20 (m, 1H), 3.90 (m, 1H), 3.00 (m, 4H), 1.60 (s, 3H), 1.35 (s, 3H), 1.00 (t, J = 4.5 Hz, 6H). MS (*m*/*z*): MH^+ 481.

O-linked regio-isomer: ^{1}H NMR: ($CDCl_3$) δ 7.80 (s, 1H), 7.70 (d, *J* = 5.5 Hz, 2H), 7.50 (m, 2H), 7.40 (m, 1H), 7.00 (d, *J* = 5.0 Hz, 1H), 5.90 (d, *J* = 4.5 Hz, 1H), 4.15 (m, 1H), 3.80 (d, *J* = 2.0 Hz, 1H), 3.20 (m, 4H), 1.50 (s, 3H), 1.35 (s, 3H), 1.10 (t, *J* = 4.5 Hz, 6H). MS (*m*/*z*): MH^+ 481.

5.1.35. (3*S*,4*R*)-6-Chloro-2-(6,7-dichloro-3-hydroxy-2,2-dimethyl-chroman-4-yl)-benzo[*d*]isoxazol-3-one (13j)

The same procedure for **8a** was followed to give the title compounds as white solids in ~1:1 ratio. Total yield, 72%.

Compound **13j**: ^{1}H NMR: ($CDCl_3$) δ 7.50 (d, *J* = 4.0 Hz, 1H), 7.45 (s, 1H), 7.40 (d, *J* = 4.0 Hz, 1H), 7.25 (m, 1H), 6.80 (d, *J* = 5.0 Hz, 1H), 5.85 (d, *J* = 1.0 Hz, 1H), 4.30 (m, 1H), 3.70 (d, *J* = 2.0 Hz, 1H), 1.55 (s, 6H). MS (*m*/*z*): MH^+ 414.

O-linked regio-isomer: ^{1}H NMR: ($CDCl_3$) δ 7.50 (d, *J* = 5.0 Hz, 1H), 7.35 (d, *J* = 5.0 Hz, 1H), 7.15 (m, 2H), 6.80 (d, *J* = 5.0 Hz, 1H), 5.65 (d, *J* = 4.5 Hz, 1H), 4.80 (br, 1H), 4.20 (d, *J* = 4.5 Hz, 1H), 1.55 (s, 3H), 1.35 (s, 3H). MS (*m*/*z*): MH^+ 414.

5.1.36. (3*S*,4*R*)-2-(6-Benzoyl-3-hydroxy-2,2-dimethyl-chroman-4-yl)-6-chloro-4-benzo[*d*]isoxazol-3-one (13k)

The same procedure for **8a** was followed to give the title compounds as white solids in ~1:1 ratio. Total yield, 70%.

Compound **13k**: ^{1}H NMR: ($CDCl_3$) δ 7.75 (m, 1H), 7.60 (m, 3H), 7.45 (m, 2H), 7.25 (m, 2H), 7.20 (m, 2H), 6.95 (d, *J* = 4.5 Hz, 1H), 5.70 (m, 1H), 4.20 (d, *J* = 5.0 Hz, 1H), 3.80 (br, 1H), 1.60 (s, 3H), 1.40 (s, 3H). MS (*m*/*z*): MH^+ 450.

O-linked regio-isomer: ^{1}H NMR: ($CDCl_3$) δ 8.05 (s, 1H), 7.75 (m, 3H), 7.60–7.40 (m, 5H), 7.30 (d, *J* = 4.5 Hz, 1H), 6.95 (d, *J* = 4.5 Hz, 1H), 6.15 (m, 1H), 4.45 (m, 1H), 2.30 (m, 1H), 1.60 (s, 3H), 1.40 (s, 3H). MS (*m*/*z*): MH^+ 450.

5.1.37. (3*S*,4*R*)-6-Chloro-[2-hydroxy-6-(4-methoxy-benzene-sulfonyl)-2,2-dimethyl-chroman-4-yl]-benzo[*d*]isoxazol-3-one (13l)

The same procedure for **8a** was followed to give the title compounds as white solids in ~1:1 ratio. Total yield, 58%.

Compound **13l**: ^{1}H NMR: ($CDCl_3$) δ 7.70 (m, 4H), 7.50 (s, 1H), 7.20 (d, *J* = 5.0 Hz, 1H), 7.10 (s, 1H), 6.90 (d, *J* = 5.0 Hz, 1H), 6.85 (d, *J* = 5.5 Hz, 2H), 5.60 (d, *J* = 6.5 Hz, 1H), 4.15 (m, 1H), 3.80 (s, 3H), 1.55 (s, 3H), 1.30 (s, 3H). MS (*m*/*z*): MH^+ 516.

O-linked regio-isomer: ^{1}H NMR: ($CDCl_3$) δ 7.90 (s, 1H), 7.80 (m, 3H), 7.50 (m, 2H), 7.30 (m, 1H), 6.90 (m, 3H), 5.85 (d, *J* = 4.5 Hz, 1H), 4.15 (m, 1H), 3.85 (m, 1H), 3.80 (s, 3H), 1.50 (s, 3H), 1.35 (s, 3H). MS (*m*/*z*): MH^+ 516.

5.1.38. (3*S*,4*R*)-6-Chloro-2-[6-(4-chloro-benzenesulfonyl)-3-hydroxy-2,2-dimethyl-chroman-4-yl]-benzo[*d*]isoxazol-3-one (13m)

The same procedure for **8a** was followed to give the title compounds as white solids in ~1:1 ratio. Total yield, 60%.

Compound **13m**: ^{1}H NMR: ($CDCl_3$) δ 7.70 (m, 4H), 7.55 (s, 1H), 7.35 (d, *J* = 5.0 Hz, 2H), 7.20 (d, *J* = 5.0 Hz, 1H), 7.10 (s, 1H), 7.00 (d, *J* = 5.5 Hz, 1H), 5.60 (d, *J* = 6.5 Hz, 1H), 4.15 (m, 1H), 4.00 (m, 1H), 1.55 (s, 3H), 1.30 (s, 3H). MS (*m*/*z*): MH^+ 504.

O-linked regio-isomer: ^{1}H NMR: ($CDCl_3$) δ 8.00 (s, 1H), 7.80 (m, 3H), 7.50 (m, 4H), 7.30 (m, 1H), 7.00 (d, *J* = 5.5 Hz, 1H), 5.90 (d, *J* = 4.5 Hz, 1H), 4.15 (m, 1H), 3.80 (d, *J* = 1.0 Hz, 1H), 1.50 (s, 3H), 1.35 (s, 3H). MS (*m*/*z*): MH^+ 521.

5.1.39. (3*S*,4*R*)-6-Chloro-2-[6-(3-fluoro-benzenesulfonyl)-3-hydroxy-2,2-dimethyl-chroman-4-yl]-benzo[*d*]isoxazol-3-one (13n)

The same procedure for **8a** was followed to give the title compounds as white solids in ~1:1 ratio. Total yield, 56%.

Compound **13n**: ^{1}H NMR: ($CDCl_3$) δ 7.75 (d, J = 5.5 Hz, 1H), 7.65 (d, J = 5.0 Hz, 1H), 7.5 (m, 2H), 7.45 (m, 2H), 7.20 (m, 2H), 7.10 (s, 1H), 7.00 (d, J = 5.5 Hz, 1H), 5.60 (d, J = 6.5 Hz, 1H), 4.15 (m, 1H), 1.50 (s, 3H), 1.35 (s, 3H). MS (m/z): MH^+ 504.

O-linked regio-isomer: ^{1}H NMR: ($CDCl_3$) δ 8.00 (s, 1H), 7.80 (d, J = 5.5 Hz, 1H), 7.70 (d, J = 5.0 Hz, 1H), 7.55 (d, J = 5.0 Hz, 1H), 7.50 (m, 2H), 7.25 (m, 3H), 7.00 (d, J = 5.5 Hz, 1H), 5.90 (d, J = 4.5 Hz, 1H), 4.15 (m, 1H), 3.90 (m, 1H), 1.50 (s, 3H), 1.35 (s, 3H). MS (m/z): MH^+ 504.

5.1.40. (3*S*,4*R*)-4-(1,3-Dioxo-1,3-dihydro-pyrrolo[3,4-c]pyridin-2-yl)-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile 13p

(3*S*,4*R*)-4-Amino-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile **5** (220 mg, 1 mmol) and furo[3,4-*c*]pyridine-1,3-dione(150 mg, 1 mmol) in toluene (5 mL) in sealed tube was heated at 120 °C for 10 h. The reaction was cooled down and the solvent was removed. The residue was purified by silica gel chromatography with hexanes: ethyl acetate 1:1 to yield the title compound as a yellow solid (200 mg, 55%).

^{1}H NMR: ($CDCl_3$) δ 9.05 (d, J = 6.0 Hz, 1H), 9.00–9.10 (br s, 1H), 7.45 (d, J = 7.5 Hz, 1H), 7.15 (s, 1H), 6.95 (d, J = 7.5 Hz, 1H), 5.35 (d, J = 8.2 Hz, 1H), 4.52 (dd, J = 8.0, 5.8 Hz, 1H), 3.32 (s, 1H), 1.61 (s, 3H), 1.38 (s, 3H). MS (m/z): MH^+ 350.

5.1.41. (3*S*,4*R*)-4-(5-Chloro-1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-hydroxy-2,2-dimethyl-chroman-6-carbonitrile 13o

The same procedure for **13p** was followed to give the title compound as a white solid. Yield, 66%.

^{1}H NMR: ($CDCl_3$) δ 7.80–7.70 (m, 3H), 7.40 (m, 1H), 7.15 (s, 1H), 6.90 (m, 1H), 5.30 (m, 1H), 4.45 (m, 1H), 4.35 (m, 1H), 1.55 (s, 3H), 1.30 (s, 3H). MS (m/z): MH^+ 351.

6. Biological evaluations

All animal studies were approved by the Johnson & Johnson Pharmaceutical Research & Development Animal Care and Use Committee and were performed in accordance with the guidelines of the Animal Welfare Act and the American Association for Accreditation of laboratory Animal Care.

6.1. In vitro studies

6.1.1. FLIPR assay

TE671 and β-TC cells were obtained from ATCC and grew in desired media. The cells were seeded in black 96-well plates at 50–60 k per well the day before. On the day of assaying, media was discarded and 100 μL of FLIPR buffer (20 μM HEPES, pH 7.4, 120 μM NaCl, 2 μM KCl, 2 μM $CaCl_2$, 1 μM mg Cl_2, and 5 μM Glucose) was added to each well. Equal volume of membrane potential dye (Molecular Devices) prepared in FLIPR buffer was also added to each well. The plate was incubated at room temperature for 15–30 min before placed in FLIPR. The fluorescence was monitored before and after the addition of various concentrations of standards and test compounds. The maximal decrease in fluorescence intensity obtained by pinacidil and P-1075 after subtracting buffer control was designated as 100%. EC50 values were determined by the concentration of compounds required to produce 50% of the maximal response induced by pinacidil and P-1075. Glyburide, a K_{ATP} channel blocker, was added 3 min after the compound addition to a final concentration of 5 μM.

SUR2A was expressed in COS-7 cells transient transfection. The day before the transfection, COS-7 cells were plated into 96-well black well plates at 20 K/well in DMED media without antibiotics. Transfection was carried out with Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. The amount of DNA used per well was 240 ng (SUR2A: Kir6.2 = 210 ng:30 ng). The plates were incubated at 37 °C with 5% CO_2 for 48 h. FLIPR assay was carried out as described above.

6.1.2. Rat bladder strips organ bath assay

Longitudinal strips (2 mm × 1 cm) of rat bladder were dissected and hung in organ chambers containing Krebs buffer (NaCl, 118.2 μM; KCl, 4.7 μM; $MgSO_4$, 1.2 μM; KH_2PO_4, 1.2 μM; $CaCl_2$, 2.5 μM, $NaHCO_2$, 25 μM; glucose, 11.1 μM). The solution was maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. After the optimal tension was reached through repeated stretching and carbachol (1 μM) challenge, the strips were pre-contracted with 1 μM carbachol. When the contraction was stabilized, an accumulative dosage curve was constructed for each compound. The stock solutions were prepared at 100 μM in DMSO and further diluted in saline. The maximum tension induced by carbachol minus baseline was defined as 100% tension. The relaxation was expressed as percent tension.

6.2. In vivo studies

6.2.1. Rats acetic acid model

Female Sprague-Dawley rats 180–220 g (Charles River, Wilmington, MA) were used. The anesthesia was induced by exposure to isoflurane (induction at 3–4% isoflurane + oxygen) briefly (about 10 min). The anesthesia is maintained by s.c. or i.p. injection of 1.0–1.2 g/kg urethane. Body temperature is maintained at 36–38 °C using a warming blanket. The urinary bladder is exposed through a midline incision of the abdomen and urine is emptied by application of light pressure. A PE 50 catheter is inserted through the apex of the bladder dome and secured by a purse-string silk suture (5-O). The tubing was attached (using a 'T' connector) to a Statham pressure transducer (Model P23Db) and to a Harvard infusion pump. The right jugular vein was cannulated for compound administration. After the preparation was rested for I h, cystometric evaluations were initiated by infusing saline containing 0.25% acetic acid into the bladders at 2.4 mL/h. The infusion continued for 60–90 min until consistent micturation pattern was obtained. Vehicle or test compounds dissolved in 10% PEG300/20% HPBCD was delivered intravenously. Bladder infusion was continued for another 60 min. Micturition parameters were continuously monitored and recorded on a on a GOULD Ponemah Physiology Platform.

6.2.2. Rats partial outlet obstruction model

Female Sprague-Dawley rats (Charles River, Wilmington, MA; 190–210 g) were anesthetized with isoflurane and through a midline incision the bladder and urethra were exposed. A 4–0 silk ligature was tied around the proximal urethra in the presence of a stainless steel rod (1 mm diameter). The rod was then removed resulting in a partial occlusion. The abdominal musculature was closed using 3–0 silk and the skin was closed with surgical staples. Each rat received 150,000 U of bicilin C-R, i.m. (Wyeth Laboratories, Philadelphia, PA). After six weeks, the ligature was removed under isoflurane anesthesia, and a flared catheter (PE60) was placed in the dome of the bladder and secured with a purse-string suture. The catheter was tunneled under the skin and exteriorized through an opening in the back of the neck. The abdominal incision was sutured and the free end of the catheter was sealed. Two days after catheter implantation, the animals were used for cystometric evaluations. The night before testing the animals were placed in the metabolic cages, the catheter was connected to a Harvard infusion pump, and bladders were perfused overnight with saline at 2 mL/h. The next morning the bladder catheter was attached (using a 'T' connector) to a Statham pressure transducer (Model P23Db)

and to a Harvard infusion pump. Urine was collected and measured through a force displacement transducer (Grass FTO3). The cystometric evaluation of bladder function was started by infusing saline (20 mL/h) and after the first micturition the infusion was maintained for 20 min. Two hours after the first cystometry period, the rats were dosed orally with the test compound and a second cystometry was performed between 30 min and 4 h after administration of test compound. The appropriate vehicle [polyethylene glycol 200 (PEG200)] was similarly administered to groups of rats that served as controls and the cystometry was performed at the respective time point.

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