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Novel K_V7 ion channel openers for the treatment of epilepsy and implications for detrusor tissue contraction

Mark A. Seefeld[a,*], Hong Lin[a,b,1], Joerg Holenz[a], Dave Downie[c], Brian Donovan[d], Tingting Fu[e], Kishore Pasikanti[a], Wei Zhen[f], Matthew Cato[g], Khuram W. Chaudhary[g], Pat Brady[d], Tania Bakshi[h], Dwight Morrow[b,2], Sridharan Rajagopal[i], Swapan Kumar Samanta[i], Naveena Madhyastha[i], Bharathi Mohan Kuppusamy[i], Robert W. Dougherty[j], Ravi Bhamidipati[i], Zainuddin Mohd[i], Guy A. Higgins[k], Mark Chapman[l], Céline Rouget[m], Philippe Lluel[m], Yasuji Matsuoka[a,*]

[a] Neuroscience Virtual-Proof-of-Concept Discovery Performance Unit, GlaxoSmithKline, Collegeville, PA, United States
[b] Regenerative Medicine Discovery Performance Unit, GlaxoSmithKline, Collegeville, PA, United States
[c] Screening Profiling & Mechanistic Biology, Platform Technology Sciences, GlaxoSmithKline, Stevenage, UK
[d] Screening, Profiling and Mechanistic Biology, Platform Technology Sciences, GlaxoSmithKline, Collegeville, PA, United States
[e] Mechanistic Safety & Disposition, Product Development and Supply, Platform Technology Sciences, GlaxoSmithKline, Shanghai, China
[f] Integrated Biological Platform Sciences, Product Development and Supply, Platform Technology Sciences, GlaxoSmithKline, Shanghai, China
[g] In Vitro/In Vivo Translation, Safety Pharmacology, Platform Technology Sciences, GlaxoSmithKline, King of Prussia, PA, United States
[h] Pattern Recognition Receptor Discovery Performance Unit, GlaxoSmithKline, Collegeville, PA, United States
[i] Jubilant Biosys, Bangalore, Karnataka, India
[j] Jubilant Discovery Services, Malvern, PA, United States
[k] InterVivo Solutions, Toronto, ON, Canada
[l] Icagen, Durham, NC, United States
[m] Urosphere SAS, Toulouse, France

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ABSTRACT

Neuronal voltage-gated potassium channels, K_V7s, are the molecular mediators of the M current and regulate membrane excitability in the central and peripheral neuronal systems. Herein, we report novel small molecule K_V7 openers that demonstrate anti-seizure activities in electroshock and pentylenetetrazol-induced seizure models without influencing Rotarod readouts in mice. The anti-seizure activity was determined to be proportional to the unbound concentration in the brain. K_V7 channels are also expressed in the bladder smooth muscle (detrusor) and activation of these channels may cause localized undesired effects. Therefore, the impact of individual K_V7 isoforms was investigated in human detrusor tissue using a panel of K_V7 openers with distinct activity profiles among K_V7 isoforms. *KCNQ4* and *KCNQ5* mRNA were highly expressed in detrusor tissue, yet a compound that has significantly reduced activity on homomeric $K_V7.4$ did not reduce detrusor contraction. This may suggest that the homomeric $K_V7.4$ channel plays a less significant role in bladder contraction and further investigation is needed.

Neuronal voltage-gated potassium channels composed of the following heterodimeric K_V7 isoforms, $K_V7.2$, 7.3, 7.4, and 7.5, are coded by *KCNQ2-5*, respectively. K_V7 ion channels are the molecular mediators of the M current and regulate membrane excitability in the central and peripheral neuronal systems.[1] Genetic mutations in *KCNQ3* and *KCNQ4*, are associated with excitability disorders including multiple forms of epilepsy and deafness.[2,3] Therefore, K_V7 channel opening is a potential therapeutic approach for aberrant neuroexcitation conditions.[4]

Previously reported small molecule K_V7-openers suppress neuronal activity at sub-micromolar concentrations *in vitro*. Suppression of neuronal activities with K_V7-openers translate to functional endpoints in a

* Corresponding authors.
E-mail addresses: Mark.A.Seefeld@gsk.com (M.A. Seefeld), Yasuji.Y.Matsuoka@gsk.com (Y. Matsuoka).
[1] Current affiliation: Prelude Therapeutics, Wilmington, DE, United States.
[2] Current affiliation: Magenta Therapeutics, Cambridge, MA, Unites States.

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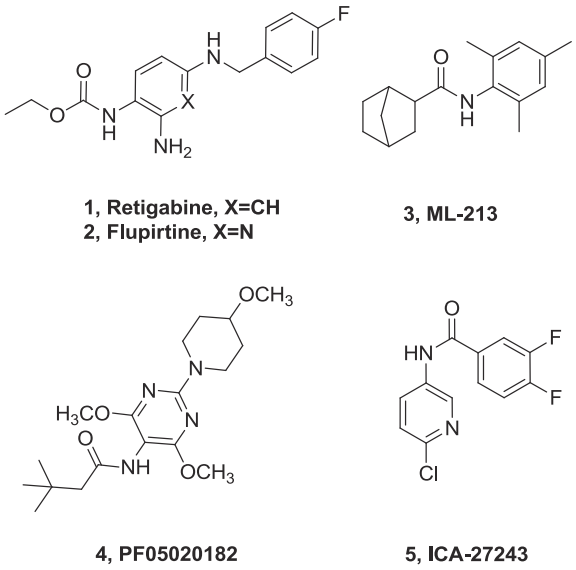
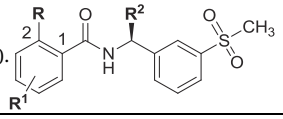


Fig. 1. Representative Kv7.2/7.3 openers.

Table 1

Benzamide series potencies (μM).

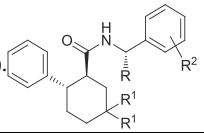


Cmpd.	R	R^1	R^2	EC_{50}[a]	Sol. (μM)[b]
15	SPh	5-CF_3	H	0.20 (175)	34
16	OPh	5-CF_3	H	0.16 (245)	23
8	OPh	5-CF_3	CH_3	0.04 (134)	57
17	OPh	5-CF_3	CH_2OH	0.08 (77)	11
18	OPh	5-SF_5	H	0.08 (185)	7
19	OPh	5-SF_5	CH_2OH	0.02 (101)	121
20	OPh	4-F,5-F	CH_3	0.13 (132)	145

[a] Measured using IWQ (% of Maximal response to ML213).
[b] Kinetic aqueous solubility measured using CAD or CLND.

Table 2

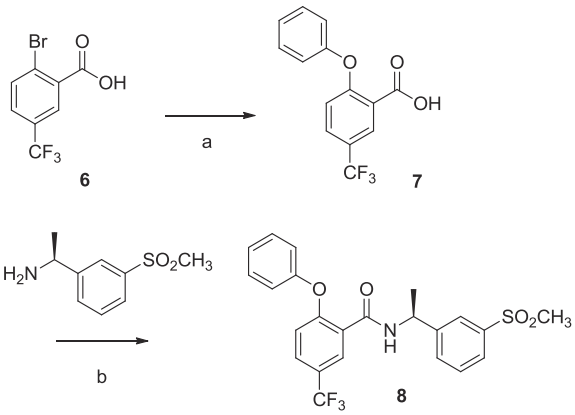
Cyclohexamide series potencies (μM).



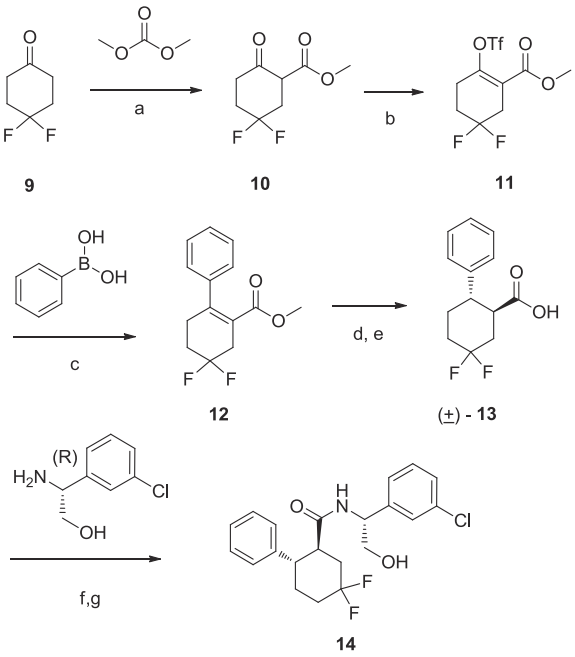
Cmpd.	R	R^1	R^2	EC_{50}[a]	Sol.(μM)[b]
21	CH_3	H	H	0.25(115)	57
22	CH_2OH	H	H	0.79(165)	455
23	CH_2OH	F	H	1.3(108)	332
24	CH_3	F	H	0.20(140)	25
14	CH_2OH	F	3-Cl	0.30(141)	318
25	CH_2OH	F	3-CF_3	0.08(96)	119

[a] Measured using IWQ (% of Maximal response to ML213).
[b] Kinetic aqueous solubility measured using CAD or CLND.

wide range of pre-clinical models.[5] In human clinical studies, Retigabine (**1**) and Flupirtine (2) (Fig. 1) that act on pan-KV7.2–7.5 channels demonstrated clinical efficacy in partial epilepsy[6] and multiple forms of pain,[7] respectively. However, these molecules have undesired effects including of discolouration to the skin, nails, lips and ocular tissues (Retigabine)[8] and hepatotoxicity (Flupirtine)[9,10] that are suspected as K_V7-unrelated actions. Furthermore, the frequency of



Scheme 1. Synthesis of benzamide derivatives. Reagents and conditions: (a) Phenol, CuI, K_2CO_3, DMF. 130 °C, 16 h; (b) HOBt, EDC, DIPEA, DMF, 60 °C, 2 h.



Scheme 2. Synthesis of aryl amide analogs. Reagents and conditions: (a) NaH, DMF, 0 °C-RT, 16h; (b) Commin's reagent, NaH, THF, 0 °C-RT, 16h; (c) Pd(dppf)Cl_2·CH_2Cl_2, Na_2CO_3, Toluene, H_2O, EtOH, 90 °C, 16 h; (d) H_2, Pd/C, MeOH, RT 16 h; (e) NaOEt, EtOH, 90 °C; (f) HATU, DIPEA, THF, RT, 16 h; (g) Chiral prep HPLC.

urinary adverse events such as urinary retention was increased in patients receiving Retigabine compared with placebo in clinical studies.[11] Urinary adverse events were suspected as target-related because some *KCNQ* isoforms are expressed in human urinary bladder smooth muscle[12] and pan-K_V7 openers affected bladder contractibility *ex vivo* in human[12] and guinea pig.[13]

While K_V7 channel openers have demonstrated clinical efficacy, improved therapeutic agents are desired. Discovery efforts leading to chemically distinct $K_V7.2/7.3$ channel opener series (1–5, Fig. 1) have been described in the literature[5,14–16] and, except for the ICA-27243 (**5**) series of compounds, all have been reported to activate human pan-K_V7-mediated currents. In this study, we aimed to discover novel Kv7 isoform-selective openers for the treatment of epilepsy and to investigate the implications of K_V7 isoform activities in human urinary bladder contraction.

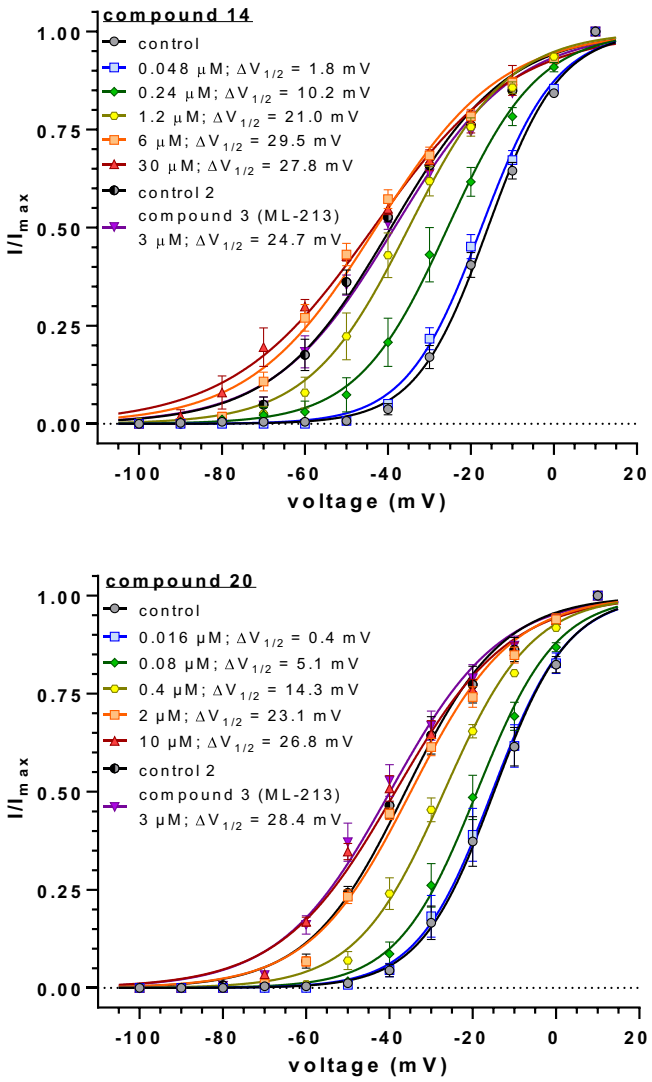


Fig. 2. Examples of IV curve.

Table 3
Pharmacokinetic parameters for selected KV7.2/7.3 channel openers following intravenous dosing in mice.

Cmpd.	Clb (mL/min/kg)	DNAUC$_{0-t}$ (μg.h/mL)	Br/Bl ratio/mg/kg
14	48 ± 2.1	0.51 ± 0.5	0.8
20	31 ± 2.6	0.33 ± 0.5	1.3

A high throughput screen of the GSK compound collection using the heterodimeric K_V7.2/7.3 channel (See Supplemental Information for methods of all biological experiments) revealed two similar, but structurally distinct lead series, Benzamide series (Table 1) and Cyclohexamide series (Table 2).

The synthesis of the benzamide series is depicted in Scheme 1. Ortho-bromo benzoic acids **6** were coupled with phenolic reagents via an SnAr reaction to provide biaryl ethers **7**. Subsequent amide coupling with chiral benzyl amines gave benzamide products (Table 1).

Cyclohexamide compounds (Scheme 2) were synthesized starting from 4,4-difluoro cyclohexanone (**9**) and dimethyl carbonate to afford ketoester **10**. Converting **10** to the corresponding enol triflate **11** and subsequent cross-coupling with phenylboronic acid gave the

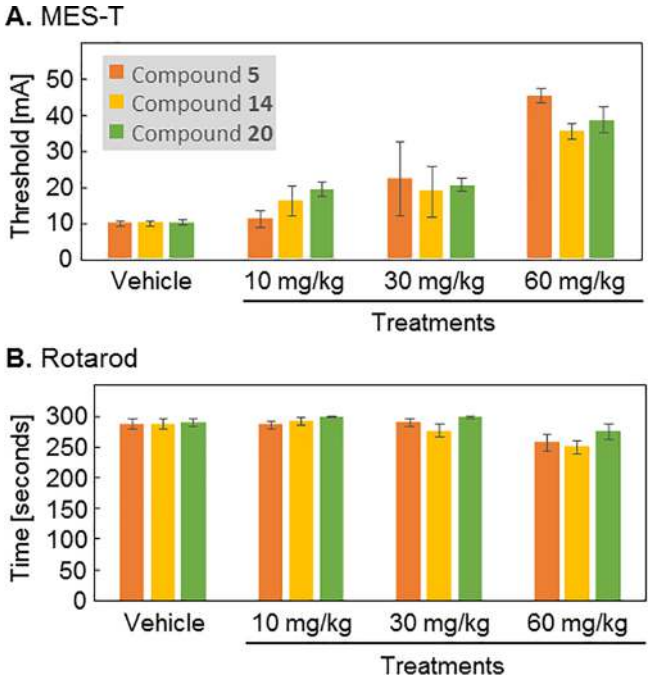


Fig. 3. MES-T seizure and Rotarod studies.

unsaturated aryl ester **12**. Hydrogenation followed by saponification under basic conditions provided the racemic *trans*-cyclohexyl carboxylic acid **13**. Amide coupling with appropriately substituted chiral benzyl amines yielded K_V7.2/7.3 openers which were separated into pure diastereomers using chiral HPLC (Table 2). Only one enantiomer in the cyclohexamide series was determined to be consistently active in the K_V7.2/7.3 primary assay and this absolute stereochemistry was confirmed by an X-ray crystal structure of compound **14** (Supplemental Fig. 1, CCDC code: 1855751).

In the benzamide series, thioether screening lead **15** (Table 1) was metabolically unstable in pooled human liver microsomes (HLM, > 3 mL/min/g liver) as were phenyl ether analogs lacking electron withdrawing groups (EWGs) at R1 (not shown). Alkyl substituents at R were tolerated, however, unsubstituted phenyl ethers provided the highest level of K_V7.2/7.3 activation. Functionality at R2 was limited to methyl or hydroxymethyl with the specific stereochemistry shown. The methyl sulfone at the 3-position of the benzylic ring remained unchanged as it provided the best combination of solubility and K_V7.2/7.3 activity.

Initially, poor aqueous solubility was observed in the cyclohexamide series. This issue was resolved by the addition of a hydroxymethyl group at position R. Again, the stereospecificity at this position was limited to that shown in Table 2. Stabilities under HLMs were compromised without EWGs on the cyclohexyl ring. *Gem*-difluoro substitution on the cyclohexyl ring provided stability and avoided the introduction of an additional chiral center. The phenyl group adjacent to the carboxamide was ideally left unsubstituted much like the phenyl group in the benzamide series. Lipophilic substituents at the *meta*-position of the benzyl ring improved target activity without sacrificing significant solubility. Methylsulfone substitution was not well-tolerated on the benzyl ring in this series.

The selection criteria for progressing compounds to *in vivo* studies were minimally: aqueous kinetic solubilities of > 100 μM, K_V7.2/7.3 channel opening $EC_{50} \leq 0.30$ μM (IonWorks™ Quattro), and HLM clearance values under 3 mL/min/g liver. All compounds had P-glycoprotein (P-gp) ratios of less than 2. Some compounds elicited complex curves wherein the currents measured were actually depressed as

Table 4
Activities in human K_V7.2–7.5 channels (μM).[a]

Cmpd.	K_V7.2/7.3	K_V7.3/7.5	K_V7.4
14	0.10	0.36	0.10
19	0.06	0.08	> 10
20	0.20	0.20	0.16
24	0.40	0.40	4.0
25	0.05	0.25	0.02
ML-213	0.04	0.50	1.6
ICA-27243	0.46	> 10	> 10

[a] Activities determined by SyncroPatch platform.

Table 5
Activities in human wildtype and mutant KV7.2/7.3 channels (μM).[a]

Cmpd.	WT K_V7.2/7.3	K_V7.2W236L/K_V7.3W265L
15	0.02	> 30
16	0.05	> 30
21	0.10	> 30
ML-213	0.04	> 11
ICA-27243	0.46	0.43–1.81
Retigabine	0.32	> 30

[a] Activities determined by ^{86}Rb efflux assay.

compared with vehicle controls at more depolarized potentials (i.e., generally higher than −10 mV). These compounds were not advanced to *in vivo* studies. Examples of IV curves of compounds in QPatch (see Supplemental section) that met with our progression criteria are indicated in Fig. 2.

Prior to *in vivo* testing in rodents, activities of representative compounds from both chemical series **14** and **20** were measured in primary cultured rat cortical neurons using a multiple-electrode array system (see Supplemental section). Both compounds inhibited spontaneous neuronal firing activities at similar concentrations to those observed with cell lines over-expressing K_V7 isoforms. IC_{50} values of 0.28 and 0.11 μM were determined for compounds **14** and **20**, respectively.

Compounds **14** and **20** were also found to be sufficiently brain penetrant (Table 3) to progress to maximum electroshock-threshold (MES-T, Fig. 3A) and Rotarod (Fig. 3B) studies that measure anti-seizure effects and CNS tolerability, respectively. Dose-dependent increases in seizure threshold with no meaningful effects in the Rotarod model were similar to the results observed with the experimental control ICA-27243 (Compound **5** in Fig. 3A and B). Similar results were obtained using the pentylenetetrazol (PTZ)-induced seizure model (Supplemental Fig. 2). In addition, pharmacodynamic (PD) effects observed in both the PTZ and MES-T mouse models correlated well with the predicted target engagement across compounds at all dose levels tested (Supplemental Fig. 3).

After determining that compounds from these two series could achieve efficacy in seizure models, we sought to understand if the compounds demonstrated isoform selectivity amongst the K_V7.2–7.5 channels. Compounds meeting the previously described selection criteria for activity and solubility were cross-screened against the heterodimeric K_V7.3/7.5 channel using the IonWorks™ Barracuda platform (Molecular Devices). All compounds demonstrated similar levels of activities in both K_V7.2/7.3 and K_V7.3/7.5 assays (Table 4). To further scrutinize the binding site interactions, select compounds were tested in a cell line that has point mutations (K_V7.2W236L and K_V7.3W265L) in the pore domain (Table 5). No compounds from these two series showed measurable activity up to 10 μM in this assay, suggesting that these compounds bind in the K_V7.2/7.3 pore domain. The activity of Icagen compound ICA-27243 (**5**) was unaffected in this mutant K_V7.2/7.3 assay, supporting reports of compound binding in the voltage sensor domain.[17]

It has been reported that K_V7.4 is implicated in pain and urinary bladder contraction. Therefore, we measured the effects of compound **19**, which has significantly reduced activity on K_V7.4, in a bladder contraction model.

Urinary adverse events were reported more frequently in patients receiving the pan-Kv7 activator Retigabine (**1**), as compared with placebo, although most patients were able to continue with treatment.[11] *KCNQ* isoforms are expressed in human detrusor and K_V7 openers were shown to influence bladder contraction.[12,18] In this study, we have examined expression of *KCNQ* isoforms (Supplemental Fig. 4) and effects on human bladder contraction *ex vivo* (Fig. 4). We tested compounds that have diverse *KCNQ* isoform activity profiles to determine how different isoform profiles influence bladder contraction. While the inhibition of bladder contraction was clearly associated with K_V7 activation, it wasn't clear how individual K_V7.2–7.5 isoforms may contribute. Compound **19**, which has significantly reduced homomeric K_V7.4 activity, did not reduce impact on bladder contraction, and this may suggest that the homomeric K_V7.4 channel plays a less critical role in bladder contraction. Further studies are needed to conclude a role of K_V7 isoforms in bladder contraction.

In the present study, we have identified novel pan-K_V7 openers, that are free from structural alerts. Representatives of novel K_V7 openers demonstrated anti-seizure effects in a dose-dependent manner without

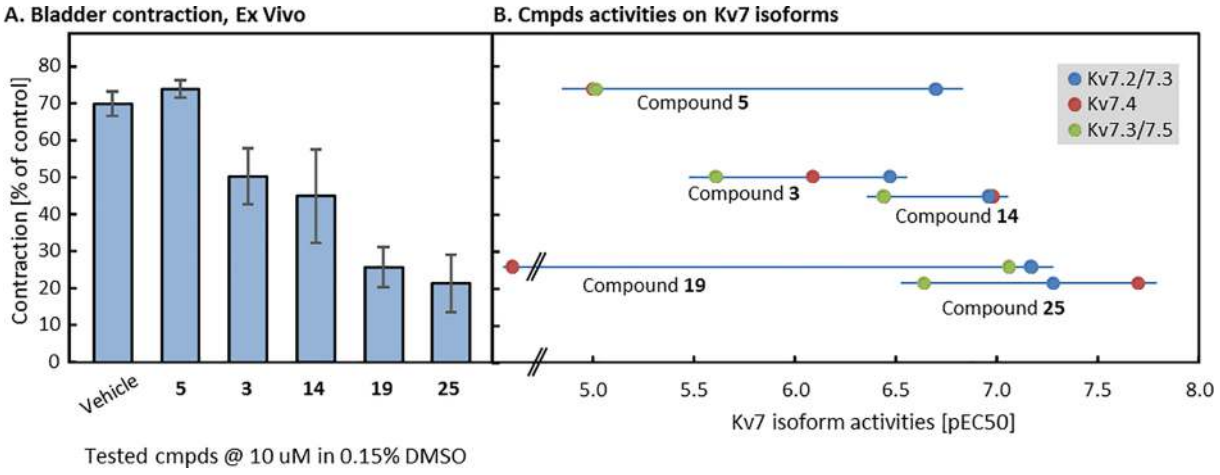


Fig. 4. Effects of K_V7 openers with differential activities on K_V7.2/7.3, 7.4 and 7.3/7.5 isoforms on carbachol-induced contraction in human bladder.

meaningful changes in the Rotarod CNS tolerability model. We found that effects on bladder contraction depend on pan-K_V7 activity, but perhaps not on homomeric $K_V7.4$ activity. Further investigation is required to conclude the roles of individual K_V7 isoforms in bladder contraction.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2018.09.036.

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