CHAPTER 4

Recent Progress in the Discovery of Kv7 Modulators

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1. INTRODUCTION

1.1. Function

The Kv7 ion channels belong to the voltage-gated ion channel superfamily [1–3]. There are to date five known members, Kv7.1–Kv7.5. These potassium-selective channel proteins are found in cardiac tissue (Kv7.1), the central and peripheral nervous system (Kv7.2, Kv7.3, and Kv7.5) and in the inner ear and some auditory nuclei (restricted to Kv7.4) [4–6]. Kv7 channels can homo- or heteromultimerize to form various tetramers

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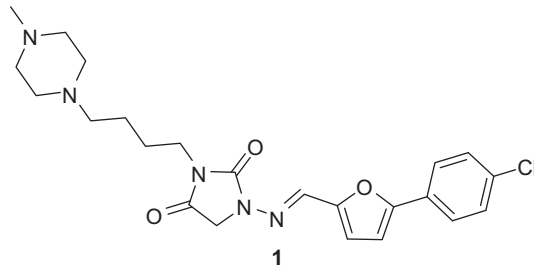
having different pharmacological properties, and can also associate with other auxiliary proteins, further diversifying the biological response. Kv7.2, Kv7.3, and Kv7.5 channels are activated at a lower-threshold membrane potential than required to initiate the firing of neurons (subthreshold activation) and underlie the M current, a non-inactivating current that hyperpolarizes the neuronal membranes. In turn, this has the effect to reduce or prevent the firing of an action potential. Thus, Kv7 ion channels play a crucial role in regulating the excitability of membranes, and in fact, Kv7.2 or Kv7.3 inherited loss-of-function mutations in humans are associated with benign familial neonatal convulsion syndrome [7], whereas Kv7.1 mutations are associated with cardiac arrhythmias and deafness, [8–10] and Kv7.4 mutations with hearing loss [11]. It is only quite recently that these channels, and in particular Kv7.2/Kv7.3, have attracted attention as potential therapeutic targets. Judging by the amount of recent publications, the current main interest consists of finding modulators that have the potential to treat epilepsy and pain.

1.2. Structural biology

All Kv7 channels share a similar structure that is formed by four subunits (homo- or heteromeric tetramers). Each of the tetramers consists of six segments (S1–S6) spanning across the membrane. The S1–S4 fragments act as the voltage-sensitive domain (VSD), wherein the fourth helix that bears many alternating positively charged arginine residues has the prime role, while the S5–S6 fragments form the potassium permeable pore [12–16].

2. Kv7.1 CHANNELS

Kv7.1 channels (KCNQ1) are mainly involved in the repolarization phase and the duration of the cardiac action potential [17]. Together with KCNE1 (MinK), an auxiliary subunit, Kv7.1 associates to form the slow delayed rectifier Iks cardiac potassium current [18]. Human mutations of Kv7.1 are associated with potentially severe conditions, such as short (gain of function) and long QT syndrome (loss of function), and atrial fibrillation (gain of function). As a therapeutic target, the blockade of Kv7.1 channels has attracted some attention, and Azimilide (**1**) has reached phase 3 in clinical development as a dual inhibitor of Iks (Kv7.1/KCNE1) and the rapid delayed rectifier Ikr (Kv11.1) for the treatment of arrhythmias. As far as openers of Kv7.1 are concerned, no recent report is found in the literature, and in fact, the only existing reports date from the late 1990s.



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3. Kv7.2–Kv7.5 CHANNELS

3.1. Function

Subunits Kv7.2 and Kv7.3 coassemble and form a tetramer that underlies the M current [19]. It is noteworthy that other subunits (Kv7.4 and Kv7.5) are also associated, albeit to a lesser extent, with M current characteristics [20,21]. The M current which is activated at a lower-threshold membrane potential than would normally activate neuronal cells, hyperpolarizes the cell membrane, and consequently reduces the firing of action potential. In other words, modulation of these channels may control neuronal excitability. Recognizing that neuronal hyperexcitability is the cause of several clinical disorders such as epilepsy and pain, modulation of these channels represents an appealing approach for the treatment of such conditions.

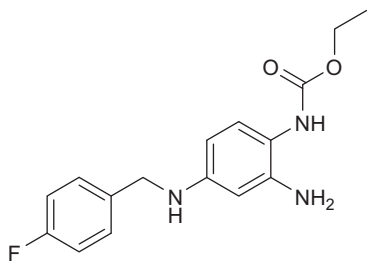
3.2. Binding sites

Even though no crystal structure has been published to date for any of the Kv7 ion channels, mutagenesis, crystallographic, and molecular docking studies have identified two distinct binding sites [13]. The first is located in the pore of the channel, namely in the S5–S6 region, wherein a conserved Tryptophan (Trp 236 in Kv7.2 and Trp 265 in Kv7.3) from Kv7.2 to Kv7.5 was shown to be crucial to maintain sensitivity to retigabine (**2**, *vide infra*), a non-subunit (Kv7.2–Kv7.5)-selective Kv7 activator [22]. Another conserved amino acid (G 301) in the S6-gating hinge region proved to be essential for the efficacy of retigabine [23].

The other binding site is located in the VSD formed by the S1–S4 helices. ICA-27243 (**4**, *vide infra*), a subtype-selective Kv7 activator was shown to bind in this binding pocket [24,25]. More precisely, the compound was shown to bind to the pocket that is formed by at least two helices, S2 and S3. The low degree of sequence homology in this region is consistent with ICA-27243 being a Kv7 subunit-selective compound.

3.3. Compounds in clinical development: Flupirtine, retigabine, and ICA105665

In the recent years, there has been an increased interest in developing positive modulators (activators) of Kv7.2–Kv7.5 channels. The first identified channel opener was retigabine [1,2]. Retigabine is a non-subtype-selective activator of Kv7.2–Kv7.5 ion channels (except Kv7.1) that was shown to be efficacious in a broad range of preclinical seizure models [26]. Recently, this year, retigabine was preapproved as an adjunctive therapy in adult epilepsy patients with partial-onset seizures (POS) [27]. Retigabine was also efficacious in various animal models of pain [28,29]. However, a phase 2a clinical trial aimed at treating pain associated with post-herpetic neuralgia failed to meet the primary end point of pain intensity reduction. The compound is still listed in the company website as being in a phase 2 clinical trial for the treatment of pain (https://www.valeant.com).



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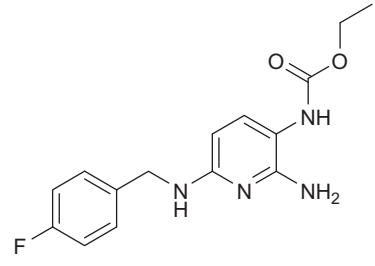

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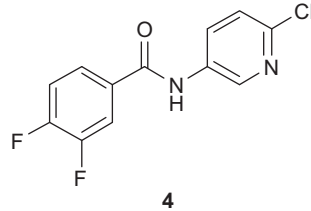
A close structural analog to retigabine that also activates Kv7.2–Kv7.5 channels, flupirtine (**3**), has been on the market for more than two decades in certain countries (Brazil, Estonia, Germany, Italy, Latvia, Lithuania, Portugal, Slovakia, and Russia) for the treatment of various types of pain [30]. Flupirtine is currently in a clinical trial (phase 2) for the treatment of fibromyalgia, the end point being the reduction of musculoskeletal pain and overall symptoms of fibromyalgia (reduction of severity of mood, fatigue, cognitive symptoms, and sleep disturbance). Flupirtine is also being investigated in a clinical trial (phase 2a) for the treatment of neuropathic pain as an adjunct to current opioid drug therapy (https://www.delevarepharma.com).

Although both these compounds modulate Kv7 channels [1,2], they lack Kv7 subtype selectivity (except for Kv7.1). Moreover, flupirtine is also an indirect NMDA receptor antagonist [30], and flupirtine and retigabine have shown GABA A agonistic effects, albeit in 10- to 100-fold higher concentrations than required to activate Kv7 channels [31–34] (plasma concentrations for flupirtine required for analgesic activity

correlate well with *in vitro* activation concentrations of 2.5–6.5 μM). In order to avoid potential side effects, current efforts consist of developing selective Kv7.2–Kv7.3 channel openers. ICA-105665, a potentially more selective compound, is currently in a clinical trial (phase 2) for the treatment of epilepsy. While the clinical trial was suspended in September 2010 following occurrence of serious adverse effects in the high dose group (600 mg), the FDA has since lifted the hold (February 2011). This compound is also being evaluated in a clinical trial for the treatment of pain. Unfortunately, in a phase 1b pain study, the ability of ICA-105665 to decrease the sensation of pain in response to the intradermal injection of capsaicin (the red chili pepper pungent irritant component) or to a UV-simulated sunburn was not observed at the dose tested of 200 mg. As of today, the structure of ICA-105665 has not been revealed.

3.4. Compounds in drug discovery

The relative success of the compounds in the clinic and the discovery of ICA-27243, a subtype-selective Kv7.x channel modulator [35], currently used as a tool compound, have boosted the interest of pharmaceutical companies to develop small molecule activators of Kv7 channels. Several preclinical drug discovery programs have emerged and these efforts can be classified into two main themes. The first consists of using the retigabine/flupirtine scaffold as the starting point for the design of new compounds, and the second uses a more rational drug design approach (starting point identified by compound screening) such as in the discovery of **4**.



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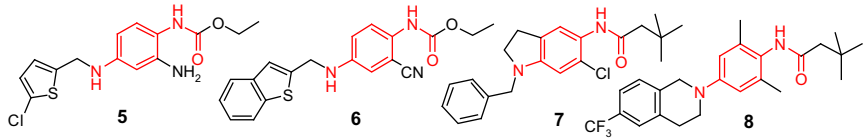
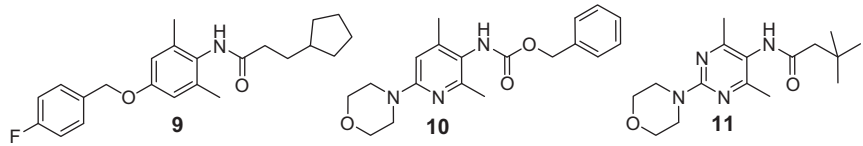
3.4.1. Efforts using retigabine/flupirtine as template

The development of Kv7 openers based upon the retigabine scaffold (**2**) is described elsewhere and only a brief summary will be given here, followed by a complementary update [2]. Generally, the 4-amino-1-carbonylaminophenyl moiety of retigabine is preserved (highlighted in structures **5**–**8**), whereas replacement of the 2-amino group with small substituents such as methyl, halogen or cyano **6**, or/and isosteric

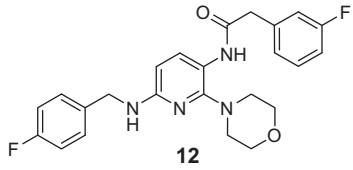
substitution of the benzylamine phenyl in retigabine such as in **5** or **6** proved to be beneficial for improving *in vitro* activity. The amino group in the 4-position has also been explored, and its incorporation into a ring such as in the indoline **7** also improves activity [36,37]. These modifications were all reported to exhibit an $EC_{50} < 2$ μM in a Kv7.2 rubidium flux assay (Rb^+ assay), and many analogs had an $EC_{50} < 200$ nM. Restriction of conformational changes by replacing the flexible benzylamine moiety with the more rigid 1,2,3,4-tetrahydroisoquinoline moiety such as in **8** [38] or with the corresponding naphthyridine analogs [39] brought a substantial increase in the *in vitro* activity, with EC_{50}s < 50 nM in a Kv7.2–Kv7.3 Rb^+ assay.



Substitution of the 4-amino moiety by a 4-ether group as in **9** yielded potent compounds with reported EC_{50}s < 10 nM in the Kv7.2–Kv7.3 Rb^+ assay [40]. It is noteworthy that the use of a second small substituent α to the amide bond such as in **8** and **9** led to compounds with a 10- to 100-fold improvement in potency. The use of flupirtine as starting point for the development of new Kv7 openers has also been fruitful. Keeping the 1,4-diamino pattern intact and replacing the benzylamine with morpholine led to pyridine **10** and pyrimidine **11**, with the best derivatives displaying EC_{50}s < 200 nM in the Kv7.2–Kv7.3 Rb^+ assay [41,42].



More recently, the 2-amino moiety was investigated and introduction of a morpholine such as in **12** led to a 35-nM compound in a thallium-sensitive assay using retigabine as a reference [43]. Furthermore, exchange of the benzylamine with an aliphatic amine such as in **13** (R = tetrahydropyranylmethyl) increases *in vitro* potency, with **13** reported to increase the amplitude of the current by 708% at 0.03 μM in a patch clamp assay using HEK-293 cells expressing Kv7.2–Kv7.3 [44]. Interestingly, incorporation of heteroaromatic five-membered ring carboxamides in place of the flupirtine ethyl carbamate, combined with the modifications previously discussed (*vide infra*), led to **14**, a 20-nM compound in the Tl^+ assay [45,46].



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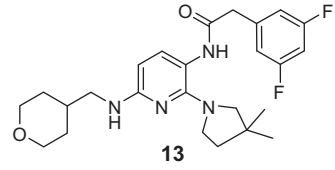

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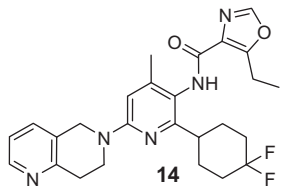

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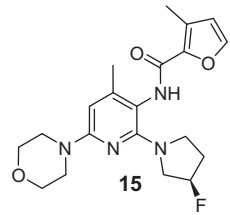

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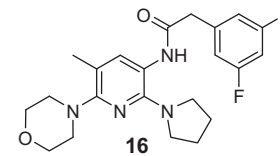

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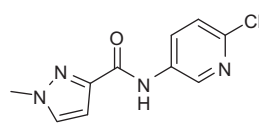
Moving the 4-Me (see **15**) to the 5-position of the pyridine as in **16** led to the most potent compound published to date with an $EC_{50} = 0.32$ nM in a Tl^+-sensitive assay using retigabine as a reference [47]. Of interest, the homomorpholine analogs were also reported active with EC_{50}'s ranging from 0.4 to 270 nM [48–50].

3.4.2. Other Kv7 activators

A very important compound for the discovery of subtype-selective Kv7 openers is ICA-27243 (*vide supra*). This compound is part of a series of compounds consisting of pyridin-3-yl benzamides that were discovered by rational drug design [51,52]. Compound **4** was shown to enhance activation of heteromeric Kv7.2–Kv7.3 but had little effect on homomeric Kv7.1, Kv7.3, and Kv7.4 channels as well as on heteromeric Kv7.3–Kv7.5 channels [24]. Derivative **4** has been extensively studied and was shown to alleviate pain as well as epileptic seizures in many animal models [35,53]. SAR analysis demonstrated that the left-hand difluorophenyl could be replaced by different heterocycles such as the methylpyrazole in **17**. The 2-Cl atom on the pyridine was the most suited substituent for activity, whereas other substitutions on the ring were detrimental for potency. Amide bond bioisosteres such as in indazole **18** or benzisoxazoles were tolerated transformations [54].



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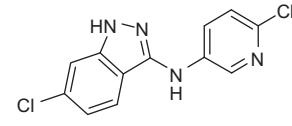

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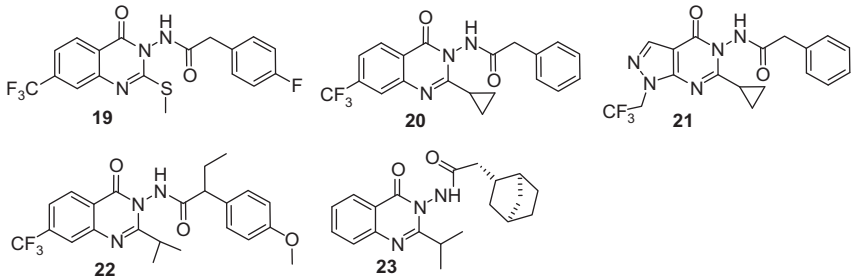
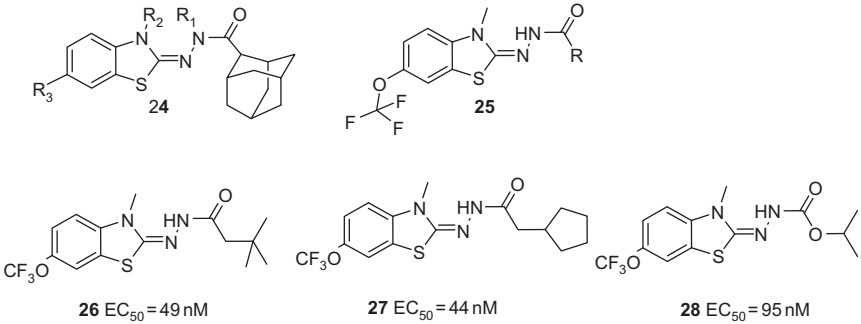
Quinazolinone derivatives were another class of compounds structurally different from the retigabine scaffold. The first series of this class to be

disclosed consisted of 2-thio quinazolinones [55], and the most potent example **19** (*vide infra*) displays an activity of 70 nM against the M current (NG-108 FLIPR assay). The potentially labile thioether group was successfully replaced by a variety of other groups such as alkyl, cycloalkyl, and aryls as in **20**–**23** [56]. The phenyl quinazolinone core could be modified to the more polar pyrazolopyrimidinone core as in **21** with a reported $EC_{50} < 50$ nM [57]. The linker between the amide and the phenyl group has been extensively explored [58,59] and led to ethyl derivative **22**, which enhances the current amplitude by 187% at 3 μM. In a more recent example, different central scaffolds were explored, however, the most potent compounds reported kept the quinazolinone in place, with the key modification being replacement of the benzylic moiety with a bicyclic aliphatic group [60]. This compound (**23**) has an EC_{50} of 17 nM against Kv7.2–Kv7.3 in a Tl^+ assay.



A different series of compounds with yet another modified central core, but retaining the acyl hydrazine moiety, was identified from an High Throughput Screening (HTS) effort [61]. The first identified hit (**24**, R1 = H, R2 = Me, R3 = H) had an EC_{50} of 27 nM against Kv7.2–Kv7.3 channels in a Rb^+ isotopic efflux assay and was 100-fold-selective against the Kv7.1–KCNE1 channel. This agonist exhibited anticonvulsant properties in epileptic animal models when administrated intraperitoneally, but due to poor bioavailability, ceased to show effects when given orally. Thus, the first chemistry efforts were aimed at evaluating the impact of small substituents on both activity and bioavailability. The different positions of the aryl substituents were evaluated and the only variation that showed both good *in vitro* EC_{50} (25 nM in the Rb^+ efflux assay) and *in vivo* efficacy (epileptic animal model) when given orally contained an OCF_3 at the R3 position (see **24**, R3 = OCF_3). It is noteworthy that methylation at the R1 position or replacement of the sulfur by an oxygen atom led to substantial loss of activity ($EC_{50} > 10$ and 2.7 μM, respectively). The medicinal chemistry effort then concentrated on the replacement of the adamantane moiety to give compounds of structure **25**. In particular, compounds **26**, **27**, and **28** proved to have good *in vitro* activity (*vide infra*) and *in vivo* efficacy in anticonvulsant assays (mouse maximal

electroshock seizure (MES) and rat subcutaneous pentylenetetrazol seizure models) with ED_{50} values <10 mg/kg. These analogs were also efficacious in animal pain models. In fact, the effect of these compounds was comparable and similar to the effect of retigabine in the rat formalin and spinal nerve ligation (SNL) assays. Moreover, **28** demonstrated a complete reduction of tactile allodynia in the SNL model when orally administrated at 10 mg/kg, whereas retigabine had no effect at the same dose.



The imidazolopyridine scaffold (**29**) was disclosed as a subtype-selective Kv7 agonist [62]. An extensive SAR exercise was conducted, and while small electronegative substituents at the R2 position proved to be beneficial for activation of the Kv7.2–Kv7.3 channels, substituents at the R1, R3 and R4 positions were detrimental (see Figure 1). Variation at positions R5 and R6 has been widely investigated, and the best groups are highlighted in Figure 1.

Compounds **30** and **31** were reported to have EC_{50}s < 500 nM in a fluorometric (imaging plate reader) assay and showed protective effects in the rat MES model. Compounds **30** and **31** exhibited potency in a nerve injury model with reported ED_{50}s of 10 and 1.9 mg/kg, respectively; **31**

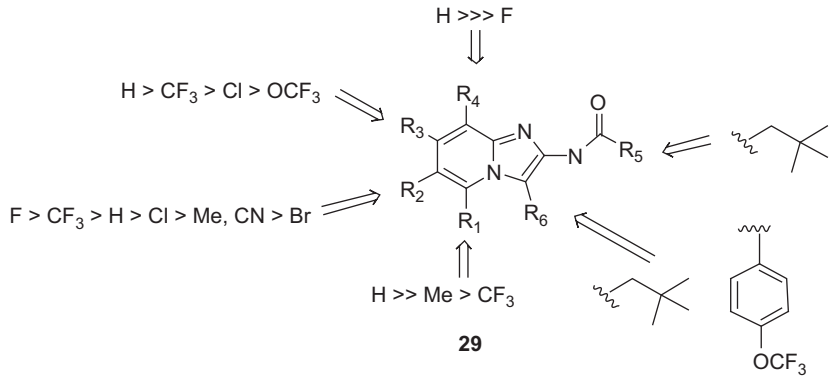
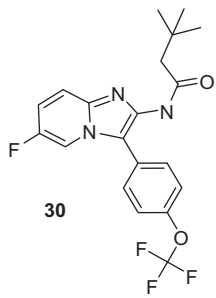


Figure 1 SAR of the imidazolopyridine series.

demonstrated potency in the carrageenan inflammatory pain model with an ED_{50} of 0.5–1 mg/kg. Further examples were disclosed [63] and revealed that the metabolic soft spot on the neopentyl moiety could be addressed by fluorination (see example **32**).



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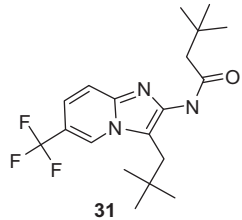

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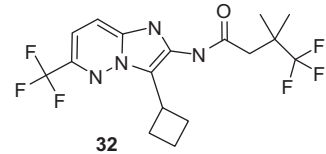

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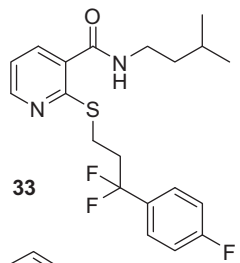
Other scaffolds were recently reported as subtype-selective agonists of Kv7.2–Kv7.3 (compounds **33**–**36**). Pyridine derivative **33** was shown to be active in an *in vitro* fluorometric assay with an EC_{50} of 224 nM, as well as *in vivo*, in a low-intensity tail flick rat model with an $ED_{50} = 2.7$ mg/kg [64]. The quinoline derivative **34** proved to have the exact same *in vivo* potency [65–69]. The fused bicyclic dihydropyrrolopyrazine analog **35** was also shown to exhibit activity *in vitro* with an EC_{50} of 2.4 nM in a fluorometric assay and *in vivo* to alleviate pain in the rat formalin model [68]. Recently, a pyrazolopyrimidinone core was disclosed and the most potent compound (**36**) displayed an EC_{50} of 60 nM in a Rb^+ assay. However, no *in vivo* experiments have been reported for these analogs [70].



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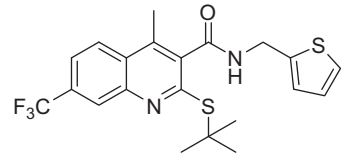

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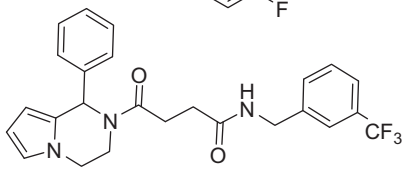

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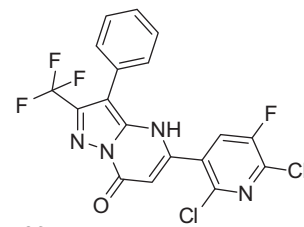

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4. CONCLUSION

Kv7 channels play a central role in regulating several critical cell functions, such as regulation of the heart beat or modulation of the neuronal activity. It is thus understandable that as therapeutic targets, this class of ion channels has attracted attention. The identification of Kv7 human mutations that lead to heart disorders (Kv7.1) and to neonatal familial epilepsy (K7.2 and Kv7.3) has raised hope in finding new treatments in cardiology and epilepsy. The advancement of retigabine and ICA-105665 in the clinic is proving the validity of the target. It is somewhat more recent that Kv7 channels have shown potential for the treatment of pain. Flupirtine, a non-opoid analgesic on the market for more than 20 years has paved the way for others to follow. Moreover, the discovery of subtype-selective activators (*e.g.*, ICA-27243) has constituted another milestone in the search for analgesics having fewer side effects. However, despite strong support from animal studies, there are still no data supporting translation to the clinic. It seems more than likely that a better understanding of the Kv7 pharmacology and the translational science will be required to address some of these issues.

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