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Discovery of a novel Kv7 channel opener as a treatment for epilepsy

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ABSTRACT

Facilitating activation, or delaying inactivation, of the native Kv7 channel reduces neuronal excitability, which may be beneficial in controlling spontaneous electrical activity during epileptic seizures. In an effort to identify a compound with such properties, the structure–activity relationship (SAR) and in vitro ADME for a series of heterocyclic Kv7.2–7.5 channel openers was explored. PF-05020182 (**2**) demonstrated suitable properties for further testing in vivo where it dose-dependently decreased the number of animals exhibiting full tonic extension convulsions in response to corneal stimulation in the maximal electroshock (MES) assay. In addition, PF-05020182 (**2**) significantly inhibited convulsions in the MES assay at doses tested, consistent with in vitro activity measure. The physiochemical properties, in vitro and in vivo activities of PF-05020182 (**2**) support further development as an adjunctive treatment of refractory epilepsy.

Five genes of the KCNQ gene family (KCNQ1-5) encoding the voltage-gated delayed rectifier K+ channels Kv7.1–Kv7.5 have been associated with inherited diseases.[1] Mutations in either KCNQ2 (encoding Kv7.2) or KCNQ3 (encoding Kv7.3) genes cause the dominantly inherited epileptic syndrome referred to as benign familial neonatal seizures (BFNS),[2] and sequence analysis has identified mutations in KCNQ2 or KCNQ3 genes in 60–70% of families with BFNS. The disorder is characterized by clusters of seizures occurring in the first days of life, and while most patients spontaneously remit by 24 months of age, 10–15% of individuals with BNFS develop epileptic seizures later in life.[3] Additionally, mutations in KCNQ2 are associated with peripheral nerve hyper excitability (PNH), neuromyotonia, and myokimia.[4]

A recent review highlights the therapeutic potential for Kv7 channel openers in epilepsy and psychiatric disease, and supports a profile of selective activity at Kv7.2/3, as opposed to Kv7.4, channels.[5] The known Kv7 channel opener retigabine (**1**) is active at all neuronal Kv7 channels with limited selectivity between the subtypes (Kv7.2–7.5), as well as significant activation of GABA(A) receptors.[6] Given its pre-clinical anticonvulsant activity and its clinical activity in treating epilepsy, it was recently approved as an adjunctive treatment of partial-onset seizures in adults with epilepsy.[7] In order to improve upon some perceived liabilities associated with **1**, including its lack of Kv7 selectivity and the aniline structural alert,[8] we investigated alternative core structures as well as replacements for the primary amine. We report herein a series of dimethoxy-pyrimidines characterized by PF-05020182 (**2**), that exhibit potent Kv7.2/7.3 channel opener activity, suitable for exploration as an anti-epileptic agent (Fig. 1).

Since certain idiosyncratic adverse drug reactions (IADRs) can be triggered by electrophilic protein-reactive metabolites that are formed in the process of drug metabolism, our design strategy centered on removing the aniline structure alert by replacing the core structure with a heterocycle. A 4,6-dimethoxypyrimidine core substituted with an amide (vs a carbamate) at the 5-possition was the optimal replacement based on a combination of potency and physical chemical properties. Compounds in this series are predicted to

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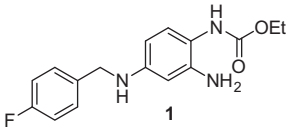


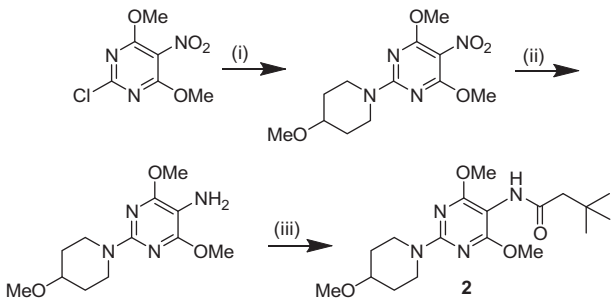
Figure 1. Structure of Kv7 channel opener retigabine (**1**).

be in good CNS drug like space (CNS MPO >5)[9] and were synthesized according to the approach outlined in Scheme 1. S_nAr addition into the known 2-chloro-4,6-dimethoxy-5-nitropyrimidine followed by reduction and acylation affords compound **2** in high yield. The remaining compounds were prepared by similar chemistry and are listed in Tables 1 and 2, along with their Kv7.2/7.3 EC_{50} values, % maximum activity, and CNS MPO.

In an effort to select the compounds with the best drug like properties we chose to progress compounds with an EC_{50} <500 nM, LipE[10] >3 (Fig. 2). Based on these criteria, 9 of the 18 compounds were further profiled via in vitro ADME and safety assays as shown in Table 3. For context, retigabine (**1**) had an EC_{50} of 536 ± 7 nM in our assay, LipE of 3.4, and CNS MPO of 5.00.

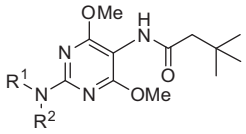
Permeability in the presence and absence of the efflux transporter protein P-glycoprotein (P-gp) was assessed in the RRCK and MDCK-MDR1 transwell assays.[11] All compounds exhibited high passive permeability and P-gp efflux ratios congruent with high brain availability. Intrinsic clearance and cytotoxicity was measured by the human liver microsomes (HLM) assay[12] and ATP depletion in the transformed human liver epithelial (THLE) cell line,[13] respectively. Compounds **3** and **6** had high intrinsic clearance in HLM whereas **14** showed rapid non-metabolic decline in HLM in the absence of nicotinamide adenine dinucleotide phosphate (NADPH) and measureable risk in the THLE toxicity assay. Further filtering by HLM clearance provided a handful of compounds with the right balance of properties from which compound **2** emerged as the most promising for further profiling.

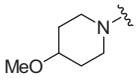
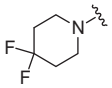
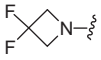
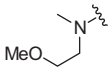
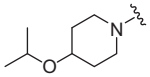
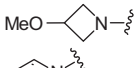
Detailed profiling of compound **2** is shown in Table 4. Functional activity was examined at several related Kv7 family voltage-gated potassium channels. Results revealed greater functional enhancement of Kv7.2/7.3 channel current than of Kv7.4. Compound **2** was selective for the neuronal channels (Kv7.2/7.3, Kv7.4, and Kv7.3/7.5) over the cardiac Kv7.1/KCNE1 channels,[15] providing a presumed safety margin against possible cardiac effects. Only a small reduction in current was seen in the cardiac channels at up to 100 μM. Table 4 reports very similar potencies and efficacies were seen with compound **2** in both human and rat channels. In addition to its on-target activity, compound **2** did not exhibit any significant activity on an array of targets assessed in vitro against a broad panel of pharmacological targets including GABA.[16] The improved selectivity of compound **2** when compared



Scheme 1. Preparation of compound **2**. Reagents and conditions: (i) 4-methoxypiperidine, $EtN(iPr)_2$, THF, 88%; (ii) H_2, 20% Pd-C, MeOH; (iii) 3,3-dimethylbutanoyl chloride, Et_3N, EtOAc; 79% over two steps.

Table 1
SAR for amino analogues in the dimethoxypyrimidine series



Compd	NR^1R^2	Kv7.2/7.3, EC_{50}[a] (nM)	Kv7.2/7.3 % Max[b]	CNS MPO
2		334 ± 48	147 ± 6	5.74
3		24 ± 2	92 ± 4	5.43
4		50 ± 4	99 ± 2	5.64
5		62 ± 7	85 ± 4	5.83
6		64 ± 9	182 ± 10	5.24
7		110 ± 9	103 ± 3	5.52
8		164 ± 11	95 ± 4	5.83
9		206 ± 42	166 ± 23	5.45
10		358 ± 47	145 ± 11	5.10
11		961 ± 356	103 ± 6	5.83
12		1887 ± 347	101 ± 6	5.83
13		9187 ± 1590	106 ± 9	5.83

[a] EC_{50} value with SEM.
[b] % maximum activation with SEM.

to retigabine includes a decreased potential for modulation of GABAergic activity.[6b]

These combined attributes warranted further characterization of compound **2** in vivo in a rat corneal maximal-electroshock-induced (MES) model,[17] a well validated preclinical test that predicts drugs effective against generalized seizures of the tonic–clonic (grand mal) type.[18] The model measured a compound's ability to protect against convulsions triggered by shock via corneal electrodes (60 Hz sinusoidal current; 150 mA; 0.2 s). Rats were scored for the presence or absence of maximal seizures of their hind limbs, with tonic extension as the endpoint of the test. A rat was considered fully protected from convulsion if it did not have a full hind-limb extension. Plasma and brain samples were collected from the animals and the concentration of compound was measured from both compartments.[11a] Binding to rat plasma and/or rat brain was determined by a previously described equilibrium dialysis method:[19] rFu plasma 0.184 and rFu brain 0.130. As indicated in Figure 3 and Table 5, compound **2** readily crosses the blood brain barrier and is effective in the MES model at free brain and plasma concentrations below its rat Kv7.2/7.3 EC_{50} thus demonstrating that **2** has efficacy in an epilepsy model.