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Design and evaluation of pyrazolopyrimidines as KCNQ channel modulators

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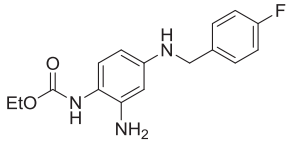
ABSTRACT

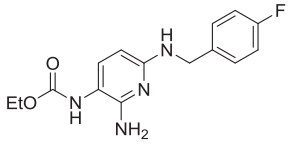
Effective treatments of neuropathic pain have been a focus of many discovery programs. KCNQ (kv7) are voltage gated potassium channel openers that have the potential for the treatment of CNS disorders including neuropathic pain. Clinical studies have suggested agents such as Retigabine to be a modulator of pain-like effects such as hyperalgesia and allodynia. In this paper, we describe the discovery and evaluation of a series of novel pyrazolopyrimidines and their affinity for potassium channels KCNQ2/3. These pyrazolopyrimidines have also shown good efficacy in the capsaicin-induced acute and secondary mechanical allodynia model and excellent pharmacokinetic properties, which may be superior to Retigabine.

Potassium channels are membrane-bound proteins responsible for regulating the flow of potassium ions through a cell membrane. The KCNQ (or K_v7) family is an important class of potassium channels that play a key role in the control of neuronal excitability.[1] The drug Flupirtine had been used as an analgesic for several decades while its mechanism of action was not known. However, it was later identified as a KCNQ2/3 channel opener,[2] a subtype of the KCNQ family. Flupirtine, a drug commonly used for post-surgical pain and other nociceptive pain states, was later found to reduce fibromyalgia pain.[3] The antiepileptic drug Retigabine is a closely related KCNQ2/3 channel opener and has also shown good efficacy in preclinical models of diseases associated with neuronal hyperexcitability.[4] Although both Flupirtine and Retigabine are effective analgesics, their efficacy is associated with side effects including dizziness and nausea with several additional side effects at elevated doses. In addition, the side effects of these two KCNQ openers may be attributable to known off-targets as they exhibit relatively poor selectivity versus other KCNQ subtypes.[5–9]

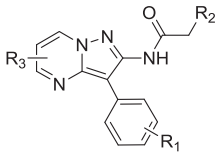
Our Hit-to-Lead strategy was focused on identifying novel, orthogonal and quality lead series with a clear SAR that demonstrated efficacy in the inflammatory and neuropathic pain models. In addition, the series had to possess an improved safety profile over existing KCNQ channel openers. Multiple chemical series of potent KCNQ2/3 openers were identified from an HTS campaign. The triage process led to the identification of a large number of hits that were clustered by structural types into multiple series including the pyrazolopyrimidine series **1**. To identify novel KCNQ2/3 channel openers in a high-throughput format, a FLIPR-based assay using thallium (Tl^+) influx was developed. Human KCNQ2/3 cDNAs were cloned and a HEK293 cell line stably expressing KCNQ2/3 was constructed. In the assay, thallium passes through the KCNQ channel and binds to a thallium-detecting dye previously loaded into the cells. When thallium enters the cells, an increase in fluorescence occurs which reflects KCNQ channel activity. Thallium influx is dependent on the expression of KCNQ2/3 and the resulting fluorescence signal can be enhanced by known KCNQ2/3 openers (e.g. Retigabine and Flupirtine). Our compound collections were screened as mixtures using the KCNQ2/3-mediated thallium influx assay in 384-well format. The identified hits were confirmed by a retest and the EC_{50} values were calculated using multipoint concentration-response curves. A series of pyrazolopyrimidines were chosen based on their favorable potency and physiochemical properties.



Flupirtine



Retigabine



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Multiple compounds of structural type **2** were identified during the HTS triage and subsequently led to the identification of compound **3** as an excellent KCNQ lead molecule (Fig. 1). Compound **3,** with KCNQ2/3

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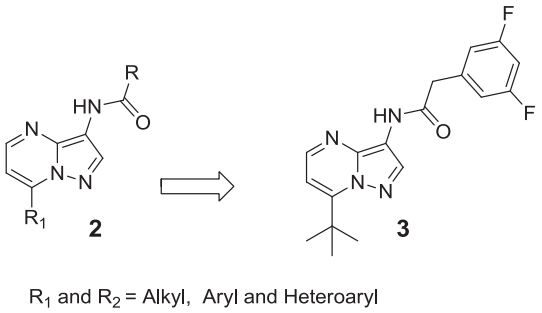


Fig. 1. Optimization of HTS hits led to compound **3**.

Table 1
Compound **3** compared to Retigabine.

Compounds	KCNQ2/3 EC_{50} (μM)	KCNQ2/3 Max Control (%)[a]
3	0.91	149
Retigabine	0.56	112
Flupirtine	4.38	99

[a] % efficacy relative to that of 10 μM Retigabine.

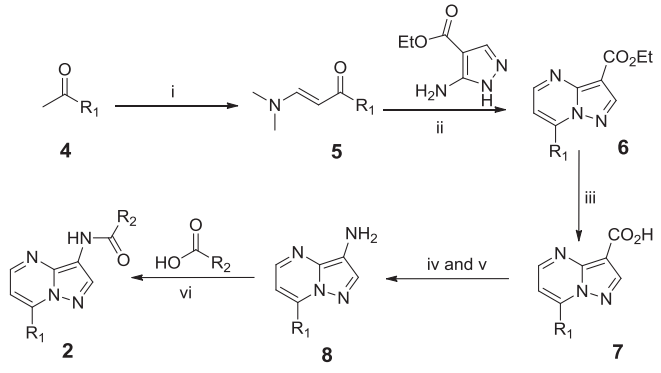
activity of 0.91 μM, compared favorably to Retigabine and Flupirtine (Table 1) and was considered an excellent starting point for further investigation.

The general synthesis of compounds **2** is shown in Scheme 1. Condensation of ketones **4** with DMF-DMA afforded the corresponding enones **5**, which were reacted with ethyl 5-amino-1H-pyrazole-4-carboxylate under acidic conditions to give the pyrazolopyrimidine esters **6**.[10] The esters **6** were converted to the acids **7** followed by a Curtius rearrangement reaction and a deprotection step to afford the amines **8**. The amines **8** were then subjected to amide coupling conditions with various acids to yield compounds **2**.

Further chemistry efforts focused on the optimization of compound **3** where considerations were given to modifications of the substitution patterns around the pyrazolopyrimidine core (Fig. 2). The substituents R1 and the entire amide group bearing R2 were moved around the ring system as shown in compound **9**. This exercise afforded the 2,3-substitued pyrazolopyrimidines **10** as a group of novel KCNQ2/3 modulators.

Structural investigation of scaffold **10** revealed the optimal R2 to be 4-substitued phenyls, with 4-triflouromethyl and 4-fluoro phenyls being the most active analogs. Additionally, alkyls and cycloalkyls were the optimal R2 representatives. R3 substituents were introduced to the scaffold as represented in structure **11**. However, no substituents larger than a methyl group were tolerated.

Compounds **11** were easily assessable via an efficient 5-step



Scheme 1. Synthesis of compounds **2** i) DMF-DMA, Et_3N, 95 °C, ii) AcOH, 100 °C, iii) 1 N NaOH, THF, 65 °C, iv) diphenylphosphoryl azide, Et_3N, 110 °C, v) 4 N HCl in 1,4-dioxane, vi) HATU, DIEA, DCM, 25 °C.

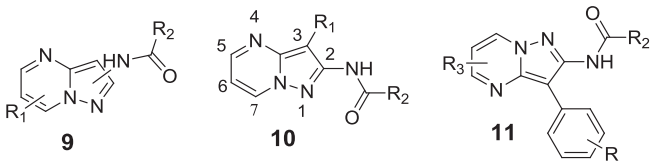
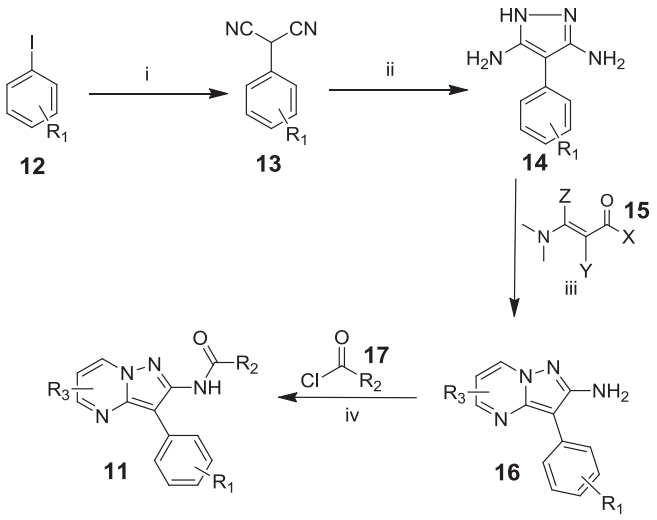


Fig. 2. 2,3 pyrazolopyrimidines as KCNQ2/3 modulators.



Scheme 2. Synthesis of compounds **11** i) malonitrile, bis(triphenylphosphine) palladium(II) chloride, NaH, THF, 0 °C to reflux, ii) hydrazine monohydrate, BuOH, 125 °C, iii) 15, AcOH, EtOH, 140 °C, iv) 17, pyridine, DCM, 25 °C.

convergent synthesis as shown in Scheme 2. Palladium catalyzed cross-coupling of aryl iodides **12** with malononitrile gave the corresponding phenylmalononitriles **13**, followed by a reaction with hydrazine to give the pyrazole-3,5-diamines **14**. The pyrazole-3,5-diamines were then reacted with various substituted enones **15** (installation of R3 substituent) to afford **16**. Numerous compound libraries of compound **11** were generated. Representative SAR for compound **18** (R3 = H, Fig. 3) is shown in Table 2.

The general SAR on the pyrazolopyrimidines shows that hydrophobic groups are well tolerated for R1 and R2. 4-substitued phenyls consistently gave good potency. The 4-triflouromethyl group gave consistent and robust activity across a variety of R2 substituents with the ethyl-cyclopentane compound **32** showing a superior potency of 0.05 μM amongst the initial group of compounds studied at the Hit-to-Lead stage. The 4-fluoro (**26**) and 4-trifluromethoxy (**29**) analogs showed reasonable KCNQ2/3 activity, at 0.14 μM and 0.31 μM, respectively. Although the unsubstituted phenyl compound **19** was inactive, the introduction of larger R2 groups such as methyl-cyclohexane (**21**) and ethyl-cyclohexane (**22**), gave good activity of 0.29 μM and 0.13 μM, respectively. In addition, the inactivity of compounds **20** and **24** revealed the need for the R2 substituent to have at least one methylene spacer when compared to compounds **21** and **25**.

Further investigation to probe the pyrazolopyrimidine SAR by substituting around the core gave further improvements of KCNQ2/3 activity. As mentioned earlier, substituents larger than a methyl group in any of the 5, 6 or 7 positions gave significant reduction in potency (Fig. 4).

For example, compound **33**, with a *t*-butyl substitution at the 7 position, exhibits an EC_{50} of > 32 μM. Representatives of compound **34** with methyl substitutions at the 5, 6 and 7 positions are shown in Table 3. In general, methyl substitution at the 6 position or 5,7-dimethyl substitution appears to significantly boost potency when compared to their corresponding unsubstituted analogs. Compounds **35** and **38** with no 5, 6 or 7 substitutions are generally weaker than their 6-

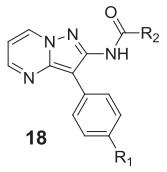


Fig. 3. Compound 18 SAR.

Table 2
SAR analysis of compound **18**.

Compound	R1	R2	KCNQ2/3 EC_{50} (μM)	KCNQ2/3 Max Control (%)[a]
19	H		–	26
20	H		–	8
21	H		0.29	133
22	H		0.13	137
23	F		6.8	109
24	F		> 32	20
25	F		1.0	100
26	F		0.14	139
27	OCF3		0.41	143
28	OCF3		0.19	88
29	OCF3		0.31	114
30	CF3		0.14	113
31	CF3		–	28
32	CF3		0.05	115

[a] % efficacy relative to that of 10 μM Retigabine.

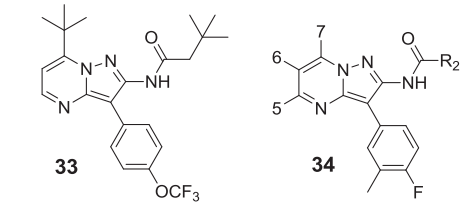


Fig. 4. Substituted pyrazolopyrimidines SAR.

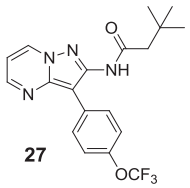


Fig. 5. Compound **27** pharmacokinetics CLp (iv, 1 mpk) = 0.8 L/h/kg, $t_{1/2}$ (iv, 1 mpk) = 1.9 h, F (po, 1 mpk) = 56%, AUC (po, 1 mpk) = 706 ng-h/ml, Brain/Plasma = 3.8.

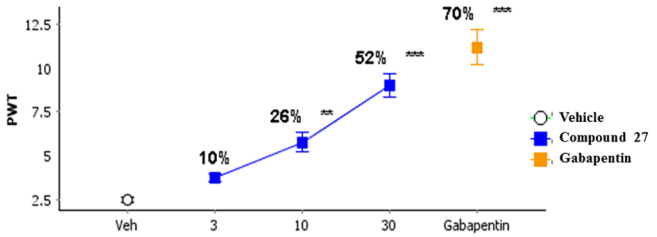


Fig. 6. Effects of Compound **27** (KCNQ, ip) on Capsaicin – SMH Compound 27 (KCNQ, ip) at 3, 10, and 30 mg/kg in 10% DMSO/PEG, 2 mL/kg 30 min pretreatment time. Gabapentin at 100 mg/kg in water, ip 2 mL/kg. PWT = Paw Withdrawal Threshold. **p < 0.1, ***p < 0.001 vs vehicle (n = 6).

substituted counterparts, compounds **36** and **39**. Furthermore, in addition to the observed boost in potency from compound **35** to 6-methyl analog **36**, a much larger shift in activity was observed with the 5,7-dimethyl substituted analog **37** when compared to its unsubstituted counterpart (**35**). An additional upward shift in the Max control at 177% relative to Retigabine was also observed in conjunction with an improved EC_{50} value for the 5,7-dimethyl substituted analog **37**. When the ethyl-cyclopentane group at R2 was combined with the 6-methyl and 5,7-dimethyl substitutions to create compounds **42** and **43**, the expected positive shift in potency was observed. Most notably, the di-substituted compound **43** exhibited a KCNQ2/3 EC_{50} of 0.07 μM and a Max control of 205%, which are significant improvements over Flupirtine and Retigabine.

At an early phase of our Hit-to-lead efforts, we identified compound **27** for further evaluation due to its favorable pharmacokinetic properties (Fig. 5). Although compound **27** had a modest KCNQ2/3 EC_{50} of 0.4 μM, it exhibited a superior Max control of 139%. The combination of its good plasma clearance of 0.8 L/hr/kg, favorable oral bioavailability of 56% and high brain to plasma ratio of 3.8, made this compound a candidate for further evaluation in the *in vivo* capsaicin-induced secondary mechanical hyperalgesia (SMH) inflammatory pain model.[11–13]

Table 3
SAR analysis of compound **34**.

Compounds	5-position	6-position	7-position	R2	KCNQ2/3 EC_{50} (μM)	KCNQ2/3 Max Control (%)[a]
35	H	H	H		2.31	135
36	H	CH3	H		0.59	159
37	CH3	H	CH3		0.05	177
38	H	H	H		1.17	139
39	H	CH3	H		0.14	168
40	CH3	H	CH3		0.07	165
41	H	H	H		0.18	169
42	H	CH3	H		0.08	99
43	CH3	H	CH3		0.07	205

[a] % efficacy relative to that of 10 μM Retigabine.

The capsaicin-SMH result shows that compound **27** produced effects of 10%, 26% and 52% at 3, 10 and 30 mg/kg, respectively. The dose-dependent response was statistically significant at 10 and 30 mg/kg when compared to the positive control Gabapentin (100 mg/kg), which produced a 70% effect in this study (Fig. 6).

In conclusion, the Hit-to-lead campaign successfully identified a novel pyrazolopyrimidine series as KCNQ channel openers. Several compounds, including compound **27**, have shown favorable pharmacokinetic properties and the desired pharmacological effects in several inflammatory and neuropathic pain models. These compounds represent promising leads for novel pain treatment with minimal abuse potential.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.08.007.

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