Identification and Biological Characterization of 6-Aryl-7-isopropylquinazolinones as Novel TRPV1 Antagonists that Are Effective in Models of Chronic Pain

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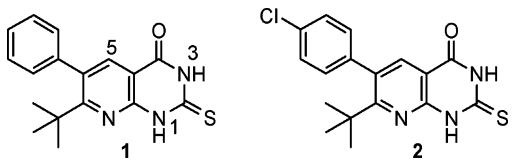
Abstract: Vanilloid receptor 1 (VR1, TRPV1) is a cation-selective ion channel that is expressed on primary afferent neurons and is upregulated following inflammation and nerve damage. Blockers of this channel may have utility in the treatment of chronic nociceptive and neuropathic pain. Here, we describe the optimization from a high throughput screening hit, of a series of 6-aryl-7-isopropylquinazolinones that are TRPV1 antagonists in vitro. We also demonstrate that one compound is active in vivo against capsaicin-induced hyperalgesia and in models of neuropathic and nociceptive pain in the rat.

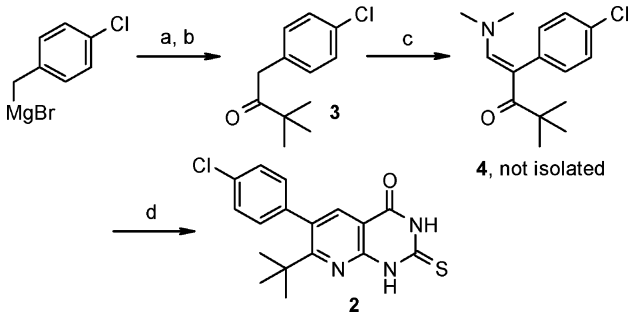
Vanilloid receptor 1 (VR1, TRPV1) is the best known member of the transient receptor potential ion channel family, a group of channels that are widely expressed in mammalian tissues and have a key role in sensory processes. TRPV1 is present on polymodal primary sensory neurons and is activated by low pH, noxious heat, and chemical mediators such as capsaicin (the pungent principle of chili peppers) and resiniferatoxin, an extreme irritant from the sap of *Euphorbia resinifera*.[1] Studies on vanilloid receptors have been facilitated by the discovery in these laboratories of capsazepine,[2] a competitive antagonist of capsaicin-induced responses. The ability of capsazepine to inhibit responses to capsaicin has been used as a diagnostic feature in vanilloid research and played an important role in the characterization of the cloned vanilloid receptor.[3]

Evidence suggests that TRPV1 is a key integrator of the pain response because it is upregulated following inflammation and nerve damage.[4,5] Chronic pain sufferers endure a debilitating condition for which there is often no satisfactory treatment. Neuropathic pain, in particular, is poorly served by standard therapies—NSAIDs, opioids, and tricyclic antidepressants either work poorly or not at all, or produce unacceptable side effects.[6] Even the current gold standard treatment, the anti-convulsive agent gabapentin, requires a relatively high, carefully titrated dose, is ineffective in some cases, and leads to side effects such as dizziness and somnolence in some patients.[7] Hence, there remains a great medical need for new medicines for the treatment of pain.

Chart 1. Pyridopyrimidine Hit and Close Analogue



Scheme 1. Synthesis of Pyridopyrimidines[a]



[a] (a) Pivalonitrile, xylene, reflux, 18 h (77%); (b) 2M HCl, reflux (78%); (c) $tBuOCH(NMe_2)_2$, 110 °C (quantitative by NMR); (d) 6-amino-2-thioxo-2,3-dihydro-1H-pyrimidin-4-one, AcOH, EtOH, water (1:1:8), reflux, 18 h (29% after recrystallization).

Since the cloning of TRPV1 by Caterina et al.,[3] there has been an upsurge in research activity in the TRP channel area. Capsazepine[8] and other small molecule TRPV1 antagonists[9,10] have been shown to be antihyperalgesic in animal models of chronic pain, opening a new avenue in the search for innovative pain drugs.[11,12] In this letter, we divulge a new class of small molecule TRPV1 antagonists, illustrating their evolution from a high throughput screening hit and their characterization as novel antihyperalgesic agents, effective against both nociceptive and neuropathic pain states.

The pyridopyrimidine derivative **1**[13] (Chart 1) was identified as a hit from a high throughput screen for blockers of capsaicin activation of the TRPV1 receptor. This compound inhibited the Ca^{2+} influx evoked by capsaicin in vitro in both human (IC_{50} 100 ± 18 nM) and rat (IC_{50} 90 ± 16 nM) TRPV1-expressing cells and was also able to block low pH activation of human (IC_{50} 49 ± 13 nM) and rat (IC_{50} 25 ± 10 nM) TRPV1 responses.

A preliminary appraisal of **1** showed that replacement of the thione with oxo led to a >5-fold loss of potency in the human low pH assay[17] (IC_{50} 716 ± 166 nM). Replacing the ^{t}butyl group with ethyl while retaining the thione gave an approximately equipotent analogue (IC_{50} 172 ± 59 nM). N-1 of **1** could be methylated without loss of activity, but methylation of N-3 gave inactive compounds. Removal of the phenyl ring at C-6 was detrimental. It became clear that the 2-thioxo-2,3-dihydro-1H-pyrido[2,3-d]pyrimidin-4-one core was optimal and that a *tert*-butyl group in the 7-position was best. Potency could be enhanced by suitable substitutions to the C-6 aryl ring, and it was through changes to this region that we identified **2**, the synthesis of which is shown in Scheme 1.

Reaction of pivalonitrile with 4-chlorobenzylmagnesium bromide followed by acidic hydrolysis of the imine intermediate gave ketone **3**.[14] This was reacted with Bredereck's reagent to give the enamine **4**, which could be combined with commercially available 6-amino-2-thioxo-2,3-dihydro-1H-pyrimidin-

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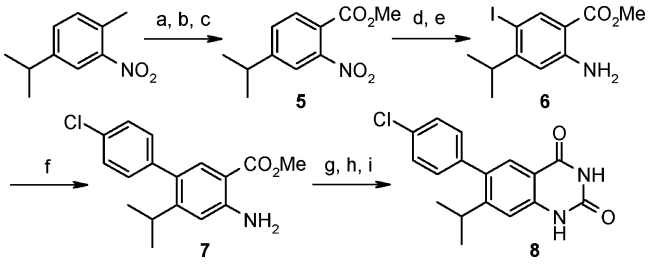
Scheme 2. Synthesis of Quinazolinedione **8**[a]



[a] (a) $tBuOCH(NMe_2)_2$, 110 °C (quantitative, product not isolated); (b) $KMnO_4$, KOAc, tBuOH/water (93%); (c) $(COCl)_2$, DCM, cat. DMF then MeOH (96%); (d) H_2, 10% Pd on C (100%); (e) I_2, Ag_2SO_4, EtOH (87%); (f) 4-chlorophenylboronic acid, $Pd(Ph_3P)_4$, Na_2CO_3, water/DMF, 80 °C (75%); (g) diphosgene, DCM, RT; (h) ammonia solution (sg 0.88); (i) NaOH, MeOH/water, reflux (80% overall).

4-one to give **2** in moderate overall yield. **1** was prepared by the same procedure but using benzylmagnesium bromide.

4′-Chloro substitution of the pendant phenyl ring of **1** to give **2** increased potency to provide a functional antagonist of human TRPV1 expressed in CHO cells when activated by low pH (IC_{50} 18 ± 1 nM). Compound **2** had no agonist activity in a $^{45}Ca^{2+}$ uptake assay.[15]

Compound **2** displayed suboptimal physicochemical and pharmacokinetic properties—in particular, exceedingly low thermodynamic aqueous solubility (0.15 mg/L at pH 6.8, 25 °C), a relatively long half-life in the rat ($t_{0.5}$ 7.5 h; 3 mg/kg p.o. in 20% cremophor EL/pbs) which might translate into an unacceptably long half-life in man, and low micromolar interactions with several members of the cytochrome P_{450} family.

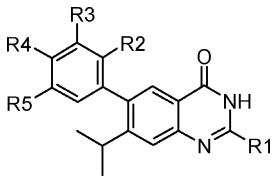
We decided to attenuate the half-life of **2** by introduction of a metabolically labile group. First, though, we opted to modify the core of **2** to see if we could influence its overall physicochemical properties. Our primary aims for this phase were to remove the sulfur atom at C-2 (toxicology concerns) and improve the aqueous solubility relative to **2**.

The first breakthrough arrived with the discovery that the sulfur atom could be replaced with oxygen if the pyridine nitrogen was concomitantly replaced by carbon. This gave the quinazolinedione (**8**), the synthesis of which is shown in Scheme 2. In **8**, an isopropyl group was employed in the 2-position as it provided a ca. 35-fold improvement in potency compared with the *tert*-butyl analogue.

Commercial 2-nitro-*p*-cymene was treated with Bredereck's reagent at 110 °C to give an enamine that was oxidized directly to the benzoic acid without prior isolation. This acid was esterified using standard methods to give the nitro ester **5**. The ester was reduced to the corresponding methyl anthranilate and iodinated to give the key intermediate **6**. Suzuki coupling, in this case using 4-chlorophenylboronic acid to give **7**, was followed by cyclization to the quinazolinedione using a three-step, one-pot procedure.

Initial profiling of **8** suggested that it was suitable as a new lead structure: e.g., MW 314.77, clogP 4.27, reaching 5317 ± 352 pmol/mL in plasma at 1 h after a 3 mg/kg p.o. dose (20% cremophor/water) to male Wistar rats, selective for TRPV1 over 30 other receptors. Although it had excellent in vitro potency (IC_{50} 6 ± 1 nM), it retained the poor solubility of **2** and returned a positive result in the mouse lymphoma chromosome aberration ("micronucleus") test. Analogue synthesis around this new lead commenced, with the aim of introducing solubilizing groups to the pendant aromatic ring and evaluating promising compounds in the micronucleus test. It did not prove possible to achieve an acceptable balance between in vitro activity and solubility

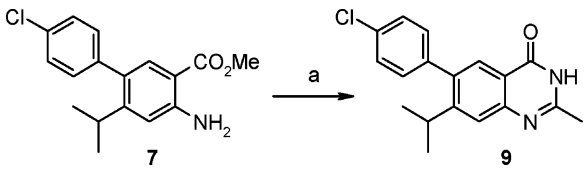
Table 1. IC_{50} Values of Selected Quinazolinones in the Human Low pH Assay[a]



cmpd	R1	R2	R3	R4	R5	IC_{50} (nM)
9	Me	H	H	Cl	H	13
10	Me	H	F	Cl	H	23
11	Me	H	OMe	Cl	H	14
12	Me	H	OCH_2CO_2H	Cl	H	>1000
13	Me	H	$O(CH_2)_2NMe_2$	Cl	H	487
14	Et	H	H	Cl	H	>1000
15	NH_2	H	H	Cl	H	133
16	Me	Cl	H	Cl	OEt	37
17	Me	H	OH	Cl	H	1343
18	Me	H	OMe	H	Cl	100
19	Me	H	OEt	Cl	H	12
20	Me	H	O^{n}Pr	Cl	H	40
21	Me	H	O^{n}Bu	Cl	H	82
22	Me	H	$O(CH_2)_2OMe$	Cl	H	216
23	Me	H	$O(CH_2)_2OH$	Cl	H	557
24	Me	H	OEt	OMe	H	286
25	Me	H	$-OCH_2O-$		H	31
26	Me	H	$OCH_2{}^c$Pr	Cl	H	50
27	Me	H	$OCH_2{}^c$Bu	Cl	H	144
28	Me	H	O^{i}Pr	Cl	H	201
29	Me	H	Cl	Me	H	42
			capsazepine			334

[a] IC_{50} values are average values of three determinations; see Supporting Information for statistical analysis.

Scheme 3. Synthesis of Quinazolinone **9** from Key Intermediate **7**[a]



[a] (a) HCl (g), MeCN, reflux (70%)

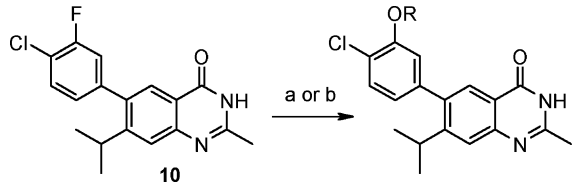
with this compound class, although the undesirable micronucleus activity could be removed by methylating the N-1 position. When the C-2 carbonyl was replaced with a methyl group to give the 2-methylquinazolinone (**9**), there were signs that the equilibrium solubility could be slightly improved (4 mg/L, pH6.8) while retaining in vitro activity (see Table 1). The synthesis of quinazolinone **9** via intermediate **7** is shown in Scheme 3.

With this new lead in hand, our strategy was to introduce solubilizing groups to the pendant aryl ring, or effect disruption of the crystal packing by introduction of bulky and/or flexible chains at the same position. Use of an alkoxy group fulfilled two roles, providing a handle for modulation of half-life via dealkylation while the additional oxygen atom was expected to marginally improve the solubility.

Late-stage variation of a 3′-alkoxy substituent was possible via thermal or microwave assisted S_NAr reactions of the 4′-chloro-3′-fluoro derivative **10** with various alkoxides, as shown in Scheme 4.

Compound **10** was prepared by Suzuki coupling of **6** (Scheme 2) with 4-chloro-3-fluorobenzeneboronic acid and subsequent acid mediated ring closure in acetonitrile, analogous to the procedure shown in Scheme 3. Other derivatives were accessed

Scheme 4. Variation of 3′-Substituent by S_NAr Displacement[a]



10

[a] (a) NaH, ROH, NMP, RT then microwave 160 °C, 10 min; (b) NaH, ROH, NMP, 60 °C, 2 h.

by Suzuki coupling of **6** with the appropriate boronic acids and subsequent ring closure, as previously described.

IC_{50} values of representative quinazolinones are shown in Table 1. In the 2-position (R1), a methyl group is optimal—extension to higher alkyl (compound **14**) or substitution with polar functionality (e.g., compound **15**) resulted in much reduced TRPV1 activity. In the 3-position an H-bond donor is essential for activity. The SAR of substitutions around the 6-position phenyl group was extensively investigated. Ortho substitutions (R2) alone are generally detrimental unless additional substitutions are made in the 3′- and 4′-positions (compound **16**).

In the 4′-position (R4) small, hydrophobic groups such as Cl, Me (**29**), OMe are tolerated. A variety of alkoxy groups (but not the parent hydroxyl, **17**) can be introduced at the meta position (R3) provided there is also a substituent at the 4′-position. A para, meta arrangement is superior to a meta, meta arrangement (compare compounds **11** and **18**). Better activity is obtained if the alkoxy chain is relatively short (compare **19**, **20**, **21**). Branching adjacent to the oxygen atom is not favorable (e.g., compare compounds **19** and **28**). Small carbocycles are tolerated by the channel, the smaller cyclopropyl ring being superior to cyclobutyl (**26**, **27**), but polar atoms other than the linking oxygen are generally not accommodated (e.g., **12**, **13**, **22**, **23**). A combination of 4′-chlorine atom with 3′-alkoxy group is superior to alkoxy groups in the corresponding positions (compare **11** and **24**) unless the alkoxy groups have additional constraint (compound **25**).

Although **26** was not the most potent member of the series and it did not have markedly improved solubility (0.8 mg/L at pH6.8, 25 °C) compared with **2**, a cross section of other properties led us to carry out further investigations in animal models of pain. In particular, **26** maintained comparable activity in the rat low pH assay (IC_{50} 105 ± 3 nM) and the intrinsic clearance values for **26** in liver microsomes in vitro predicted a moderate hepatic clearance in vivo for both man (CL_{int} 49 μL/min/mg) and rat (CL_{int} 76 μL/min/mg). A Caco-2 permeability study showed that **26** crossed the membrane passively with P_m 26.2 × 10^{-5} cm/min. **26** showed high plasma protein binding (99.4%) at a single concentration in rat plasma but was significantly less bound to human plasma proteins (94%). Compound **26** was assessed for its binding interactions with a wide range of GPCRs, kinases, transporters, and ion channels. It had significant activity at the dopamine transporter (1.1 μM), the norepinephrine transporter (2.8 μM), and at the 5HT1A (7 μM) and 1B (4.4 μM) receptors. **26** was clean in the Ames and micronucleus tests.

Selected pharmacokinetic parameters for **26** are shown in Table 2. Additionally, there was no evidence of accumulation of **26**, and absorption was shown to be linear after increasing the oral dose to 10 mg/kg.

In naïve rats, compound **26** (administered i.v. as a solution in 20% cremophor EL/saline) inhibited mechanical hyperalgesia (induced by injection of capsaicin into the hind paw) in a dose-dependent manner, indicating that in vivo it acts as an antagonist of the TRPV1 receptor (Figure 1).

Table 2. Selected Pharmacokinetic Parameters for **26**

parameter	**26** i.v. 1 mg/kg	**26** p.o. 3 mg/kg
$C_{t=2min}$ pmol/mL	3543 ± 271	
$C_{maxplasma}$ pmol/mL		1912 ± 150
$T_{maxplasma}$ h		4.0
$C_{maxbrain}$ pmol/g	1079 ± 32	1666 ± 228
$T_{maxbrain}$ h	0.5	4.0
$t_{1/2terminal}$ h	3.48	3.5
CL_{tot} mL/min/kg	5.25	
V_{ss} L/kg	1.55	
BAV		75%

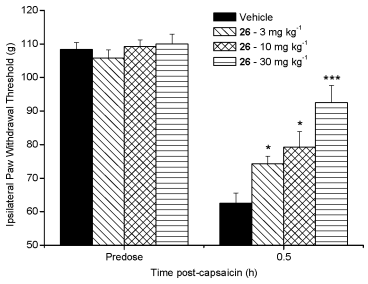


Figure 1. Inhibition of capsaicin-induced mechanical hyperalgesia by compound **26** in the rat. ***P < 0.001, *P < 0.05 compared to vehicle by ANOVA followed by Tukey's HSD test.

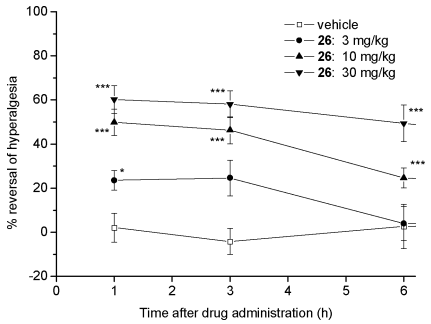


Figure 2. Activity of **26** against inflammatory hyperalgesia in the rat. ***P < 0.001, *P < 0.05 compared to vehicle by ANOVA followed by Tukey's HSD test carried out on withdrawal threshold data.

In a rat model of persistent nociceptive pain, **26** (administered p.o. as a solution in 20% cremophor/water 24h after CFA injection into the hindpaw) reversed established mechanical hyperalgesia with a ED_{50} value[16] of 4.7 mg/kg at 1 h and maximal efficacy of 60% (Figure 2).

A similar profile was observed in a rat model of neuropathic pain. Compound **26** (administered p.o. as a solution in 20% cremophor EL/water 11−15 days following partial sciatic nerve ligation) reversed established mechanical hyperalgesia in a dose dependent fashion (Figure 3). The maximal reversal was 57% with a ED_{50} of 2.6 mg/kg at 1 h.

Following repeated administration (10 and 30 mg/kg p.o., twice daily for 5 days) there was no evidence for the development of tolerance to the anti-hyperalgesic effect of **26**, and no obvious side effects.

Since compound **26** readily penetrates the blood−brain barrier, the accelerating rotarod was used to assess changes in coordination or motor function following oral administration to rats. These data indicate that **26**, at doses at least 10 times greater than those producing an anti-hyperalgesic effect, does not produce ataxia, sedation, or spasmolysis, which would result in a loss of motor coordination.

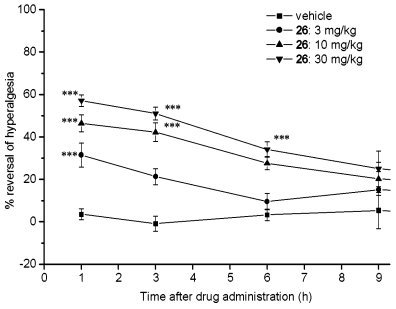


Figure 3. Oral activity of **26** against neuropathic mechanical hyperalgesia in the rat. ***$P < 0.001$ compared to vehicle by ANOVA followed by Tukey's HSD test carried out on withdrawal threshold data.

We believe that these data show that TRPV1 antagonists have great promise as new treatments for a broad range of pain states and that the 6-arylquinazolinones described are useful prototypes in the search for clinically useful compounds.

Acknowledgment. We thank Chris Brain for the original resynthesis of **1**, Alfred Schmidt, David Ruckstuhl, and Sandro Nocito for providing a large quantity of intermediate **6**, Rochdi Bouhelal for coordinating the high throughput screening effort, Christian Guenat and Eva Bürgin for HR-MS measurements, and LeighAnn Alexander, Gian Camenisch, Hans-Georg Capraro, Peter End, Bernard Faller, Stephen Fowler, Eric Francotte, Willi Frieauff, Bruno Galli, Susanne Glowienke, Michael Mutz, Appu Subramanian and Steven Whitebread for profiling **26**.

Supporting Information Available: ^{1}H NMR, HPLC, and HRMS characterization data for all compounds, elemental analysis for **26**, and details of in vitro and in vivo assays including expanded pharmacokinetic data. This information is available free of charge via the Internet at http://pubs.acs.org.

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(16) ED_{50} is defined here as the dose required to bring about 50% of the maximal antihyperalgesic effect.

(17) Subsequent IC_{50} values refer to human low pH data unless stated otherwise. Blockade of low pH induced TRPV1 activation may be predictive of antihyperalgesic activity in vivo; see ref 9.

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